Prospective Study of Hyperhomocysteinemia as an Adverse Cardiovascular Risk Factor in End-Stage Renal Disease

Ali Moustapha, MD; Arabi Naso, MD; Maher Nahlawi, MD; Anjan Gupta, MD; Kristopher L. Arheart, EdD; Donald W. Jacobsen, PhD; Killian Robinson, MD; Vincent W. Dennis, MD

Background—Retrospective and case-control studies show that hyperhomocysteinemia is an independent risk factor for atherosclerosis in patients with end-stage renal disease. We studied prospectively the association between total homocysteine and cardiovascular outcomes.

Methods and Results—In all, 167 patients (93 men, 74 women; mean age, 56.3±14.7 years) were followed for a mean duration of 17.4±6.4 months. Cardiovascular events and causes of mortality were related to total homocysteine values and other cardiovascular risk factors. Cox regression analysis was used to identify the independent predictors for cardiovascular events and mortality. Fifty-five patients (33%) developed cardiovascular events and 31 (19%) died, 12 (8%) of cardiovascular causes. Total plasma homocysteine values ranged between 7.9 and 315.0 μmol/L. Levels were higher in patients who had cardiovascular events or died of cardiovascular causes (43.0±48.6 versus 26.9±14.9 μmol/L, P=.02). The relative risk (RR) for cardiovascular events, including death, increased 1% per μmol/L increase in total homocysteine concentration (RR, 1.01; CI, 1.00 to 1.01; P=.01).

Conclusions—These prospective observations confirm that hyperhomocysteinemia is an independent risk factor for cardiovascular morbidity and mortality in end-stage renal disease, with an increased RR of 1% per μmol/L increase in total homocysteine concentration. Interventional studies are needed to evaluate the possible effects of modifying this risk factor in these patients. (Circulation. 1998;97:138-141.)

Key Words: homocysteine ■ risk factors ■ kidney ■ atherosclerosis ■ thrombosis

The 1-year mortality rate for patients on dialysis in the United States between 1991 and 1993 was 23%, with cardiovascular and cerebrovascular diseases accounting for ~47% of these deaths.1 Case-control studies show that high total plasma homocysteine (tHcy) concentrations (>14.5 μmol/L) increase the risk for vascular events in these patients independent of other known risk factors such as diabetes, hypertension, hypercholesterolemia, and smoking.2-4 This study examines prospectively the association between tHcy and cardiovascular events in patients with end-stage renal disease (ESRD).

Methods

Subjects
We studied 176 patients with ESRD on dialysis for at least 90 days. One hundred thirty were on hemodialysis and 46 on peritoneal dialysis. We previously reported the association between homocysteine values and vascular events in these patients by use of a case-control design.1

Total Plasma Homocysteine
Baseline predialysis tHcy values were determined between March and April 1995 by the method of Jacobsen et al.3

Risk Factors for Vascular Disease
Total fasting cholesterol concentrations were measured on the same samples as used for measurement of homocysteine. Hypercholesterolemia, hypertension, diabetes mellitus, and smoking status were defined in our previous report.4

Diagnostic Criteria for Vascular Events
All vascular events that occurred after homocysteine concentrations were originally measured in this patient population were documented. Clinical criteria were used for the diagnosis of these events, which were confirmed by standard tests.

Thromboembolic Episodes
Device-related or venous thrombosis was diagnosed by contrast angiography and/or duplex ultrasound. Unexplained ischemic stroke was diagnosed by clinical presentation, CT, and MRI.
Atherosclerosis

Coronary Artery Disease Events
New coronary artery disease events that developed after the measurement of the baseline homocysteine concentration were diagnosed in the presence of (1) documented myocardial infarction or unstable angina, (2) a stenosis of ≥70% of at least one major epicardial coronary vessel at coronary angiography, (3) an abnormal cardiac functional test defined as a detectable wall motion abnormality on dobutamine echocardiography or the presence of reversible perfusion defects on perfusin or sestamibi thallium tests, or (4) a requirement for coronary revascularization by percutaneous angioplasty or bypass surgery.

Peripheral Vascular Disease
Peripheral vascular disease was diagnosed by the development of intermittent claudication, accompanied by diminished pulses on clinical examination and combined with measurements of peripheral vascular resistance and/or angiography.

Cerebrovascular Disease
New-onset cerebrovascular disease was always suspected in patients with recent onset of new neurological symptoms, eg, aphasia, focal deficits, or unilateral paresis and was confirmed by CT or MRI.

Cardiovascular Death
Cardiovascular death was confirmed from death certificates as well as hospital and other medical records and observers’ accounts. Sudden death was included as a cardiovascular event.

Statistical Analysis
Descriptive statistics are reported as frequency and percent for categorical data and as mean and SD or median and interquartile range for continuous data. Percentages were compared by Pearson’s chi-square test or Fisher’s exact test, depending on the frequencies. Student’s t test was used to compare continuous variables. Cox regression was used to evaluate the significance of risk factors for cardiovascular events and for a composite end point of cardiovascular events and cardiovascular mortality. To ascertain the effect of arteriovenous fistula thrombosis on the risk attributable to tHcy, we built two separate models. Age, sex, and homocysteine were always included. A stepwise procedure was used to choose other significant risk factors for the model from a set of variables that included smoking, diabetes, hypertension, total cholesterol, LDL cholesterol, and creatinine. Risk ratios and 95% confidence limits are reported. Statistical test results having a probability of ≤.05 are considered statistically significant.

Results
There were 176 patients with ESRD at baseline. Nine (5%) were lost to follow-up. The remaining 167 patients were followed for a mean duration of 17.4±6.4 months. Their mean age was 56.3±14.7 years, and 93 (56%) were men. Hypertension and diabetes were present in 142 patients (85%) and 55 patients (33%), respectively. Fifty-nine patients (35%) had a history of current smoking or of ever having smoked. Mean cholesterol and creatinine concentrations were 189.3±71.2 and 12.2±4.4 mg/dL, respectively. Mean tHcy concentration was 32.7±32.2 μmol/L, and median concentration was 25.4 μmol/L (interquartiles, 19.7 and 33.5 μmol/L). Ninety percent of patients were hyperhomocysteinemic, with values >14.5 μmol/L. The demographic data for patients in each quartile of tHcy are shown in Table 1.

Relationship of Homocysteine to Cardiovascular Outcomes
Of the 167 patients, 55 (33%) had one or more cardiovascular complications within the follow-up period. Overall, 31 patients (19%) died, 12 (8%) of cardiovascular causes (see Table 2). tHcy concentrations were higher, on average, in patients with cardiovascular complications than in those without (43.4±50.6 versus 27.4±14.9 μmol/L, P=.03). Similarly, mean tHcy concentration was higher in the group of patients who had adverse cardiovascular events (complication or death) than in those who did not (43.0±48.6 versus 26.9±14.9 μmol/L, P=.02). Patients in the upper quartile of homocysteine values (>33.6 μmol/L) were more likely to develop a cardiovascular complication than patients in the lower three quartiles (18 of 40, 45%, versus 37 of 127, 29%; P=.06). Similarly, cardiovascular deaths were more common in patients in the upper quartile of tHcy values than in those in the lower three quartiles (7 of 40, 18%, versus 5 of 116, 4%; P=.01). Cox regression analysis showed that tHcy (RR, 1.01; CI, 1.00 to 1.01; P=.01) and diabetes mellitus (RR, 2.38; CI, 1.43 to 3.96; P<.01) were independent predictors for cardiovascular complications or death. This corresponds to an increase in relative risk for cardiovascular events or death of 1%.

### TABLE 1. Demographic and Clinical Characteristics of Study Patients in Each Quartile of Total Homocysteine Concentration

<table>
<thead>
<tr>
<th>Variable</th>
<th>Quartile 1 (n=41)</th>
<th>Quartile 2 (n=42)</th>
<th>Quartile 3 (n=42)</th>
<th>Quartile 4 (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57.4±12.6</td>
<td>56.9±12.7</td>
<td>57.6±15.0</td>
<td>53.4±18.0</td>
</tr>
<tr>
<td></td>
<td>(50.0-67.0)</td>
<td>(47.0-67.0)</td>
<td>(45.0-73.0)</td>
<td>(37.0-71.0)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>46</td>
<td>52</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>90</td>
<td>86</td>
<td>86</td>
<td>79</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>34</td>
<td>36</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>Ever smoked, %</td>
<td>66</td>
<td>21</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Total homocysteine, μmol/L</td>
<td>15.1±3.1</td>
<td>22.5±1.6</td>
<td>28.7±2.4</td>
<td>64.0±52.5</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>10.0±4.2</td>
<td>12.1±3.7</td>
<td>13.2±4.2</td>
<td>13.5±4.7</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>212.5±87.4</td>
<td>183.5±64.0</td>
<td>173.9±60.1</td>
<td>177.5±66.3</td>
</tr>
<tr>
<td></td>
<td>(146.0-246.0)</td>
<td>(147.0-234.0)</td>
<td>(131.0-194.0)</td>
<td>(121.0-211.0)</td>
</tr>
</tbody>
</table>

Values are mean±SD and median (Q25-Q75).
TABLE 2. Cardiovascular Complications and Causes of Cardiovascular Death in Study Patients

<table>
<thead>
<tr>
<th>Cardiovascular Outcome</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular complications</td>
<td>76 (100)*</td>
</tr>
<tr>
<td>Arteriovenous fistula thrombosis</td>
<td>22 (29)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>16 (21)</td>
</tr>
<tr>
<td>Stroke</td>
<td>14 (18)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>14 (18)</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>5 (7)</td>
</tr>
<tr>
<td>Arterial thrombosis</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Coronary artery bypass graft surgery</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Cardiovascular death (ICD.9 code)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Myocardial infarction (410.9)</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Sudden death (798.1)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Stroke (436)</td>
<td>1 (8)</td>
</tr>
</tbody>
</table>

*Fifty-five patients suffered a total of 76 complications.

per μmol/L increase in tHcy concentration. When arteriovenous fistula thrombosis events were excluded from the cardiovascular events, analysis showed that tHcy (RR, 1.01; CI, 1.00 to 1.01; \( P = .03 \)) and diabetes (RR, 2.27; CI, 1.23 to 4.20; \( P = .01 \)) remained independent predictors for cardiovascular complications or death. The Figure depicts the probability for event-free survival for patients with mean homocysteine concentrations of 10, 33, and 100 μmol/L.

**Discussion**

This study extends our previous case-control analysis and confirms prospectively that there is a relationship between homocysteine concentrations and vascular complications in patients with ESRD. There was a high incidence of cardiovascular complications, including death, in this population, which is characterized by high prevalences of hypertension, hypercholesterolemia, diabetes, and smoking. In both retrospective and prospective studies, hyperhomocysteinemia is a common and independent risk factor for cardiovascular disease, and case-control studies indicate a similar association in ESRD.

In this study, patients who developed cardiovascular complications had higher baseline homocysteine concentrations, on average, than those who did not develop cardiovascular events. Independent predictors for cardiovascular complications or death included increased homocysteine concentration, diabetes mellitus, and increasing age. The relative risk for the occurrence of cardiovascular events or death increased 1% for each μmol/L increase in homocysteine concentration.

The mechanisms by which homocysteine enhances thrombosis and atherosclerosis remain uncertain. The major focus of current studies, however, is on endothelium as the site of initiation of vascular damage. Postulated mechanisms include direct endothelial cytotoxicity or encouragement of smooth muscle cell growth and inhibition of endothelial cell proliferation. Oxidant stress may play a role in endothelial cell damage. In monkeys, diet-induced hyperhomocysteinemia is associated with altered endothelium-dependent vascular function, and in humans, homocysteine may inhibit endothelium-dependent dilation, suggesting interference with nitric oxide.

Limitations of the present study include the relatively short duration of follow-up (18 months) and the small patient numbers, which did not permit analysis of subsets such as women or the elderly. Assessment of the relative contribution of homocysteine levels to the risk of thrombotic compared with atherosclerotic events was also difficult. Nevertheless, the data demonstrate the expected rates of overall cardiovascular events according to prevailing homocysteine levels in ESRD and will be useful in developing interventional studies.

In summary, prospective observations confirm that patients with increased homocysteine concentrations are more likely to develop fatal or nonfatal thrombotic or atherosclerotic complications. Because homocysteine concentrations can be reduced by the administration of folic acid either alone or combined with vitamin B₆ or B₁₂, interventional studies are now justified to evaluate such treatment.

**Acknowledgments**

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**References**


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