

# Increased Plasma S-Nitrosothiol Concentrations Predict Cardiovascular Outcomes among Patients with End-Stage Renal Disease: A Prospective Study

ZIAD A. MASSY,<sup>\*†</sup> CHRISTINE FUMERON,<sup>‡</sup> DIDIER BORDERIE,<sup>§</sup>  
PHILIPPE TUPPIN,<sup>||</sup> THAO NGUYEN-KHOA,<sup>\*¶</sup> MARIE-ODILE BENOIT,<sup>#</sup>  
CHRISTIAN JACQUOT,<sup>\*\*</sup> CLAUDE BUISSON,<sup>‡</sup> TILMAN B. DRÜEKE,<sup>\*</sup>  
OHVANESSE G. EKINDJIAN,<sup>§</sup> BERNARD LACOUR,<sup>¶</sup> and  
MARIE-CHRISTINE ILIOU<sup>††</sup>

<sup>\*</sup>INSERM U507, Necker Hospital, Paris, France; <sup>†</sup>Divisions of Clinical Pharmacology and Nephrology, Faculty of Pharmacy, University of Picardie and Centre Hospitalier Universitaire Amiens, Amiens, France; <sup>‡</sup>Hemodialysis Unit, Association pour L'Utilisation du Rein Artificiel, Paris, France; <sup>§</sup>Biochemistry Laboratory A, Cochin Hospital, Paris, France; <sup>||</sup>French Transplantation Agency, Paris, France; <sup>¶</sup>Biochemistry Laboratory A, Necker Hospital, Paris, France; <sup>#</sup>Biochemistry Laboratory and <sup>\*\*</sup>Division of Nephrology, Hopita Européen - Goerges Pompidou, Paris, France; and <sup>††</sup>Cardiac Rehabilitation Centre, Broussais Hospital, Paris, France.

**Abstract.** The plasma concentrations of S-nitrosothiols, which are circulating nitric oxide metabolites with potential biologic activity, are increased among patients undergoing chronic hemodialysis (HD). However, the ability of S-nitrosothiols to release nitric oxide at physiologically relevant sites may be reduced among HD patients, because of impaired availability and/or activity of factors involved in S-nitrosothiol breakdown. The resultant lack of S-nitrosothiol bioavailability could contribute to the high cardiovascular risk for such patients. A possible relationship between plasma S-nitrosothiol levels and cardiac outcomes, as well as all-cause mortality rates, was investigated in a cohort of 250 chronic HD patients and who were undergoing regular dialysis three times per week were monitored for 1 yr. During that follow-up period, major cardiac events and all-cause deaths were prospectively recorded. At baseline, high plasma S-nitrosothiol levels ( $>2 \mu\text{M}$ , corre-

sponding to the top quartile of all measured values) were independently associated with pulse pressure in an adjusted multivariate analysis (odds ratio, 1.03; 95% confidence interval, 1.01 to 1.05;  $P = 0.007$ ). During the follow-up period, 36 patients died (16 as a result of cardiac causes) and 33 patients experienced major adverse cardiac events. In an adjusted Cox proportional-hazards model, high plasma S-nitrosothiol concentrations (*i.e.*, the top quartile *versus* the three other quartiles) were an independent predictor of cardiac events (hazard ratio, 3.30; 95% confidence interval, 1.61 to 6.76;  $P = 0.001$ ) but not of all-cause death. Therefore, among chronic HD patients, markedly elevated plasma S-nitrosothiol levels are associated with pulse pressure and predict cardiovascular outcomes. These findings support the hypothesis that impaired S-nitrosothiol bioavailability in uremia is an important factor for the excessive cardiovascular risk among HD patients.

Premature atherosclerosis is one of the primary causes of morbidity and death among patients with ESRD (1,2). Among several classic and nonclassic mechanisms conferring increased cardiovascular risk, impairment of nitric oxide (NO) bioavailability has emerged as a potentially important mechanism. Impaired NO-mediated, endothelium-dependent vasodilation, an early marker of atherosclerosis, has been observed among patients with predialysis chronic renal failure (3) and

patients with ESRD (4,5). The impaired endothelium-dependent vasodilation could be linked to an absolute deficit of NO production. Indeed, increased levels of asymmetric dimethyl-arginine (ADMA), an active endogenous inhibitor of NO synthase, have been observed to be associated with the severity of atherosclerosis, independently of other vascular risk factors (6), and with concentric left ventricular hypertrophy and left ventricular dysfunction among patients with ESRD (7). Moreover, associations between ADMA levels and overall mortality or cardiovascular outcome rates were recently demonstrated among patients with ESRD (8).

However, the evidence for deficient NO synthesis among such patients remains inconclusive. Schmidt and Baylis (9) demonstrated that 24-h urinary nitrite/nitrate excretion was low among patients with chronic kidney disease, compared with healthy control subjects with similar dietary NO intake, suggesting that net endogenous NO production was decreased.

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Correspondence to Dr. Ziad A. Massy, INSERM U507, Necker Hospital, 161, rue de Sèvres, 75015, Paris, France. Phone: +33-1-4449-5234; Fax: +33-1-4566-5133; E-mail: massy@necker.fr

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However, elevated extrarenal NO production has been observed among patients with ESRD (10). Furthermore, it was recently reported that the plasma of patients undergoing chronic hemodialysis (HD) contained significantly higher S-nitrosothiol levels than did the plasma of healthy control subjects (11). S-Nitrosothiols, which are the result of reactions of NO with molecules containing functional sulfhydryl groups, are considered a NO pool with a potential for vasodilatory effects (12). This points to the presence of circulating NO metabolites with potential biologic activity and argues against an absolute quantitative NO deficiency among patients with ESRD. It should be noted, however, that elevated plasma S-nitrosothiol concentrations among HD patients could also be attributable to peroxynitrite detoxification via a reaction with thiols, to decrease nitrosative stress (11). It was recently demonstrated that nitrosothiol formation *in vivo* depends not only on the availability of NO and O<sub>2</sub> but also on the degree of oxidative stress via changes in the steady-state concentration of thiyl radicals (13). There are also other explanations for elevated plasma S-nitrosothiol concentrations among HD patients, such as thiol retention of NO attributable to the accumulation of thiol molecules (14). The half-lives and renal or extrarenal clearances of plasma S-nitrosothiols in the general population and among patients with ESRD are still unknown. The issue seems even more complicated because of the heterogeneity of circulating S-nitrosothiol molecules.

The pathophysiologic consequences of high plasma S-nitrosothiol concentrations are unknown. It is possible that the ability of S-nitrosothiols to release NO at physiologically relevant sites is reduced among patients with ESRD because of impaired availability and/or activity of factors involved in S-nitrosothiol breakdown, such as ascorbate and several enzymes, including plasma glutathione peroxidase (GSH-Px) (15,16). Marked ascorbate deficiencies and low levels of plasma GSH-Px activity have been observed among patients with ESRD (17). Ascorbate deficiency was recently suggested as a potential cause of elevated plasma S-nitrosothiol concentrations in preeclampsia (18). Therefore, an enhanced vasodilatory action of high S-nitrosothiol levels is improbable. On the contrary, a lack of S-nitrosothiol bioavailability might be involved in the pathogenesis of the impaired NO-mediated, endothelium-dependent vasodilation observed among patients with ESRD, favoring hypertension, left ventricular hypertrophy, and cardiovascular events. With these pathophysiologic considerations in mind, the aim of our study was to examine a possible association between plasma S-nitrosothiol levels and cardiac outcomes, as well as overall survival rates, in a cohort of chronic HD patients.

## Materials and Methods

### Study Protocol

We included in the study cohort all patients at least 18 yr of age who attended one of three large Parisian HD centers. All of the patients included had been undergoing intermittent HD treatment for >3 mo, with three sessions per week. They gave informed consent, according to local internal review board rules, and did not meet any of the following exclusion criteria: predialysis hemoglobin concentration

of <8 g/dl, surgical treatment or severe infectious episode in the 8 d preceding the start of the study, acute coronary syndrome, acute cerebrovascular event, arterial revascularization in the 3 wk preceding the start of the study, or refusal to participate.

### Patients

Medical data were recorded for each patient with routine computerized screening. We analyzed the history of ischemic heart disease, cardiovascular risk factors, and dialysis modalities. A diagnosis of ischemic heart disease was made with stringent criteria, such as a history of myocardial infarction, coronary artery revascularization, and/or significant stenosis on coronary artery angiograms. Patients were considered to be diabetic if they demonstrated fasting blood glucose levels of >126 mg/dl or were being treated with insulin. BP during the week of blood sample collection was determined by averaging three semiautomatic measurements before each HD session. Pulse pressure was calculated as the difference between systolic and diastolic BP. Patients were considered to have hypercholesterolemia if they exhibited serum total cholesterol concentrations of >200 mg/dl or were receiving lipid-lowering drugs. Information concerning current tobacco use was obtained with a questionnaire. Clinical and laboratory data were obtained for 302 patients. Exclusion criteria included recent initiation of dialysis, severe anemia, refusal or inability to participate, or blood samples unsuitable for S-nitrosothiol measurements. Clinical characteristics were not significantly different for the patients who participated in the study *versus* those who did not. Two hundred fifty patients were judged eligible for the study and included.

Echocardiography was performed according to the recommendations of the American Society of Echocardiography, on a dialysis-free day, in the 3 mo before or after inclusion. Left ventricular hypertrophy was defined as a left ventricular mass index of at least 134 g/m<sup>2</sup> for men and 110 g/m<sup>2</sup> for women (19).

The duration of the HD sessions and the type of membranes used for dialysis were not modified during the study; such choices were left to the discretion of the referring physician. Kt/V was calculated according to the second formula described by Daugirdas (20).

### Biochemical Determinations

Blood samples were collected before the first dialysis session of the week. Blood samples (from an arteriovenous fistula) were collected in vacuum tubes. Blood hemoglobin levels were determined with a Coulter MAXM analyzer (Beckman Coulter, Villepinte, France). For the S-nitrosothiol assay, samples were centrifuged at 3500 rpm for 15 min immediately after collection, and serum and plasma were frozen in aliquots at -80°C. Plasma S-nitrosothiol concentrations were determined with a fluorometric method described previously (11), with a minor modification (21). Although the addition of ammonium sulfamate at neutral pH neutralized nitrite for the vast majority of HD patients, limited amounts of residual nitrite remained in the plasma of some patients. In those instances, we corrected the plasma S-nitrosothiol concentration by subtracting the residual nitrite level. The mean and the upper limit of normal plasma S-nitrosothiol concentrations were 0.45 and 1.57 μM, respectively. The assay demonstrated a within-assay variation coefficient of 7.7% and a between-assay variation coefficient of 7.9% for a mean S-nitrosothiol concentration of 2 μM. The detection limit of the assay was 0.1 μM.

### Follow-Up Observations

At the 1-yr follow-up assessments, patient outcomes were evaluated (by chart review) by the nephrologists in charge of patient

treatment, who were unaware of the *S*-nitrosothiol values. The primary endpoints were the first occurrence of a fatal or nonfatal major cardiac event and death from all causes. Major adverse cardiac events included cardiac death, nonfatal myocardial infarction, and unstable angina requiring coronary revascularization (angioplasty or bypass surgery). For patients who died, information concerning the circumstances and date of death was obtained and medical reports were procured. Heart failure attributable to fluid overload, which could usually be corrected with hemofiltration for patients without a history of heart failure, was not considered a cardiac event. Patients who received kidney transplants were monitored for 1 yr; they were not censored.

### Statistical Methods

Baseline differences between groups with elevated *versus* normal *S*-nitrosothiol levels were assessed with the  $\chi^2$  test and *t* test for univariate analyses. Multiple logistic regression models were used with the backward selection procedure to identify independent factors significantly associated with elevated plasma *S*-nitrosothiol values. The Kaplan-Meier product-limit method was used to examine survival rates. The equality of survivor function across groups was tested with the log rank test. The Cox proportional-hazards regression model was used with a stepwise procedure to identify the factors that were significant and predictive of cardiac and global mortality rates at 1 yr. The SAS computer package (SAS Institute, Cary, NC) was used for all statistical analyses.  $P < 0.05$  was considered to be significant. Data are expressed as mean  $\pm$  SD.

## Results

### Population Cohort

The main characteristics of the 250 patients included in the study are presented in Table 1. Some of the patients were treated with aspirin (26.8%),  $\beta$ -receptor blockers (31.2%), calcium antagonists (33.6%), angiotensin-converting enzyme inhibitors (24%), and/or lipid-lowering agents (27%). Seventy-six percent of the patients received subcutaneous erythropoietin therapy ( $106 \pm 75$  IU/kg per wk).

### Biochemical Findings

The mean plasma *S*-nitrosothiol concentration was  $1.77 \pm 0.32$   $\mu$ M and the median was 1.78  $\mu$ M. These values were three times higher than the normal mean plasma *S*-nitrosothiol concentration (11). According to the normal distribution of *S*-nitrosothiol levels, we divided the population into quartiles (<1.57, 1.57 to 1.78, 1.78 to 2.0, and >2.0  $\mu$ M). The top quartile ( $n = 60$ ) was considered “high titer,” compared with the three other quartiles (“low titer”). Parameters observed to be associated with elevated plasma *S*-nitrosothiol levels are presented in Table 2. Of note, no significant difference in Kt/V values was observed between the two groups (Table 2).

High *S*-nitrosothiol titers were significantly associated with elevated systolic BP and elevated pulse pressure, compared with low titers (Table 2). Because pulse pressure is highly correlated with systolic BP, we included only pulse pressure in the multivariate analysis. Pulse pressure remained independently predictive of elevated plasma *S*-nitrosothiol levels after adjustment for age, time on dialysis, history of ischemic cardiac disease, left ventricular mass, and hemoglobin levels.

Table 1. Main characteristics of the patient population<sup>a</sup>

No. of patients	250
Age (yr)	60.4 $\pm$ 14.9
Gender (men/women)	145/105
Underlying renal diseases	
glomerulonephritis	59 (23.6%)
interstitial nephropathy	43 (17.2%)
vascular nephropathy	31 (12.4%)
diabetes mellitus	39 (15.6%)
polycystic kidney disease	21 (8.4%)
other	20 (8%)
unknown	37 (14.8%)
Dialysis modalities	
time on dialysis (yr)	7.54 $\pm$ 7.22
type of dialysis membrane at time of study	
modified cellulose	87 (34.8%)
synthetic	163 (65.2%)
dialysis dose, as assessed by urea Kt/V <sup>b</sup>	1.46 $\pm$ 0.36
dialysis time per week (h)	12.45 $\pm$ 1.02
bicarbonate dialysis	223 (89.2%)
acetate-free biofiltration	27 (10.8%)
One-yr follow-up findings	
global death	36 (14.4%)
major adverse cardiac events	33 (13.2%)

<sup>a</sup> Data are expressed as mean  $\pm$  standard deviation. For noncontinuous values, results are numbers of patients.

<sup>b</sup> Urea Kt/V was calculated according to the second Daugirdas formula.

### One-Year Follow-Up Findings

All of the patients included in the study were monitored for 1 yr or until death. During the 1-yr follow-up period, 36 patients (14.4%) in the study population died, with 16 (6.46%) dying as a result of cardiac causes. Thirty-three patients (13.2%) experienced one or more major adverse cardiac events. Patients with elevated *S*-nitrosothiol levels demonstrated a significantly higher cardiac mortality rate, compared with patients with low levels (13.3% *versus* 4.2%,  $P = 0.012$ ), and experienced more major adverse cardiac events (23.3% *versus* 10.0%,  $P = 0.009$ ) (Figure 1). No difference in all-cause mortality rates according to *S*-nitrosothiol levels was observed (21.7% *versus* 12.1%,  $P = 0.068$ ) (Figure 1).

The results of the Cox proportional-hazards analysis are presented in Table 3. All-cause mortality rates depended on age and hemoglobin levels but not on *S*-nitrosothiol levels. Age (relative risk, 1.07), prior ischemic heart disease (relative risk, 3.93), and plasma *S*-nitrosothiol levels of >2.0  $\mu$ M (*i.e.*, the top quartile *versus* the three other quartiles) (relative risk, 3.30) were significant independent predictors of major adverse cardiac events.

In view of the limited number of events (33 patients experienced one or more major adverse cardiac events), we repeated the Cox proportional-hazards analysis and included only covariates with a significance of  $\leq 0.01$  in univariate analyses. The relative risk value for plasma *S*-nitrosothiol

Table 2. Factors associated with elevated plasma S-nitrosothiol levels<sup>a</sup>

	Low Titer (n = 190)	High Titer (n = 60)	Unadjusted Odds Ratio (95% CI)	P Value	Adjusted Odds Ratio (95% CI)	P Value
Age (yr)	59.8 ± 15.3	62.6 ± 13.6	1.01 (0.99 to 1.03)	0.21		
Time on dialysis (yr)	7.4 ± 7.2	7.9 ± 7.2	1.01 (0.97 to 1.05)	0.67		
Urea Kt/V	1.46 ± 0.38	1.46 ± 0.32	0.68 (0.30 to 1.56)	0.36		
S-Nitrosothiol level (μM)	1.64 ± 0.24	2.19 ± 0.14 <sup>b</sup>				
Systolic BP (mmHg)	141 ± 26.1	151 ± 20.6	1.02 (1.00 to 1.03)	0.01		
Diastolic BP (mmHg)	76 ± 16.8	79 ± 14.6	1.01 (0.99 to 1.03)	0.18		
Pulse pressure (mmHg)	64.8 ± 15.8	71.2 ± 16.0	1.03 (1.01 to 1.05)	0.007	1.03(1.01to1.05)	0.007
Hemoglobin (g/dl)	11.1 ± 1.2	11.1 ± 1.0	0.96 (0.74 to 1.24)	0.73		
Log ultra-sensitive CRP (mg/L)	1.56 ± 1.24	1.57 ± 1.21	1.01 (0.79 to 1.29)	0.95		
Diabetes mellitus (%)	20.5	16.7	0.77 (0.36 to 1.66)	0.51		
Hypercholesterolemia (%)	40.0	35.0	0.81 (0.44 to 1.48)	0.49		
Active smokers (%)	12.6	15.0	1.22 (0.53 to 2.80)	0.64		
Indexed ventricular mass (g/m <sup>2</sup> )	112 ± 37	123 ± 35	1.01 (1.00 to 1.02)	0.06		
Left ventricular hypertrophy (%)	33.5	42.9	1.49 (0.81 to 2.74)	0.20		
Prior ischemic cardiac disease (%)	23.7	18.3	0.72 (0.35 to 1.51)	0.39		
Death (%)	12.1	21.7	2.01 (0.95 to 4.30)	0.07		
Cardiac death (%)	4.2	13.3	3.50 (1.25 to 9.78)	0.016		
Major adverse cardiac events (%)	10.0	23.3	2.74 (1.28 to 5.88)	0.01		

<sup>a</sup> Data are summarized as means ± SD or percentages. The low-titer and high-titer groups were defined on the basis of the fourth quartile of S-nitrosothiol values. Urea Kt/V was calculated according to the second Daugirdas formula. CRP, C-reactive protein; CI, confidence interval.

<sup>b</sup> *t* test, *P* < 0.001.

levels adjusted for age and prior ischemic heart disease remained unchanged at 3.30 (95% confidence interval, 1.61 to 6.76).

## Discussion

Our findings demonstrate that high plasma S-nitrosothiol concentrations are associated with high pulse pressure and are predictive of negative cardiac outcomes among chronic HD patients. These findings support the hypothesis that impairment of S-nitrosothiol bioavailability is involved in the impairment of NO-mediated, endothelium-dependent vasodilation among patients with ESRD, consequently favoring hypertension and cardiovascular events.

This work confirms in a large cohort our previous observation of elevated plasma S-nitrosothiol concentrations among chronic HD patients (11). Recent work by Wlodek *et al.* (22) demonstrated that plasma S-nitrosothiol levels were higher among patients with chronic renal failure who were not yet undergoing dialysis, compared with healthy control subjects, and levels were slightly but not significantly higher among patients undergoing HD. In that report, however, the authors subsequently expressed S-nitrosothiol amounts per milligram of plasma protein and actually noted lower S-nitrosothiol levels for their HD patients, compared with healthy control subjects.

The rationale for this expression is difficult to follow, because S-nitrosothiol molecules are not only peptide-linked, and the elevation of plasma S-nitrosothiol concentrations among patients with ESRD may be attributable to nonpeptidic, low-molecular weight molecules. Furthermore, Wlodek *et al.* (22) observed plasma S-nitrosothiol concentrations different from those observed in our study, but they used L-cysteine to obtain a standard calibration curve, whereas we used reduced glutathione to obtain the calibration curve. A direct comparison of the results obtained with the two methods is therefore difficult. Determinations of S-nitrosothiol levels have yielded highly variable results in various laboratories (23). We think that, in the absence of a standard method, any direct comparison of S-nitrosothiol concentrations measured in biologic fluids should be limited to results obtained with same methodologic procedure.

Recent epidemiologic studies demonstrated that pulse pressure is associated with the relative risk of cardiovascular events and all-cause death among chronic HD patients (24,25). Arterial stiffness and early pulse wave reflection are the principal determinants of elevated systolic and pulse pressures among patients with ESRD and are associated with left ventricular hypertrophy (26). Recent animal and human data demonstrated a close relationship between reduced NO bioavailability and

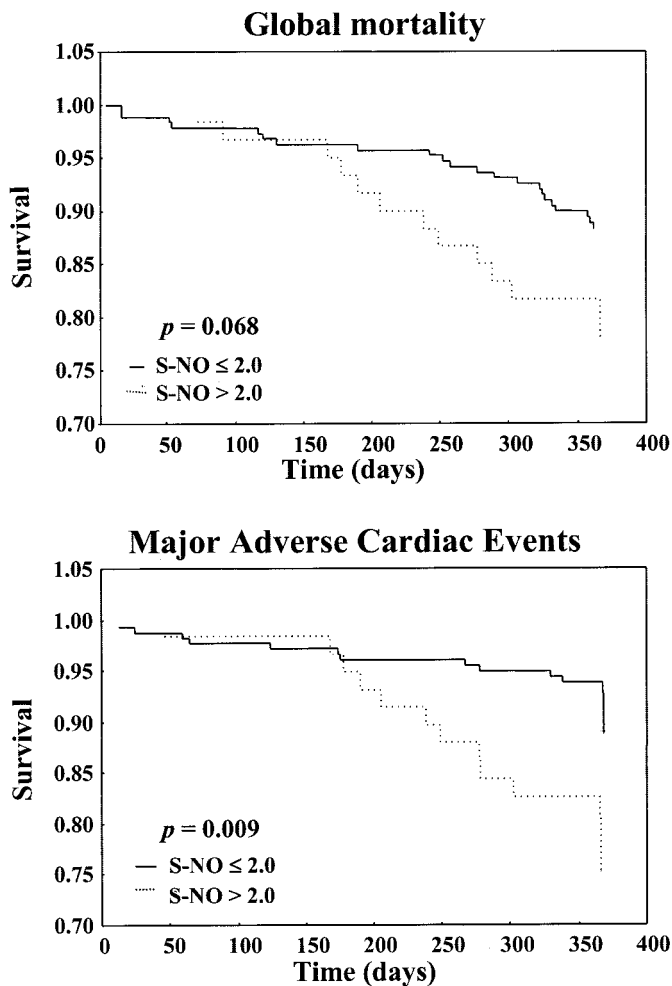


Figure 1. Kaplan-Meier survival curves for global death and major adverse cardiac events. Findings were compared for hemodialysis (HD) patients with plasma *S*-nitrosothiol (S-NO) concentrations in the top quartile and HD patients with *S*-nitrosothiol concentrations in the three other quartiles.

large-artery distensibility, leading to increased arterial stiffness (27–30). The relationship between *S*-nitrosothiol levels and pulse pressure observed in this study does not indicate a causal association, because it remains possible, at least theoretically, that the observed increase in pulse pressure is responsible for an increase in NO release (31). In any case, the association between *S*-nitrosothiol levels and pulse pressure among patients undergoing HD may help link the impairment of NO-mediated, endothelium-dependent vasodilation to the generation of hypertension and consequently the increased frequency of cardiovascular events among patients with ESRD. We are currently undertaking a study to directly test the relationship between plasma *S*-nitrosothiol concentrations and endothelium-dependent functions, as well as arterial stiffness.

In addition to vascular remodeling, reduced *S*-nitrosothiol bioavailability may be involved in the genesis of cardiovascular events through other mechanisms, such as increased platelet activation, adhesion, and aggregation (15). Deficiencies of bioactive NO have been observed to be associated with arterial

thrombosis in animal models, among individuals with endothelial dysfunction, and among patients with low extracellular GSH-Px activity (15). It was recently demonstrated that low-molecular weight plasma thiols (*e.g.*, *S*-nitrosoglutathione) play important roles in the formation and activation of *S*-nitrosoalbumin reservoirs, potentiating NO-mediated inhibition of platelet aggregation (32). The overall decrease in glutathione concentrations among chronic HD patients (17), in association with a relative platelet selectivity of *S*-nitrosoglutathione (33), may greatly contribute to the compromised hemostasis of chronic renal failure.

The link between elevated plasma *S*-nitrosothiol concentrations and the development of cardiovascular complications has not been extensively evaluated. Previously, workers postulated that elevated plasma *S*-nitrosothiol concentrations among women with preeclampsia reflect insufficient nitrosothiol decomposition and NO release at sites that are critical for normal regulation of vascular tone, because of ascorbate deficiency (18). In the latter study, a correlation between *S*-nitrosothiol concentrations and systolic BP was observed, although it was at the limit of significance ( $r = 0.43$ ,  $P = 0.06$ ). Therefore, to the best of our knowledge, this work is the first systematic demonstration of an association between plasma *S*-nitrosothiol levels and cardiovascular disease in the clinical setting.

Patients with ESRD have high plasma ADMA concentrations, and the latter were observed to be associated with cardiovascular outcomes (8). They were also correlated with increased BP in different populations (34,35). ADMA, an active endogenous inhibitor of NO synthase (36), could influence circulating *S*-nitrosothiol levels. Because we did not evaluate plasma ADMA levels, we cannot exclude the possibility of an additional increase in plasma *S*-nitrosothiol concentrations among HD patients when the ADMA-associated inhibition of NO production is lacking. However, that would not modify the impairment of *S*-nitrosothiol bioavailability among HD patients. If elevated plasma *S*-nitrosothiol concentrations reflect the intensity of nitrosative stress among HD patients, then they could be the trigger for high ADMA levels. Indeed, intracellular concentrations of ADMA are highly dependent on the activity of the enzyme dimethyldiaminohydrolase, which transforms ADMA into citrulline, and the activity of this enzyme is blocked by oxidative stress (37). This complex relationship is also currently under investigation in our laboratory.

We recognize that many pathways exist for the formation of *S*-nitrosothiols, which may play a significant role in the cardiovascular system (38). The elevation of circulating *S*-nitrosothiol levels could reflect a protective mechanism against nitrosative stress (15). In line with this view, we observed normal plasma nitrotyrosine levels among HD patients (11). In that case, *S*-nitrosothiols (and ADMA) may represent useful markers of cardiovascular disease in ESRD. However, *S*-nitrosothiols are considered to be potent vasodilators, the action of which is commonly associated with the ability to release NO at physiologically relevant sites (12,39). We previously reported marked simultaneous decreases in ascorbate levels and plasma GSH-Px activity, together with increased plasma *S*-nitrosothiol

Table 3. Unadjusted and adjusted relative risks for global death, cardiac death, and major adverse cardiac events at 1 yr of follow-up monitoring<sup>a</sup>

	Relative Risk (95% CI)	P Value	Adjusted Relative Risk (95% CI)	P Value
Global death				
age	1.05 (1.03 to 1.08)	<0.001	1.05 (1.02 to 1.08)	0.0003
hemoglobin	0.65 (0.48 to 0.89)	0.007	0.62 (0.45 to 0.86)	0.004
pulse pressure	1.01 (0.99 to 1.03)	0.26		
prior ischemic cardiac disease	2.11 (1.09 to 4.08)	0.03		
left ventricular hypertrophy	1.88 (0.98 to 3.61)	0.06		
S-nitrosothiol levels of >2 μM	1.88 (0.96 to 3.71)	0.07		
Major adverse cardiac events				
age	1.07 (1.03 to 1.1)	<0.0001	1.07 (1.04 to 1.11)	0.0002
hemoglobin	0.76 (0.56 to 1.05)	0.1		
pulse pressure	1.01 (0.99 to 1.04)	0.17		
prior ischemic cardiac disease	3.50 (1.79 to 6.87)	0.0003	3.93 (1.94 to 7.97)	0.0001
left ventricular hypertrophy	2.01 (1.01 to 3.98)	0.04		
S-nitrosothiol levels of >2 μM	2.47 (1.24 to 4.93)	0.01	3.30 (1.61 to 6.76)	0.001

<sup>a</sup> CI, confidence interval.

concentrations, among patients with ESRD (17). Those findings suggest that, at least under those conditions, an enhanced vasodilatory action of S-nitrosothiols is improbable. It is possible that supplementation with molecules favoring S-nitrosothiol breakdown (e.g., ascorbate) could enhance the release of NO from S-nitrosothiols and thus correct the bioactive NO deficiency among patients with ESRD. In line with this hypothesis, Cross *et al.* (40) recently demonstrated that the acute administration of vitamin C reduced oxidative stress among patients with chronic renal failure and improved NO-mediated resistance vessel dilation. The effects of ascorbate supplementation on plasma S-nitrosothiol concentrations among HD patients remain to be tested in prospective studies.

In conclusion, elevated plasma S-nitrosothiol concentrations among HD patients are associated with high pulse pressure and prospectively predict cardiac outcomes. These findings support our theory of impaired S-nitrosothiol bioavailability, which might be an important risk factor for the excessive cardiovascular death rates among such patients. Intervention trials are needed to investigate the hypothesis that supplementation with molecules that enhance S-nitrosothiol breakdown could lead to increased NO release and favorably affect cardiovascular outcomes among patients with ESRD.

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