“New” cardiovascular risk factors in patients with chronic kidney disease: Role of folic acid treatment

BEATRIZ BAYÉS, MARI CRUZ PASTOR, JORDI BONAL, and RAMÓN ROMERO

Department of Nephrology, and Department of Clinical Biochemistry, Hospital Universitari “Germans Trias I Pujol,” Badalona, Spain


Cardiovascular disease (CVD) is the principal cause of mortality in patients with chronic renal disease undergoing hemodialysis. In addition to the CVD risk factors, a new hypothesis has recently been aroused related to “new” factors involved in the development of atherosclerosis in the uremic patient; worthwhile mentioning are the homocysteine, inflammation, and oxidative stress, among others. The potential utility of the folic acid in the hyperhomocysteinemia control is well known, although its mechanism of action, either as antioxidant or anti-inflammatory, has not been established.

Our results confirm that the patients undergoing dialysis demonstrate hyperhomocysteinemia, an increased inflammatory status, and an increase of the lipid peroxidation markers. The administration of IV folinic acid induces a reduction of homocysteine levels subordinate to the inflammatory status of the patient. Additionally, although no inflammatory effects were shown, the results provide evidence for the antioxidant effect of IV folinic acid administration by reducing the lipid peroxidation marker levels.

The statistic analysis demonstrates no correlation among the 3 markers, in spite of its higher levels in these particular patients. Homocysteine does not independently predict mortality in patients taking oral folic acid. Nevertheless, the PCR (an inflammation marker) and the antibody antioxidative-LDL (a lipidic peroxidation marker) show a good prediction of mortality at the 24-month follow-up analysis.

The knowledge of these “new” CV risk factors, as well as the factors that influence them, could be useful to prevent the development of atherosclerosis in patients with chronic renal disease.

Cardiovascular disease (CVD) is very common in kidney patients, and is, in fact, the main cause of morbidity and mortality in this population. Classic risk factors (diabetes mellitus, smoking, dyslipidemia, high blood pressure, sedentary lifestyle, and obesity) and cardiovascular risk factors related to uremia (hypervolemia, anemia, hyperdynamic status induced by vascular access, and secondary hyperparathyroidism) do not fully explain the high incidence of cardiovascular disease in these patients [1]. More data continue to arise which suggest that other factors, known as “emergent,” “new,” or “nonclassic,” must play a role [2]. Among these “new” cardiovascular risk factors, homocysteine, inflammation markers (C-reactive protein), and oxidative stress should be emphasized.

“NEW” CARDIOVASCULAR RISK FACTORS: HOMOCYSTEINE, LIPID PEROXIDATION, AND INFLAMMATION

Homocysteine is an amino acid derived from the conversion of methionine into cysteine. The participation of different vitamins is required for its metabolism. In the trans-sulfuration route, homocysteine is transformed into cysteine by 2 vitamin B6-dependent reactions. In the remethylation route, homocysteine is methylated to form methionine by 2 independent metabolic routes in which the enzymes 5-methyltetrahydrofolate-homocysteine S-methyltransferase (methionine synthase) and betaine:homocysteine methyltransferase participate. Methionine synthase requires the 5-methyltetrahydrofolic acid as co-substrate and methylcobalamin as a cofactor.

Kidney patients present hyperhomocysteinemia as a result of delayed elimination and altered metabolism (i.e., remethylation). Thus, while the prevalence of hyperhomocysteinemia (plasma homocysteine levels >15 μmol/L) in the general population is approximately 5%, in dialysis patients it reaches 80% to 90% [3, 4].

Several studies have analyzed the role of homocysteine in atherosclerosis development, and it has recently been suggested that moderate hyperhomocysteine may damage endothelial cell function in various ways. A possible cellular and molecular mechanism of endothelial lesion is oxidative damage [5]. Hyperhomocysteinemia may favor oxidative damage, since homocysteine oxidizes rapidly when released into plasma, and, as a consequence of the auto-oxidation, a great amount of oxygen-free radicals—radical superoxide and hydrogen peroxide—are generated [6]. These oxygen-free radicals are believed to cause

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the endothelial cytotoxicity of homocysteine, partly because they favor lipid peroxidation, an effect that occurs on the surface of endothelial cells and plasma lipoprotein particles [7].

Lipid peroxidation and particularly, oxidative modifications of LDL, are important factors in atherosclerosis development and, indeed, sufficient data exist indicating that LDL oxidation is present in human atherosclerotic lesions [8]. Malondialdehyde (MDA) is the simplest usual marker among those used to assess lipid peroxidation in vivo, but it has the disadvantage of being rather nonspecific [9]. Oxidated anti-LDL antibody titer is a widely used specific lipid peroxidation marker to identify and quantify oxidative stress.

The uremic patient presents chronic inflammatory status resulting from various factors: raised cytokine production and reduced renal clearance, AGE accumulation, presence of chronic heart failure, arteriosclerosis, per se, diverse inflammatory diseases, nondetected persistent infections, and dialysis-related factors, such as bioincompatibility of dialysis membranes, or exposure to endotoxins and other substances of the dialyzer that induce cytokine release [10]. C-reactive protein (CRP) is the first acute-phase reactant described, and is one of the most important of those presenting an increase during the inflammatory process.

The relationship between hyperhomocysteinemia and inflammation has long been the object of research [11]. Several studies on investigating the molecular consequences of hyperhomocysteine on the vessel wall found nuclear factor kappa B (NF-κB) activation through an oxidative mechanism. Low free radical concentrations (H₂O₂-hydrogen peroxide) activate enzymes that phosphorylate the inhibitory protein, and the active form of NF-κB remains, which stimulates the target genes. NF-κB is a transcription factor necessary for remodeling cell proinflammatory response [12]. Control of cytokine release and adhesion molecules (VCAM-1) is known to be partially mediated by NF-κB activation [13]. Other inflammation markers, such as TNF-α, may potentiate the proinflammatory effect of hyperhomocysteinemia and stimulate adhesion molecule (VCAM-1, ICAM-1, e-selectin) activation [14] (Fig. 1). Adhesion molecules and proinflammatory cytokines constitute important parts in the binding of inflammatory mononuclear cells to the vessel wall. These markers have been detected in atherosclerosis, unstable angina, or acute myocardial infarction, and correlate with plasma CRP concentrations [15].

**ROLE OF THE FOLIC ACID AND RELATION WITH THE “NEW” CARDIOVASCULAR RISK FACTORS**

Different treatments to control hyperhomocysteinemia (betaine, serine, and N-acetylcysteine [16]) have been assayed; however, many studies have shown vitamin supplements to be the most effective. Antioxidants have proved to neutralize hyperhomocysteine-induced endothelial dysfunction [17]. In a previous study, we analyzed for the first time the relationship between oxidative stress and homocysteine in hemodialysis patients and the role of folic acid in these “new” CVD risk factors. We showed that hemodialysis patients presented moderate-severe hyperhomocysteinemia (mean homocysteine levels pretreatment 38 μmol/L) that, following the intravenous administration of 10 mg of folic acid 3 times a week, dropped by 44% (mean homocysteine levels posttreatment 21 μmol/L), but without reaching normal levels. This same study showed that, further to the reduction in homocysteine levels, a statistically significant reduction in lipid peroxidation was achieved: a 40% decrease in MDA concentration, and 13% in oxidized anti-LDL antibody titer [18] (Fig. 2). These results suggest that folic acid exerts an indirect antioxidant effect by reducing the prooxidant action of homocysteine and, consequently, lowering oxidative stress.

Despite the high prevalence of inflammation in patients with chronic kidney failure, recommendations as to whether this should be the object of treatment, and how this should be carried out, remain to be established. Folic acid is known to reduce hyperhomocysteine-induced adhesion molecule expression by 50% [14]. We analyzed the effect of inflammatory status on homocysteine response to vitamin supplements in 15 stable patients on hemodialysis who had received 75 mg of IV folic acid at the end of each dialysis session. Serum CRP concentrations did not change following folic acid administration (Table 1). No correlation was found between plasma homocysteine concentration and serum CRP levels; however, the study shows that a decreased percentage of homocysteine levels with IV folic acid supplements depends on the serum
Fig. 2. Effect of IV folinic acid on plasma homocysteine concentration and lipid peroxidation marker (malondialdehyde). Abbreviations are: MDA, malondialdehide (μmol/L); Hcy-homocysteine (μmol/L); Basal-HD, at start of the study; Folinic-HD, 10 mg of folinic acid intravenously 3 days per week. Statistic analyses were performed using the nonparametric Wilcoxon test for paired data. *P* < 0.05 between control and basal HD. P < 0.05 between basal and folinic HD.

Table 1. Serum folic acid concentrations, serum C-reactive protein (CRP), and plasma homocysteine (Hcy) concentrations in the 3 study periods

<table>
<thead>
<tr>
<th></th>
<th>Folinic acid 25 mg/wk</th>
<th>Washout</th>
<th>Folinic acid 75 mg/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folic mg/mL</td>
<td>8.49 ± 2.29</td>
<td>5.52 ± 4.54	extsuperscript{a}</td>
<td>15.95 ± 4.07	extsuperscript{b}</td>
</tr>
<tr>
<td>CRP mg/dL</td>
<td>10.10 ± 7.59</td>
<td>12.26 ± 9.80	extsuperscript{a}</td>
<td>13.57 ± 9.80</td>
</tr>
<tr>
<td>Hcy μmol/L</td>
<td>19.34 ± 4.18</td>
<td>22.92 ± 6.90	extsuperscript{a}</td>
<td>18.00 ± 4.80	extsuperscript{b}</td>
</tr>
<tr>
<td>Δ% Hcy</td>
<td>+16.91</td>
<td>−20.43</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations are: CRP, C-reactive protein; Hcy, homocysteine; Δ% Hcy, homocysteine variation. Pearson’s correlation coefficient was used to analyze the quantitative variables.

	extsuperscript{a}P < 0.05 vs. folic acid 25 mg/week.

	extsuperscript{b}P < 0.05 vs. wash-out.

The relationship between homocysteine and atherosclerosis in the dialysis population has been reflected in several works. In 1997, Bostom et al [19] presented a prospective study of 73 subjects on dialysis receiving vitamin supplements in whom they demonstrated a relationship between homocysteine concentration and cardiovascular disease. These data were later confirmed by other studies [20, 21]. In any event, other works exist in which no relationship between homocysteine concentrations and cardiovascular disease [22], or even an inverse correlation [23], was found.

The role of CRP in cardiovascular disease development in dialyzed patients is controversial, and CRP has frequently been considered an epiphenomenon, rather than a pathogenic mechanism [24]. However, CRP has been isolated in the inflammatory lesion of atherosclerotic vessels and myocardial infarcted zones, which suggests that CRP and activation of the complement (classic pathway) play a pathogenic role [25]. Several published studies show that CRP is an independent predictor of mortality in hemodialysis patients regardless of other factors, even after a follow-up period of 4 years [26].

On the other hand, the role of oxidized anti-LDL antibody titer and its relationship with atherosclerosis in kidney failure patients has been less studied. Stenvinkel et al [27] demonstrated that patients with carotid plaque have a statistically significant high oxidated anti-LDL antibody titer compared with those without atheromatous plaque. By contrast, Shoji et al [28] reported that, in hemodialysis patients, a higher oxidated anti-LDL antibody titer is a low mortality risk factor for cardiovascular disease, and

folic acid concentration (r = −0.60; P = 0.003) and the patient’s basal CRP concentration (r = −0.66; P = 0.007) (Fig. 3). When adjusted in a linear regression analysis by serum folic acid values of the washout period by each increase in 1 mg/dL of CRP concentration, the decreased percentage of homocysteine dropped by 1.29%. Thus, we can state that patients with lower inflammatory status are those who present better response to folic acid treatment [abstract; Bonal J, J Am Soc Nephrol 13:212A, 2002].

**RELATION BETWEEN THE “NEW” CARDIOVASCULAR RISK FACTORS AND MORTALITY**

When these 3 “new” CV risk factors were analyzed together, the relationship between homocysteine, inflammation, oxidative stress, and cardiovascular disease in the dialyzed population was found to be complex and, on occasion, the studies yielded conflicting results.
they found no relationship with noncardiovascular disease mortality. The reasons for such discrepancies among studies are unknown; however, most observations in the general population suggest that the oxidated anti-LDL antibody titer is raised in cases of advanced atherosclerosis [29–31].

We studied these “new” cardiovascular risk factors in 94 patients on hemodialysis treated with oral vitamin supplements (folic acid and vitamin B complex) and followed-up for 24 months. The patients presented chronic inflammatory status, high oxidated anti-LDL antibody titer, and moderate hyperhomocysteinemia. Although a relationship between the 3 CVD risk factors was theoretically possible, we found no correlation between these “new” CVD risk factors studied. Cox analysis revealed that homocysteine is not a mortality risk factor in this study population supplemented with vitamins. Statistical analysis confirmed that inflammation (CRP) and oxidative stress, together with age, are the main risk factors for mortality in this population (Table 2) [32]. The reduction in homocysteine levels achieved after vitamin supplementation may lead to patients presenting very similar homocysteine concentrations, and to losing importance as a cardiovascular risk factor and predictor of mortality. These results, together with the reduction in lipid peroxidation achieved after folic acid administration, underline the importance of vitamin administration in kidney patients to avoid deficits, and neutralize the elevated oxidative stress frequently presented.

**CONCLUSION**

The rapid development of atherosclerosis in uremia is a complex, multifactorial process; however, it should be emphasized that the role of inflammation and oxidative stress in its etiopathogenesis is increasingly clear. Knowledge of these “new” risk factors may help to prevent the development of atherosclerosis in these patients.

**REFERENCES**


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**Table 2.** Analytic parameters and age according to vital status at the end of follow-up: Unadjusted mortality-related risk factors

<table>
<thead>
<tr>
<th></th>
<th>Death</th>
<th>Survival</th>
<th>OR (95% CI)</th>
<th>Lower-Higher</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years</td>
<td>74 ± 7</td>
<td>63 ± 16</td>
<td>1.05</td>
<td>1.01–1.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP mg/L</td>
<td>9.72</td>
<td>4.86</td>
<td>3.28</td>
<td>1.14–9.44</td>
<td>0.03</td>
</tr>
<tr>
<td>HCY μmol/L</td>
<td>(0.39–88)</td>
<td>(0.35–87)</td>
<td>1.01</td>
<td>0.97–1.06</td>
<td>0.51</td>
</tr>
<tr>
<td>IgG</td>
<td>191 ± 85</td>
<td>147 ± 68</td>
<td>1.01</td>
<td>1.002–1.010</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Abbreviations are: CRP: C-reactive protein; Hyc: homocysteine; IgG: oxLDL-Ab, plasma copper oxidized anti-LDL antibodies. Risk of death during follow-up was analyzed by a Cox proportional risk model.


