Relationship Between Homocysteine and Mortality in Chronic Kidney Disease

Vandana Menon, MD; Mark J. Sarnak, MD; Tom Greene, PhD; Xuelei Wang, MS; Arema A. Pereira, MD; Gerald J. Beck, PhD; John W. Kusek, PhD; Jacob Selhub, PhD; Allan J. Collins, MD; Andrew S. Levey, MD; Michael G. Shlipak, MD

Background—The relationship between total homocysteine (tHcy) and outcomes has not been investigated in patients with chronic kidney disease stages 3 to 4.

Methods and Results—The Modification of Diet in Renal Disease Study was a randomized, controlled trial of 840 patients. Serum tHcy was measured in frozen samples collected at baseline (n=804). Survival status and cause of death were obtained from the National Death Index. To evaluate its association with all-cause and cardiovascular disease (CVD) mortality, tHcy was evaluated both as tertiles (<14.7, 14.7 to 19.5, ≥19.6 μmol/L) and as a continuous variable (per 10/μmol/L). Participants had a mean age of 52±12 years and glomerular filtration rate (GFR) of 33±12 mL/min per 1.73 m²; 60% were male, and 85% were white. During a median follow-up of 10 years, 195 (24%) died from any cause, and 118 (15%) from CVD. The level of GFR was lower and proteinuria higher in the highest tHcy tertile. There was no association between the highest tertile of tHcy and all-cause (hazard ratio [HR]; 95% confidence interval [CI], 1.32, 0.94 to 1.85) or CVD (HR; 95% CI, 1.50, 0.96 to 2.34) mortality in univariate analyses; this association was further attenuated by adjustment for GFR (HR; 95% CI all-cause, 1.04, 0.72 to 1.51; CVD, 1.20, 0.73 to 1.95). There was no association between tHcy as a continuous variable and all-cause (0.98, 0.83 to 1.16) or CVD (1.04, 0.85 to 1.27) mortality.

Conclusions—Hyperhomocystinemia does not appear to be a risk factor for all-cause or CVD mortality in the Modification of Diet in Renal Disease Study. Prior studies demonstrating an association between tHcy and CVD risk may have inadequately adjusted for the confounding effects of kidney function. (Circulation. 2006;113:1572-1577.)

Key Words: kidney ■ mortality ■ epidemiology
study the effect of dietary protein restriction and blood pressure control on the progression of kidney disease. Details of the study have been published previously. In brief, 585 patients with a baseline GFR of 25 to 55 mL/min per 1.73 m² were randomized in study A, and 255 patients with a baseline GFR of 13 to 24 mL/min per 1.73 m² were randomized in study B. Patients in study A and study B were combined for the current analyses.

As described previously, levels of total Hcy were measured in frozen samples collected at baseline from 804 participants of the MDRD Study cohort. Samples were assayed for tHcy by using high-performance liquid chromatography with fluorometric detection. GFR was assessed by the kidney clearance of [125I]-iothalamate.

Survival status and cause of death were ascertained from the National Death Index. A death was ascribed to CVD if the primary cause of death was International Classification of Diseases, 9th Revision (ICD-9) code 390 to 459 (n = 96) or if kidney disease was listed as the primary cause of death and CVD was the secondary cause (n = 22). Survival time was defined as time from randomization to death or end of follow-up (December 31, 2000). Data collection procedures were approved by the Cleveland Clinic and Tufts–New England Medical Center Institutional Review Boards.

Statistical Analysis
Demographic, CVD risk factors, and kidney disease factors were compared across tertiles of tHcy through the use of the χ² test for categoric data, ANOVA for approximately normally distributed continuous variables, and the Kruskall–Wallis test for skewed continuous variables.

Incidence rates for mortality (per 1000 person-years) were calculated for tertiles of tHcy. Unadjusted and 3 adjusted Cox proportional hazards models were conducted in sequence to evaluate the association of tHcy with all-cause and CVD mortality. Model 1 adjusted for GFR only; model 2 added age, gender, race, and random assignments to protein diets and blood pressure targets; model 3 added proteinuria, cause of kidney disease, history of coronary artery disease and diabetes, and body mass index. We calculated the proportional change in the Cox regression coefficients for tHcy after addition of GFR to quantify its attenuation.

To maximize statistical power to examine the relationship between tHcy and mortality, continuous variable analyses were conducted with hazard ratios (HR) presented per tHcy higher by 10 μmol/L. Given the non-normal distribution of tHcy, we repeated the analyses by using log-transformed values for tHcy. Because the results from these analyses were very similar to the ones using untransformed tHcy, we present the latter for ease of interpretation. We also evaluated interactions between tHcy and study (study A versus study B) and between tHcy and GFR to test for any potential differential effects of severity of kidney disease on the relationships examined.

Proportional hazards assumptions were tested by using log-minus-log survival plots and plots of Schoenfeld residuals versus survival time.

Additional Analyses
The fully adjusted Cox models (model 3) were repeated for all-cause and CVD mortality after the exclusion of individuals with prevalent CVD (defined as history of coronary artery disease, n = 77).

Because dietary protein intake may modify tHcy levels and use of random assignments to protein diet to represent protein intake may not fully account for this effect, we repeated the fully adjusted Cox model (model 3) for all-cause and CVD mortality using adjusted protein intake estimated from urine urea nitrogen excretion at 1 year and with tHcy as a continuous variable.

C-reactive protein (CRP) and albumin may be related to mortality in CKD; therefore, we repeated model 3 with the addition of CRP and albumin to assess any potential effect of these covariates on the association between tHcy as a continuous variable and mortality. Homocysteine may be related to progression of kidney disease, and kidney failure may modify the relationship between tHcy and mortality outcomes. Therefore, Cox models (corresponding to model 3) were repeated with tHcy as a continuous variable and using two additional outcomes. Cox regression was performed with the outcome of death before reaching kidney failure (need for renal replacement therapy with dialysis or transplantation) with censoring at kidney failure or end of follow-up and using a composite outcome of death or kidney failure.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Baseline Characteristics
The study cohort had an average age of 52 ± 12 years, GFR of 33 ± 12 mL/min per 1.73 m², and median (interquartile range) tHcy of 16.8 (8) μmol/L. The sample was predominantly white (91%); 60% were male, and 15% were current smokers. Higher tHcy was associated with male gender, history of coronary artery disease, lower body mass index, and lower prevalence of diabetes (Table 1). Glomerular filtration rate was lower and level of proteinuria higher in the higher tHcy groups; however, there was no difference in the cause of kidney disease.

Outcomes
Median follow-up for survival analyses was 10 years (range, 3 to 140 months). All-cause mortality rate was 24% (n = 195), and CVD mortality rate was 15% (n = 118). There were 60 (22%), 58 (22%), and 77 (29%) deaths from all causes and 33 (12%), 37 (14%) and 48 (18%) deaths caused by CVD in the respective tertiles. Incidence rates for all-cause and CVD mortality, per tertile, are presented in the Figure.

There was a nonsignificant positive association between the highest tertile of tHcy and both all-cause and CVD mortality in unadjusted analyses that was largely attenuated by adjustment for GFR (Table 2). Additional adjustment for random assignments, demographic, CVD, and other kidney disease factors had little incremental effect on attenuating the association between the high tHcy tertile and each mortality outcome.

After adjustment for GFR, the Cox regression coefficient for high tHcy and all-cause mortality decreased from 0.278 to 0.037, a proportional change of 87%. Similarly, for CVD mortality adjusting for GFR decreased the Cox regression coefficient from 0.406 to 0.179, a proportional change of 56%.

The Cox model was repeated evaluating tHcy as a continuous variable (Table 3). Again, there was no association between tHcy and either all-cause or CVD mortality in unadjusted or adjusted analyses. In models examining all-cause and CVD mortality, respectively, interaction terms between study (study A versus study B) and tHcy (P = 0.70; P = 0.69) and GFR and tHcy (P = 0.73, P = 0.93) were not significant.

Additional Analyses
There was no association between tHcy and all-cause (0.89, 0.69 to 1.15) or CVD (0.82, 0.57 to 1.18) mortality in the subgroup of participants without history of prevalent CVD.

Substituting adjusted protein intake at 1 year for random assignments to low protein diet in model 3 did not alter the results for all-cause (1.00, 0.85 to 1.18) or CVD mortality (1.05, 0.86 to 1.29). The addition of CRP and albumin also as covariates did not
appreciably alter the HR for tHcy in models examining all-cause mortality (1.00, 0.85 to 1.17) or CVD mortality (1.05, 0.87 to 1.27).

Sixty-five participants died before reaching kidney failure. There was no association between tHcy and death before kidney failure in a univariate Cox model (1.06, 0.81 to 1.39). We did not perform multivariate analyses because of limited power, given the small number of events.

Five hundred ninety-three participants died or reached kidney failure by December 31, 2000. There was no association between tHcy and the composite outcome of kidney failure or death in the fully adjusted Cox model (1.02, 0.94 to 1.11).

Discussion

In this cohort of patients with CKD stages 3 to 4, tHcy does not appear to be an independent predictor of all-cause or CVD mortality. In unadjusted analysis, a nonsignificant positive association was observed between elevated tHcy and the mortality outcomes that was largely attenuated with adjustment for GFR. These findings raise the question of whether prior studies that found tHcy to be a CVD risk factor adequately adjusted for kidney function.

The results of prospective cohort studies investigating hyperhomocysteinemia as an independent risk factor for the development of CVD have been conflicting. A recent study found that hyperhomocysteinemia, defined as tHcy levels >15 μmol/L, was an independent predictor of CVD mortality in a large cohort of patients with type 2 diabetes. In a population-based cohort of women, participants in the highest quintile for Hcy were at increased risk for fatal and nonfatal acute myocardial infarctions. Homocysteine was an independent risk factor for all-cause mortality in Jewish men and women aged 50 years and older, in patients with angiographically confirmed coronary artery disease, and in the Physicians Health Study. However, other studies suggest that the relationship between Hcy and CVD exists only in specific subgroups such as those with a previous history of CVD, in the elderly, in patients with type 2 diabetes, and in patients with hypertension.

None of the studies that demonstrated an association between tHcy and outcomes adjusted for level of kidney function with the gold standard of GFR as in this study. One study used estimated creatinine clearance, some used serum creatinine, and others did not adjust for any marker of kidney function. The prevalence of CKD may have been high in several of these studies because they included the elderly, hypertensive patients, and patients with heart disease and type 2 diabetes, all populations at high risk for kidney disease. Underlying kidney dysfunction may thus have confounded the association of tHcy and cardiovascular out-

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics by Tertiles of Homocysteine</th>
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<tbody>
<tr>
<td>Homocysteine, μmol/L*</td>
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<tr>
<td>Demographic factors</td>
</tr>
<tr>
<td>Age, y†</td>
</tr>
<tr>
<td>Male, %</td>
</tr>
<tr>
<td>White, %</td>
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<tr>
<td>Current smoker, %</td>
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<tr>
<td>CVD risk factors</td>
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<tr>
<td>History of diabetes, %</td>
</tr>
<tr>
<td>History of coronary artery disease, %</td>
</tr>
<tr>
<td>Body mass index, kg/m²†</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg†</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL†</td>
</tr>
<tr>
<td>Kidney disease factors</td>
</tr>
<tr>
<td>GFR, mL/min per 1.73m²†</td>
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<tr>
<td>Proteinuria, g/d*</td>
</tr>
<tr>
<td>Kidney disease category, %</td>
</tr>
<tr>
<td>Glomerular disease</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

*Median (interquartile range). †Mean ± SD.
comes in these studies because serum creatinine is a less sensitive marker of kidney function.21

In data from the Hoorn Study, tHcy was associated with increased risk of coronary events in diabetic but not nondiabetic individuals.22 The association in diabetics persisted after adjustment for estimated GFR, calculated using the MDRD Study formula, but was attenuated by adjustment for history of cardiovascular disease. In a study of patients with type 2 diabetes, tHcy was a significant predictor of all-cause but not CVD mortality after adjustment for estimated GFR, calculated using the MDRD Study formula, but comes in these studies because serum creatinine is a less sensitive marker of kidney function.21

In contrast to the previously cited studies, other studies have failed to demonstrate an independent relationship between Hcy and CVD. An analysis from the Atherosclerosis Risk in Communities Study8 and a population-based study from Australia did not find an association between Hcy and CVD.24 Investigators from the Multiple Risk Factor Intervention Trial found no association between Hcy and fatal and nonfatal CVD events.25 Of these studies with a null result, two adjusted for serum creatinine,20,24 whereas two others did not include markers of kidney function in their multivariate analyses.8,25 Of note, these studies finding no association were conducted in relatively healthy cohorts that were likely to have low prevalence of CKD.26

Two meta-analyses have examined the associations between Hcy levels and ischemic heart disease. A publication from the Homocysteine Studies Collaboration using 30 prospective and retrospective studies found a 3-μmol/L lower Hcy level was associated with an 11% lower risk of ischemic heart disease and 19% lower risk of stroke.27 In another meta-analysis that included 20 prospective studies, a 5-μmol/L increase in Hcy was associated with a 32% increase in the risk of ischemic heart disease.28 Neither of these analyses adjusted for level of kidney function.

There are limited data evaluating Hcy as a risk factor for CVD in patients with CKD before reaching kidney failure. In a cohort of 147 patients with creatinine clearances between 20 and 55 mL/min per 1.73 m², high Hcy was an independent risk factor for incident CVD.29 However, tHcy was only available in a subset of 93 patients, and estimated creatinine clearance was the proxy for kidney function. In a cohort of 227 stable kidney transplant recipients (average serum creatinine, 1.9 ±0.8 mg/dL), Ducloyx et al30 found an independent association between Hcy and a composite outcome of fatal and nonfatal CVD events. However, serum creatinine was used as the marker of kidney function in this analysis, leaving the possibility that actual GFR may still confound the association. One prospective study of 733 kidney transplant recipients using a body surface area–adjusted measure of kidney function estimated using the Cockroft-Gault formula (mean=55.8 mL/min per 1.73 m²) found an association between Hcy levels and all-cause mortality that was independent of kidney function.31 Differences in the results from this study compared with our study may be attributable to cohort differences including different kidney disease severity and burden of preexisting CVD. Alternatively, Hcy may have a differential impact as a risk factor for CVD in patients who have received transplantation.

Several studies have examined the association between elevated levels of tHcy and outcomes in dialysis patients, with contradictory findings. Although a few studies have found high tHcy to be a risk factor for outcomes,32–34 others have failed to demonstrate this association,35 and some have described an inverse relationship between tHcy levels and outcomes.36–38 However, it must be acknowledged that the dialysis population is very different from patients with stage 3 to 4 CKD, and, moreover, it is not possible to study the effect of kidney function per se in this population.

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**Table 2. Relationship Between Tertiles of Homocysteine and All-Cause and Cardiovascular Mortality**

<table>
<thead>
<tr>
<th>All-Cause Mortality (HR, 95% CI)*</th>
<th>CVD Mortality (HR, 95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy Tertile ‡</td>
<td>Hcy Tertile 3‡</td>
</tr>
<tr>
<td>[14.7–19.5 μmol/L]</td>
<td>[19.6–131.0 μmol/L]</td>
</tr>
<tr>
<td>Unadjusted 0.94 (0.65–1.34)</td>
<td>1.32 (0.94–1.85)</td>
</tr>
<tr>
<td>Adjusted 0.83 (0.57–1.20)</td>
<td>1.04 (0.72–1.51)</td>
</tr>
<tr>
<td>Model 1‡ 0.88 (0.61–1.27)</td>
<td>1.14 (0.79–1.64)</td>
</tr>
<tr>
<td>Model 2‡ 0.86 (0.59–1.26)</td>
<td>0.98 (0.66–1.47)</td>
</tr>
<tr>
<td>Model 3‡ 0.86 (0.59–1.26)</td>
<td>0.98 (0.66–1.47)</td>
</tr>
</tbody>
</table>

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**Table 3. Relationship Between Serum Homocysteine and All-Cause and Cardiovascular Mortality**

<table>
<thead>
<tr>
<th>All-Cause Mortality</th>
<th>CVD Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, 95% CI*</td>
<td>P</td>
</tr>
<tr>
<td>HR, 95% CI*</td>
<td>P</td>
</tr>
<tr>
<td>Unadjusted 1.03 (0.91–1.18) 0.63</td>
<td>1.05 (0.90–1.23) 0.53</td>
</tr>
<tr>
<td>Adjusted Model 1† 0.95 (0.81–1.11) 0.52</td>
<td>0.97 (0.80–1.18) 0.78</td>
</tr>
<tr>
<td>Model 2‡ 1.02 (0.87–1.19) 0.81</td>
<td>1.07 (0.89–1.29) 0.48</td>
</tr>
<tr>
<td>Model 3§ 0.98 (0.83–1.16) 0.82</td>
<td>1.04 (0.85–1.27) 0.74</td>
</tr>
</tbody>
</table>

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*Hazard ratios and 95% CI are calculated per 10-μmol/L increase in serum homocysteine levels.
†Adjusted for GFR.
‡Adjusted for model 1 covariates plus age, gender, race, and random assignment to diet and blood pressure strata.
§Adjusted for model 2 covariates plus cause of kidney disease, proteinuria, history of coronary artery disease and diabetes, and body mass index.
We and other investigators have reported that GFR is a major determinant of Hcy levels in the general population as well as in patients with CKD.1,11,30,40 In the present study, adjustment for GFR attenuated most of the relationship between tHcy and mortality, suggesting that GFR was the primary confounder for the observed association of high tHcy levels and the mortality end points. Reduced GFR is a known important risk factor for CVD10; thus, the varied measurement and adjustment for kidney function in prior literature may largely explain the heterogeneity of study results.

It is also possible that tHcy may be a risk factor for progression of kidney disease and thereby promote the development of CVD. In support of this hypothesis, in a population-based cohort, tHcy was found to be a predictor of the development of microalbuminuria in nondiabetic individuals, and this relationship was independent of GFR.41 Conversely, studies in patients with kidney disease, including data from the MDRD Study, have failed to demonstrate any association between tHcy and progression.13,42,43 In our study, we did not find a relationship between tHcy and a composite outcome of death or kidney failure.

Another possibility is that our null findings could be specific to the population we studied. The MDRD Study cohort consists of nondiabetic, predominantly white patients, an overrepresentation of patients with polycystic kidney disease, with few older adults and a low burden of CVD. In addition, we were unable to assess nonfatal CVD events, which may have different associations with Hcy than mortality end points. As described in a previous publication,13 a 2-hour delay in sample processing may in theory result in a systematic measurement error resulting in elevated tHcy levels caused by release of Hcy from red blood cells. However, we have previously demonstrated that the range of tHcy levels, prevalence of hyperhomocysteinemia, and magnitude of associations with known variables such as folic acid and GFR in our sample are very similar to those previously reported.1 We also acknowledge that the relatively wide confidence intervals surrounding the hazard ratios preclude definitively ruling out an association between tHcy and outcomes. Finally, it must be recognized that the majority of the study participants reached kidney failure requiring dialysis during follow-up. Kidney failure is known to be associated with increased tHcy levels, and this may have influenced the associations studied. However, the lack of association between tHcy and mortality in participants who died before reaching kidney failure suggests that baseline tHcy is not a major determinant of outcomes in this cohort of patients with stage 3 to 4 CKD.

The major strength of this study is the inclusion of a large cohort of patients with precise measures of kidney function. In addition, this study sample has a wide range of tHcy levels, includes a large proportion individuals with abnormal tHcy values,1 and a high number of outcomes during follow-up. This is a relatively healthy CKD population; however, this makes it an ideal population to assess a potentially independent effect of tHcy on mortality in the absence of powerful confounders such as diabetes and preexisting CVD.

In summary, tHcy does not appear to be a risk factor for all-cause or CVD mortality in the MDRD Study cohort. Homocysteine may be a marker for severity of kidney disease rather than a mediator for the development of CVD, in patients with CKD stages 3 to 4. Prior studies demonstrating an association between tHcy and CVD risk may have been confounded by their less precise measurements of GFR.

Acknowledgments

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Disclosures

None.

References


CLINICAL PERSPECTIVE
Previous studies in the general population have suggested an association between high homocysteine levels and adverse outcomes such as cardiovascular disease and mortality. There is a close correlation between kidney function and levels of homocysteine, and kidney disease is associated with high prevalence of hyperhomocysteinemia. Reduced kidney function is recognized as an independent risk factor for cardiovascular disease and mortality. Although many of the previous studies demonstrating an association between high homocysteine levels and outcomes were conducted in populations at high risk for kidney disease, such as the elderly, patients with diabetes, and patients with preexisting cardiovascular disease, few of them adequately adjusted for kidney function. A few used imprecise measures of kidney function such as serum creatinine or estimated creatinine clearance. In this study, we examined the association between homocysteine levels and outcomes in a cohort of patients with chronic kidney disease stages 3 to 4 (before reaching kidney failure), using precise measures of glomerular filtration rate (GFR), the gold standard for measurement of kidney function. There was a modest but nonsignificant association between the highest tertile of homocysteine and all-cause and cardiovascular disease mortality in unadjusted analyses. This relationship was largely attenuated by adjustment for GFR. The observed relationship between homocysteine and outcomes may be accounted for by its association with kidney function. Prior studies demonstrating an association between homocysteine and cardiovascular disease risk may have inadequately adjusted for the confounding effects of kidney function.
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