

C-Reactive protein, oxidative stress, homocysteine, and troponin as inflammatory and metabolic predictors of atherosclerosis in ESRD

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Mortality in patients with end-stage renal disease remains high, with cardiovascular disease accounting for half of these deaths. Novel risk factors such as inflammation, oxidative stress, hyperhomocysteinemia, and high troponin levels are associated with cardiovascular risk in the general population. While there are substantial epidemiologic data confirming that these novel risk factors are associated with cardiovascular risk in end-stage renal disease patients, a causal relationship has not been established. Inflammation is readily identified by the presence of high levels of C-reactive protein, while studies of oxidative stress are hampered by the lack of a standardized test. The cause of both is unknown. Hyperhomocysteinemia results from decreased remethylation to methionine, although vitamin supplementation only partially corrects the defect, suggesting that uremic inhibition of the enzymatic process may be important. The most promising strategies for correcting oxidative stress and hyperhomocysteinemia are vitamin E and folinic acid therapy, respectively. Troponin I appears to be a more specific marker of myocardial injury than Troponin T, but troponin T retains its ability to predict cardiovascular mortality as well as all-cause mortality. Sorting out the role of each of these risk factors may be difficult since the factors may influence each other, may increase oxidative stress, and may mediate atherosclerosis through oxidative modification of lipids. *Curr Opin Nephrol Hypertens* 9:621–630. © 2000 Lippincott Williams & Wilkins.

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Abbreviations

CRF	chronic renal failure
CRP	C-reactive protein
ESRD	end-stage renal disease
HDL	high density lipoprotein
LDL	low density lipoprotein
MTHFR	methylene tetrahydrofolate reductase

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Introduction

Despite advances in dialysis, mortality of patients with end-stage renal disease (ESRD) remains high at 22% per year [1]. About half of the deaths are attributed to cardiovascular disease [1]. Therefore, it is imperative that we gain a better understanding of the process of atherosclerosis in patients with ESRD if we hope to improve their survival. Recently, epidemiologic studies have identified novel markers that are associated with an increased risk of atherosclerosis in the general population [2**] as well as in patients with ESRD. This paper will review the potential role of inflammation, oxidative stress, and homocysteine levels in the accelerated atherosclerosis observed in patients with ESRD. It will also review the utility of troponin levels in identifying the presence of atherosclerosis. Lipid abnormalities in ESRD patients will not be addressed unless they overlap with inflammation and oxidative stress.

C-reactive protein and inflammation

C-reactive protein (CRP) is a positive acute phase protein synthesized by the liver in response to inflammation and infection and serves as a marker of inflammation. Recent prospective epidemiologic studies have linked high CRP levels with atherosclerosis in the general population [2**,3–5]. Interestingly, CRP appears to be a long-term marker for cardiovascular risk since a single measurement of CRP correlates with cardiovascular events years in the future, although not all studies support this contention [6].

In patients with ESRD, hypoalbuminemia was identified in many studies as the strongest predictor of mortality and morbidity [7]. However, patients with low serum albumin died from cardiovascular disease and not from malnutrition, challenging the assumption that hypoalbuminemia resulted from underdialysis and malnutrition. Subsequent studies have shown that hypoalbuminemic patients had higher levels of inflammatory cytokines and positive acute phase proteins and that CRP was an important determinant of low serum albumin in multivariate analysis. These data suggest that low serum albumin results at least in part from inflammation and cytokine-mediated blockade of hepatic albumin synthesis and not from underdialysis and malnutrition, and may help to explain the high cardiovascular mortality in hypoalbuminemic patients.

Observational studies examining the relationship between inflammation and mortality have focused on overall mortality and have generally included albumin in the multivariate analysis [8–10,11*,12*] (Table 1). A majority of the studies found that inflammation predicted mortality, as evidenced by high CRP [10,11*,12*] or interleukin-6 (a cytokine that promotes the inflammatory response) [8] levels, whereas serum albumin level was either not important or of less importance. Owen and Lowrie [9] did not find such a relationship in the largest group of patients studied (Table 1). This discrepancy may be due to the shortest follow-up period in this group of patients, which was 0.5 years versus two years or more in the other studies. Examination of the Kaplan Meier survival curves reveals that the majority of deaths occurred in the first eight months for albumin, whereas deaths were cumulative over the entire follow-up period for CRP [12*]. Only four studies examined the relationship between inflammation and cardiovascular mortality. Yeun *et al.* [12*] and Zimmermann *et al.* [13*] both found that CRP and age were the most important factors in predicting cardiovascular mortality (Table 1). Ohashi *et al.* [15] and Koda *et al.* [14] reported that lipoprotein (a) is an important predictor of death from coronary artery disease and from cardiovascular causes. Although CRP was measured in one study and found to correlate with lipoprotein (a) levels, CRP levels were excluded from the multivariate analysis for cardiovascular mortality [14]. However, lipoprotein (a) may be viewed as a surrogate marker for inflammation given the correlation with CRP shown in this and other studies [16].

The exact mechanism of how inflammation leads to atherosclerosis is poorly understood. The current hypothesis is that inflammation causes endothelial injury or dysfunction, leading to leukocyte and platelet activation and adhesion to receptors on endothelial cells, smooth muscle proliferation, and eventual plaque formation [17*]. In addition, inflammation also may modify plasma protein and lipid composition to favor atherosclerosis. Not only do fibrinogen and lipoprotein (a) behave as

positive acute phase proteins, they have also been implicated as risk factors for atherogenesis [16]. Serum amyloid A and ceruloplasmin, additional positive acute phase proteins, may modify high density lipoprotein and render it ineffective in preventing oxidation of low density lipoprotein [18]. Ceruloplasmin can oxidize low density lipoprotein directly, enhancing its atherogenicity [19]. In support of inflammation causing atherosclerosis are the findings that a high CRP level is an independent predictor of carotid atherosclerotic plaques [20] and of carotid intima-media thickening [21**] in cross-sectional studies of 112 hemodialysis patients and 109 chronic renal failure (CRF) patients, respectively.

In some patients, the inflammation may be due to occult infection. However, in the majority of these patients the etiology of the inflammation is not evident [16,22]. There are several potential sources, including water impurities, dialyzer membrane bio-incompatibility, vascular access related subclinical infection or microbial colonization, vascular access related bio-incompatibility, and uremia itself. The observation that patients treated with peritoneal dialysis also have high CRP levels [7] makes water impurities a less likely cause of inflammation. Bio-incompatibility and vascular access remain candidates since patients treated with peritoneal dialysis also are exposed to potentially bio-incompatible substances and potential sources for subclinical infection or microbial colonization through the peritoneal dialysate and the catheter. Alternatively, the source of the inflammation may be atherosclerosis itself, rendering CRP levels a marker for the presence of atherosclerosis rather than the cause. Research efforts in this arena are focused on identifying the source of the inflammation and on disrupting the acute phase response through inhibiting the actions of pro-inflammatory cytokines such as interleukin-1 or 6 and tumor necrosis factor- α .

Oxidative stress

Oxidative stress is thought to play an important role in atherosclerosis in patients with ESRD because it

Table 1. Summary of recent studies evaluating markers of inflammation as predictors of overall and cardiovascular activity

Study	n	Modality	Follow-up (years)	Independent predictors of mortality
Overall mortality				
Bologa <i>et al.</i> [8]	90	hemodialysis	2.5	Interleukin-6 > age > albumin > body mass index
Iseki <i>et al.</i> [11*]	163	hemodialysis	7	C-reactive protein (not analyzed for other factors)
Owen and Lowrie [9]	1054	hemodialysis	0.5	Albumin > creatinine > total lymphocyte count
Noh <i>et al.</i> [10]	106	peritoneal dialysis	2	Cardiovascular disease > C-reactive protein > hematocrit
Cardiovascular mortality				
Zimmerman <i>et al.</i> [13]	280	hemodialysis	2	Age > C-reactive protein > body mass index > diabetes > albumin
Yeun <i>et al.</i> [12*]	91	hemodialysis	2.8	C-reactive protein > age
Koda <i>et al.</i> [14]	390	hemodialysis	2.3	Lipoprotein (a), age, diabetes
Ohashi <i>et al.</i> [15]	268	hemodialysis	5	Lipoprotein (a)

generates lipid peroxidation and oxidized lipoproteins [23,24], which are found consistently in fatty streaks and atherosclerotic plaques. Broadly, oxidative stress is defined as the excessive production of free radicals and depletion of antioxidants (such as selenium, zinc, vitamin C, vitamin E, and decreased activity of superoxide dismutase and glutathione peroxidase). Excessive production of free radicals in ESRD patients may result from inflammation, bio-incompatibility with dialyzer membrane or dialysis tubing, and uremia, including the accumulation of advanced glycation end products [23,24]. In patients with ESRD, depletion of antioxidants due to dialytic removal or insufficient intake and decreased activity of free-radical scavengers have been reported [22]. It is unknown why the activity of these enzymes is decreased. The increased oxidative stress may be identified by an increase in the products of lipid peroxidation (malondialdehyde and thiobarbituric acid-reactive substances), a decrease in substances that enhance oxidative resistance (plasmalogen phospholipids in red blood cell membranes), or a decrease in reducing substances (glutathione). There is evidence for all of the above in patients with CRF and ESRD [22,24,25]. However, contradicting data exist, partly due to the lack of standardized tests to determine the presence of oxidative stress, the rapid kinetics of oxidation, the presence of substances that may interfere with the assay, and the sequestration of lipid peroxidation products in cell membranes making plasma levels unreliable.

Recent studies looking for oxidative stress in patients with ESRD continue to suffer from the same methodologic problems. Whether the study is able to show the presence of oxidative stress depends on the chosen markers [26*,27,28]. Studies using malondialdehyde as a marker of lipid peroxidation have consistently found it to be elevated in patients with ESRD [26–28,29*,30]. Nguyen-Khoa *et al.* [30] demonstrated that the total peroxyl radical-trapping antioxidant potential assay, used to gauge oxidative stress, indicated less oxidative stress in hemodialysis patients because of its correlation with uric acid, triglycerides, and advanced oxidation protein products. Although these substances may scavenge peroxyl radicals involved in the *in-vitro* assay, they do not exert much antioxidant activity *in vivo* [30].

One of the proposed mechanisms for oxidative stress is the dialysis process itself. Antioxidants such as glutathione, ubiquinol-10, α -tocopherol, β -carotene, vitamin C, superoxide dismutase, catalase, and glutathione peroxidase experienced either no change or a further decline in their levels or activity after hemodialysis [27,28]. These findings would support the theory that dialysis creates oxidative stress by removing antioxidants or inhibiting their action. In contrast, markers of lipid peroxidation such as malondialdehyde, 7-ketocholesterol,

and thiobarbituric acid-reactive substances decreased after hemodialysis in some patients [27,29*] and increased in others [28]. The lower levels after hemodialysis do not support dialyzer bio-incompatibility with leukocyte activation and generation of reactive oxygen species as a source of oxidative stress in patients with ESRD. In fact, evidence exists that predialysis patients have increased oxidative stress, using low red blood cell plasmalogen levels (an antioxidant phospholipid) as a marker for oxidative stress [25]. The oxidative stress was more prevalent in malnourished patients as assessed by subjective global assessment and reversed partially with 12 months of dialysis. Taken together, these data would suggest that although hemodialysis does decrease antioxidant levels or activity, it may not be the dominant factor responsible for the increased oxidative stress.

Preliminary data suggest that other potential contributors to oxidative stress include hyperleptinemia [31*], defects in the antioxidant effect of high density lipoprotein (HDL) mediated by inflammation [32*,33*], use of tacrolimus [34], and intravenous iron therapy [26*]. Plasma levels of leptin, a protein involved in the control of body weight and appetite, are elevated in patients with ESRD [35]. In human umbilical vein endothelial cell cultures, leptin has been found to increase the accumulation of reactive oxygen species and activate the stress-activated protein kinase pathway, a process ameliorated by treatment with the antioxidant N-acetylcysteine [31*]. Recently, data have identified the oxidation of low density lipoprotein (LDL) as a key step in the initiation of atherosclerosis [17*]. Increasing evidence also suggests that HDL protects LDL from oxidation. In hemodialysis patients this protective effect is blunted [32*]. Although HDL-associated paraoxonase is possibly responsible for the antioxidant effect of HDL, the allele frequencies for paraoxonase polymorphism did not differ between 103 renal transplant patients with and 388 patients without known cardiovascular disease [33*]. An uncontrolled study in 20 transplant patients treated with tacrolimus showed a lower oxidation lag time and lower serum antioxidant activity compared with historical data in 23 neoral-treated patients, which improved with vitamin C and E supplementation [34]. The common practice of intravenous iron therapy in hemodialysis patients may contribute to oxidative stress since iron is an oxidizing agent. Superoxide dismutase and glutathione peroxidase activities did not differ before and after a single dose of intravenous iron in hemodialysis patients with ferritin levels of 600 $\mu\text{g/l}$ or less. However, patients with ferritin levels of more than 600 $\mu\text{g/l}$ had a significant decline in superoxide dismutase activity after receiving intravenous iron. All patients had higher levels of plasma lipid peroxides after intravenous iron [26*].

The data on treatment to reduce oxidative stress in patients with ESRD suffer from a similar lack of standardization in the assay [27,36–38,39,40,41,42]. The results of these studies are summarized in Table 2. The data are conflicting, at least in part due to the chosen marker of oxidative stress.

Despite the profusion of literature on oxidative stress in patients with ESRD, few studies address the issue of causality. Galle *et al* [43] demonstrated that oxidized LDL and lipoprotein (a), but not normal LDL, induced oxygen free radical formation and apoptosis in human umbilical vein endothelial cells and rabbit aorta. Apoptosis was enhanced by diethyl-dithio-carbamate, a superoxide dismutase inhibitor, and ameliorated by superoxide dismutase and catalase. Boaz *et al*. [29] found that only malondialdehyde discriminated between 44 hemodialysis patients with and 32 patients without prevalent cardiovascular disease. Traditional cardiovascular risk factors such as lipid levels, hemostatic factors, blood pressure, age, sex, and presence of diabetes were not associated with cardiovascular disease in multivariate analysis in this cross-sectional study. Using vitamin E coated dialyzers for two years in 25 hemodialysis patients, Mune *et al*. [39] found a mild decrease in the malondialdehyde content of LDL, in oxidized LDL levels, and in aortic calcification by computed tomography compared with 25 age- and sex-matched hemodialysis patients (Table 2). However, aortic calcification may be due to poor calcium and phosphorus control and not to atherosclerosis. Miyazaki *et al*. [40] showed that a single hemodialysis session with a vitamin E coated hemodialyzer prevented the

rise in post-hemodialysis levels of oxidized LDL. It also impaired the flow-mediated vasodilation observed with a noncoated cellulose dialyzer [40]. Although suggestive, none of these studies confirm causality.

Homocysteine

Homocysteine is a sulfur containing amino acid formed during the catabolism of methionine [44]. During methionine depletion, it is remethylated to methionine. Remethylation requires methyltetrahydrofolate as a methyl donor, vitamin B₁₂ as a cofactor, and the enzyme 5-methylene tetrahydrofolate reductase (5-MTHFR). An alternative but less important pathway for remethylation uses betaine as the donor. During methionine excess, homocysteine is metabolized by the transsulfuration pathway to cystathionine, 2-methylcitric acid, and methylmalonic acid through the action of cystathionine β -synthase using vitamin B₆ as cofactor. Therefore, folic acid, vitamins B₁₂ and B₆ deficiencies and defects of the responsible enzymes can all result in hyperhomocysteinemia.

The mechanism of hyperhomocysteinemia in patients with ESRD is debated since renal excretion accounts for less than one percent of homocysteine elimination [44]. A common genetic mutation of the 5-MTHFR gene (677C→T substitution) occurs with the same frequency in ESRD patients as in the general population and is therefore not responsible for the high homocysteine [45]. Although vitamin deficiencies may play a role, vitamin supplementation in patients with ESRD lowers but does not correct the high homocysteine levels [44]. Metabolic studies in patients with chronic renal failure show progressive accumulation of homocysteine, cy-

Table 2. Summary of recent studies directed at correcting oxidative stress in ESRD patients.

Study	n	Modality	Intervention	Dose	Duration	Marker of oxidative stress	Outcome
Maccarrone <i>et al</i> . [36]	13	HD	Vitamin E	300 mg IM qD	15 days	Membrane peroxidation	↓*
Islam <i>et al</i> . [37]	17/16	PD/HD	Vitamin E	800 I.U. po qD	12 weeks	LDL susceptibility to oxidation	↓*
Mune <i>et al</i> . [39]	50	HD	Vitamin E	Hemodialyzer	2 years	LDL-MDA	↓†
						Oxidized LDL	↓†
						Aortic calcification	↑†
Miyazaki <i>et al</i> . [40]	12	HD	Vitamin E	Hemodialyzer	One-time	Oxidized LDL	↓*
Ando <i>et al</i> . [38]	16/22	PD/HD	Eicosapentanoic acid	1.8 g po qD	3 months	Flow-mediated vasodilation	↑*
						Oxidized LDL	↓†
De Cavanagh <i>et al</i> . [27]	11	HD	Enalapril	10 mg po qD	≥6 months	Glutathione	↑*
						Glutathione peroxidase	↑†
						β -carotene	↑†
						Superoxide dismutase	No change†
						Catalase	No change†
						Ubiquinol-10	No change†
						α -tocopherol	No change†
Nishikawa <i>et al</i> . [42]	38	HD	Simvastatin	5 mg po qD	6 months	MDA	↓*
Martinez-Castelao <i>et al</i> . [41]	12	RT	Fluvastatin	20 mg po qD	12 weeks	LDL susceptibility to oxidation	No change*

HD, hemodialysis; MDA, malondialdehyde; PD, peritoneal dialysis; RT, renal transplant.

*Effect of intervention on oxidative stress parameter compared with baseline. †Effect of treatment on oxidative stress parameter compared with no intervention.

stathionine, methylmalonic acid, and 2-methylcitric acid with worsening renal function, culminating in the high levels seen in patients with ESRD [46]. Methionine levels remain unchanged and are only slightly higher in these patients. These changes remain marked despite vitamin repletion, suggesting that the defect in uremia is impairment of the remethylation pathway. Using ²H- and ¹³C-labeled methionine, van Guldener *et al.* [47**] confirmed that homocysteine remethylation was decreased but transsulfuration was not in four hemodialysis patients. In transplant patients, the main determinant of homocysteine levels appear to be renal function, with vitamin status playing a smaller role and cyclosporine use a negligible role [48*].

Various strategies have been used to lower homocysteine levels in patients with ESRD and these are summarized in a recent review [44]. Treatment with folic acid (1–15 mg) alone or in combination with vitamin B₆ (10–300 mg) and vitamin B₁₂ (0.012–1.0 mg) lowers homocysteine only by 30–50% and normalizes homocysteine only in 30–40% of ESRD patients. Vitamin B₆ alone and betaine alone [43] or in combination with folic acid [49,50*] (Table 3) have no effect on homocysteine levels. Both hemodialysis and peritoneal dialysis do not remove sufficient amounts of homocysteine to normalize its levels [44,57*] because 80% of it is protein bound.

However, homocysteine levels and the prevalence of hyperhomocysteinemia (67% versus 91%) appear to be lower in patients treated with peritoneal dialysis compared with hemodialysis [59]. Peritoneal dialysis may be more effective at lowering homocysteine levels, since these levels fall from 31.9±9.0 to 23.2±6.9 μmoles/l after one month of such treatment [60]. Recent studies on vitamin supplementation suggest that folic acid doses higher than 2.5 mg three times a week do not lower homocysteine levels any further [51**–53**] (Table 3). In a randomized, double-blind, multicenter study of 150 patients, Sunder-Plassmann *et al.* [53**] found that even 60 mg of folic acid a day only reduced homocysteine levels by 38% (Table 3). The lack of efficacy of higher doses of folic acid likely reflects saturation of red blood cells or impaired transmembrane transport. Increasing folic acid supplementation from 5 mg three times a week to 5–10 mg a day had little additional effect on red blood cell folate despite a continued rise in plasma folate levels [52**]. Interestingly, although 5-MTHFR polymorphism did not predict homocysteine response to folic acid treatment, homocysteine levels became normal more frequently in patients with the normal 5-MTHFR gene [53**]. The only study evaluating the role of vitamin B₁₂ alone found that it lowered homocysteine levels by 35% in B₁₂-deficient patients but also decreased plasma folate levels

Table 3. Summary of recent studies evaluating the efficacy of various vitamin regimens to lower homocysteine levels

Study	Modality	N	Folic acid dose	Other	% Change homocysteine	Comments
Dierkes <i>et al.</i> [51**]	HD/PD	70/12	2.5 mg TIW 5 mg TIW		35	No further change with higher dose
Arnadottir <i>et al.</i> [52**]	HD	14	5 mg TIW 5 mg qD		36	No further change with higher doses. Cross-over study design.
Sunder-Plassmann <i>et al.</i> [53**]	HD	150	10 mg qD		32	
			15 mg qD		30	
			30 mg qD 60 mg qD		38	
Jungers <i>et al.</i> [54]	CRF	78	5 mg TIW	B ₆ 250 mg BIW B ₁₂ 1 mg BIW	40	Homocysteine normalized in about 50%
van Guldener <i>et al.</i> [49]	PD	30	5 mg qD (3 mos) + 1 or 5 mg qD (maintenance)	± Betaine 4 g qD		Betaine had no effect on homocysteine. Homocysteine normalized in 39%. No changes in endothelium dependent vasodilation.
Kunz <i>et al.</i> [55*]	HD	63	10 mg qD		37	No changes in fibrinogen, von Willebrand factor, and plasminogen activator inhibitor type I levels.
van Guldener <i>et al.</i> [50*]	HD	41	5 mg qD (3 mos) + 1 or 5 mg qD (maintenance)	± Betaine 4 g qD	53	Betaine had no effect on homocysteine. No changes in carotid artery stiffness.
Beaulieu <i>et al.</i> [56]	RT	60	2.4 mg qD 0.4 mg qD None	B ₆ 50 mg qD (all groups) B ₁₂ 0.4 mg qD (all groups)		Homocysteine normalized in 50%. Homocysteine normalized in 9%. Homocysteine normalized in 0%.
Dierkes <i>et al.</i> [57*]	HD	14		B ₁₂ 1 mg/wk IV	35	B ₁₂ deficient patients
Touam <i>et al.</i> [58**]	HD	37		Folinic acid 50 mg/wk IV Pyridoxine 250 mg TIW IV	67	Homocysteine normalized in 78%

HD, hemodialysis; IV, intravenous; PD, peritoneal dialysis; qD, once daily; RT, renal transplant; TIW, three times a week

by 47% without affecting red blood cell folate levels [61*] (Table 3). The most impressive data to date show that intravenous folinic acid and pyridoxine decreased homocysteine levels from 37.3 ± 5.8 to 12.3 ± 5.4 $\mu\text{moles/l}$ in 37 patients treated with hemodialysis, and 78% of the patients achieved a normal homocysteine level [58**]. Folinic acid is an immediate precursor of methyltetrahydrofolate and may increase the availability of methyltetrahydrofolate when folate metabolism or absorption is impaired.

In the general population, most of the epidemiologic data support hyperhomocysteinemia as an independent risk factor for atherosclerotic disease in the coronary, cerebral, and peripheral vasculature [62]. Until recently, data on the ESRD population was sparse, consisted mainly of cross-sectional data on a small number of patients, and demonstrated conflicting results [44]. Since then, larger cross-sectional studies [63*,64] and some prospective studies [65,66,67*] have consistently demonstrated increased cardiovascular risk with hyperhomocysteinemia in patients with ESRD. These are summarized in Table 4. However, in agreement with previous studies [44], Manns *et al.* [63*] were unable to show any association between homocysteine level and atherosclerotic disease in the subset of 92 women with ESRD [60]. The inability to find such an association may be due to the small number of women and the lower homocysteine levels in women. Although hyperhomocysteinemia is associated also with vascular thrombosis in the general population, prior cross-sectional studies in hemodialysis patients showed conflicting results on the association between homocysteine levels and vascular access thrombosis [44,69,70]. In the only prospective study, Shemin *et al.* [68*] found that each 1 $\mu\text{moles/l}$ increase in homocysteine level was associated with a 4% increase in the risk of access thrombosis. They also found that homocysteine levels were higher in the 47 patients with access thrombosis compared with the 37 patients without thrombosis [68*] (Table 4).

Alternatively, the conflicting data in the ESRD population concerning homocysteine may be explained by the

influence of malnutrition and inflammation on atherosclerosis (see above) and by the effect of nutrition on homocysteine levels. Suliman *et al.* [71*] found that hemodialysis patients ($n = 70$) with cardiovascular disease had lower subjective global assessment scores, lower serum albumin, and lower homocysteine levels than those without cardiovascular disease ($n = 47$). Furthermore, those patients with homocysteine levels of less than 24 $\mu\text{moles/l}$ had a lower four-year survival than those with higher levels, contradicting the other studies. It may be that higher levels are associated with atherosclerosis only in well-nourished and non-inflamed ESRD patients. In the presence of inflammation, not only do homocysteine levels fall from cytokine-induced anorexia but also the effect of inflammation on atherosclerosis may become dominant. Therefore, the prevalence of inflammation in the ESRD patients under study may determine whether an association between homocysteine and atherosclerosis is found. Another explanation for the seeming contradiction of increased cardiovascular mortality in patients with low homocysteine and albumin levels may be altered protein binding. Vychytil *et al.* [57*] found that more homocysteine existed in the free form in 13 peritoneal dialysis patients with serum albumin of 4.1 g/dl or less, compared with 12 patients with serum albumin of more than 4.1 g/dl ($51.3 \pm 6.4\%$ versus $43.3 \pm 2.5\%$, $P = 0.0005$).

Although most epidemiologic studies in ESRD patients suggest a link between hyperhomocysteinemia and atherosclerotic and thrombotic diseases, no study has shown causality (i.e., that lowering homocysteine levels decreases the incidence of these diseases). Unlike studies in the general population [72,73], three recent studies in ESRD patients failed to demonstrate a causal relationship. They used surrogate endpoints for atherosclerosis and thrombosis, such as endothelium dependent vasodilation [49], carotid artery stiffness [50*], fibrinogen, von Willebrand factor, and plasminogen activator inhibitor type I levels, putative mediators of thrombosis and endothelial dysfunction [55*] (Table 3). There are no long term data on the effects of lowering homocysteine levels in either the general or ESRD population.

Table 4. Summary of recent studies identifying homocysteine as a cardiovascular risk factor in ESRD patients

Study	Design	n	Modality	Outcome	Homocysteine ($\mu\text{mol/l}$)		P	RR
					CVD	No CVD		
Ducloux <i>et al.</i> [64]	Cross-section	224	Transplant	CV events	25.2 ± 11.7	20.5 ± 8.9	0.005	-
Ducloux <i>et al.</i> [67*]	Prospective	207	Transplant	CV events	31.5 ± 10.3	17.8 ± 7.5	<0.001	1.06
Bostom <i>et al.</i> [65]	Prospective	73	HD, PD	CV events				3.6
Moustapha <i>et al.</i> [66]	Prospective	167	HD	CV events	43.0 ± 48.6	26.9 ± 14.9	0.02	1.01
Manns <i>et al.</i> [63*]	Cross-section	218	HD	CV events	$28.6 (25.6-31.7)$	$25.0 (23.3-26.8)$	0.036	2.12
Shemin <i>et al.</i> [68*]	Prospective	84	HD	Access	26.1 ± 11.6	22.9 ± 2.8	0.016	1.04

CV, cardiovascular; CVD, cardiovascular disease; HD, hemodialysis; PD, peritoneal dialysis

Troponin

Despite the high prevalence of cardiac disease in patients with ESRD, diagnosing myocardial infarction and ischemia may be difficult when these patients present with atypical chest pain or with an abnormal baseline electrocardiogram. The traditional marker for myocardial injury, the muscle–brain isoenzyme of creatine kinase (CK-MB), loses its specificity in the setting of renal failure because of noncardiac sources of this enzyme [74]. The availability of cardiac troponin as a specific marker for myocardial injury offered promise for improving the accuracy of such diagnoses in the ESRD patients. George and Singh [74] reviewed the initial experience with two troponin subunits, troponin T and troponin I, in ESRD patients. They concluded that troponin I is a better marker of myocardial injury than T. The poorer specificity of troponin T was attributed to subclinical myocardial injury in the setting of left ventricular hypertrophy or to uremia-induced skeletal muscle expression of the cardiac isoform of troponin T.

Some of the earlier literature must be interpreted with caution because the initial cardiac troponin T assay has some cross-reactivity with skeletal muscle troponin T [75]. Using the more specific second generation troponin T assay, 18%–56% of asymptomatic patients with ESRD or CRF had a high troponin T level (variably defined as >0.05 or $0.1 \mu\text{g/l}$) [76,77•]. The prevalence of a high troponin I level (>0.5 or $1.5 \mu\text{g/l}$) in asymptomatic patients in the more recent studies varied from 0–15% if measured by the Behring OPUS Plus immunofluorescence assay [76,77•,78,79•,80•] to up to 55% [79•] by the Stratus II immunofluorescence assay. The lower prevalence of elevated troponin I level in 144 asymptomatic hemodialysis patients compared favorably with the prevalence of elevated CK-MB (17% versus 28%) [81].

In the general population, elevated troponin T and troponin I levels predict a poor cardiac outcome even in the absence of myocardial infarction [74]. In ESRD patients, the data are conflicting. Two studies of a small number of patients found that high troponin T [78,79•] and troponin I [79•] levels were unable to predict cardiac outcome after 6–18 months of follow-up. In contrast, Roppolo *et al.* [80•] followed 49 asymptomatic hemodialysis patients for six months and reported that the baseline troponin T was elevated in half of the patients and troponin I in three patients. A cardiac event (angina, myocardial infarction, cardiac death) occurred in six patients. All six patients had an elevated troponin T level but only three had elevated troponin I level. The three patients with a high troponin I level all had a cardiac event. Stolar *et al.* [82•] found that half of 94 hemodialysis patients had at least one elevated troponin

T level. Forty-two percent of these patients died during the 12-months follow-up, compared with 8% in the low troponin T group. Interestingly, only 14 of the deaths were from cardiovascular causes. In a larger study of 172 asymptomatic hemodialysis patients, Ooi *et al.* [83•] reported very similar findings. Of the 31 patients with troponin T levels of more than $0.1 \mu\text{g/l}$, a third died during the 12-months follow-up, as opposed to 10% death in patients with low troponin T level. Only 12 patients died from cardiac events. The discrepancy among these studies probably is due to the smaller number of patients and the shorter follow-up period in the negative studies, resulting in insufficient power to detect a difference in outcome.

Prior data have suggested that troponin T is frequently positive in ESRD patients because uremia induces cardiac troponin T expression in skeletal muscle [74]. However, these findings are likely due to the use of the older and less specific troponin T assay [84]. Instead, using the second generation troponin T assay, hemodialysis patients with left ventricular hypertrophy were more likely to have elevated troponin T levels (61% versus 22%) [77•].

Conclusion

The strong association between high CRP levels and atherosclerosis as well as cardiovascular mortality in ESRD patients is intriguing, because inflammation may provide the missing link between low serum albumin and death from cardiovascular complications. However, as with other novel cardiovascular risk factors, it is too early to tell whether there is a causal relationship between inflammation and atherosclerosis. The biological basis for such a relationship is plausible, but proving causality will require studies demonstrating that amelioration of inflammation improves cardiovascular mortality. Finding the right intervention may be difficult since the source of inflammation in ESRD patients remains speculative.

Oxidative stress remains difficult to quantify, in part because of the lack of standardization of tests. Despite this difficulty, there is evidence for increased oxidative stress in ESRD patients. The etiology remains speculative. Although the most commonly proposed mechanism is that of hemodialyzer bio-incompatibility resulting in white blood cell activation and release of free radicals, the presence of oxidative stress in predialysis patients suggests that there are other factors. Thus far, the most promising treatment appears to be vitamin E administration orally or attached on the dialyzer membrane, although such studies have involved only a small number of patients. Whether reducing oxidative stress will translate to improved cardiovascular outcome remains to be seen.

Hyperhomocysteinemia is prevalent in ESRD patients and is due to decreased remethylation to methionine. Supplementation with folic acid only partially corrects it. Recent studies suggest that folic acid doses above 2.5 mg three times a week offer no additional benefit. The combination of folinic acid and pyridoxine given intravenously appears to offer the most promise for correcting hyperhomocysteinemia. Although epidemiologic data suggest an association between hyperhomocysteinemia and atherosclerosis and thrombosis in ESRD patients, lowering homocysteine levels with vitamin therapy has not improved surrogate markers for atherosclerosis and thrombosis. Prospective studies evaluating the effect of lowering homocysteine levels on cardiovascular mortality are lacking. There are some data, however, that suggest that homocysteine may not be a useful marker for atherosclerosis because hypoalbuminemic patients have the highest cardiovascular mortality yet have low homocysteine levels.

Use of troponin levels in stratifying cardiac risk is still under study. Despite the more specific troponin T assay, the prevalence of a high troponin T level in asymptomatic ESRD patients remains high, likely because of its high sensitivity in detecting subclinical myocardial injury associated with left ventricular hypertrophy. Because it is less often positive in ESRD patients, troponin I is a more specific marker of myocardial injury compared with CK-MB and troponin T but a less sensitive marker than troponin T. Combining troponin T and I may offer a better assessment of myocardial injury. Despite its high prevalence, not only has an elevated troponin T level retained its ability to predict cardiovascular mortality and to stratify cardiovascular risk, but it also predicts all-cause mortality. This ability likely resides in the findings that troponin levels are more often positive in asymptomatic patients with left ventricular hypertrophy [77•], ischemic heart disease [77•], and diabetes mellitus [80•].

Cardiovascular mortality remains high in ESRD patients, particularly in the subgroup of patients with evidence for inflammation and malnutrition. It will be difficult to distinguish between the inciting and perpetuating factors since all of these factors are intertwined. Inflammation may lead to malnutrition through appetite suppression, may enhance oxidative stress through increased free radical generation, and may directly modify antiatherogenic and proatherogenic factors, rendering them more proatherogenic. Malnutrition may decrease the ingestion of reducing agents, allowing oxidizing reactions to go unchecked, and may also result in altered protein binding of proatherogenic substances such as homocysteine. Current research efforts are directed at improving our understanding of these factors individually and in concert.

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