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Serum Myeloperoxidase and Mortality in Maintenance Hemodialysis Patients

Kamyar Kalantar-Zadeh, MD, PhD, MPH, Marie-Luise Brennan, PhD, and Stanley L. Hazen, MD, PhD

● **Background:** During inflammation, myeloperoxidase (MPO) is released, for which its measurement in systemic circulation may be used as an index of leukocyte activation and oxidant stress. MPO levels correlate with angiographic evidence of coronary atherosclerosis and cardiovascular events in subjects with chest pain within the general population. We hypothesized that serum MPO levels are associated with adverse clinical outcomes in maintenance hemodialysis (MHD) patients. **Methods:** MPO levels were determined in serum samples from 356 MHD patients at the start of a 3-year cohort. **Results:** Patients (46% women, 28% blacks, 54% with diabetes) were 54.6 ± 14.6 (SD) years old and had undergone MHD for a median period of 26 months. Measured serum MPO level was $2,005 \pm 1,877$ pmol/L (median, 1,444 pmol/L; interquartile range, 861 to 2,490 pmol/L). MHD patients with greater total body fat had greater MPO levels. MPO level had statistically significant ($P < 0.01$) and positive correlations with values for serum C-reactive protein (CRP; $r = +0.15$), interleukin 6 (IL-6; $r = +0.23$), tumor necrosis factor α (TNF- α ; $r = +0.21$), and white blood cell count ($r = +0.21$). A death hazard ratio for each 1,000-pmol/L increase in serum MPO level was 1.14 (95% confidence interval [CI], 1.03 to 1.26; $P = 0.01$) after controlling for age, race (black), diabetes mellitus, dialysis vintage, Charlson comorbidity score, history of previous cardiovascular disease, blood hemoglobin level, and serum concentrations of albumin, CRP, IL-6, and TNF- α . After dividing MPO values into 3 equal groups (tertiles), the death hazard ratio of the highest tertile (versus the middle tertile) was 1.82 (95% CI, 1.07 to 3.10; $P = 0.03$). **Conclusion:** Serum MPO levels correlate with levels of markers of inflammation and prospective mortality risk in MHD patients. *Am J Kidney Dis* 48:59-68.

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INDEX WORDS: Myeloperoxidase; hemodialysis (HD); malnutrition-inflammation complex syndrome; oxidative stress; proinflammatory cytokines.

MYELOPEROXIDASE (MPO), an abundant enzyme secreted by neutrophils, monocytes, and certain tissue macrophages during phagocyte activation, is a major component of the bactericidal armamentarium of leukocytes.¹ In acute inflammatory conditions, MPO is released in the extracellular medium and participates in innate immune defense mechanisms through formation of microbicidal reactive oxidant species. However, MPO-generated oxidants also can damage normal tissues, contributing to

inflammatory injury and potentially participating in the pathogenesis of cardiovascular disease.¹⁻⁶ Because of the many links between elevated MPO levels and atherosclerosis, recent interest has focused on a potential role for MPO and its oxidants as clinical predictors of cardiovascular risk in the general population.^{1-5,7}

Recent studies showed that MPO levels identify those among high-risk patients who are at increased risk for near-term cardiac events, highlighting the potential usefulness of MPO measure-

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ment for risk stratification in high-risk individuals.⁸⁻¹⁰ However, very few studies examined the role of MPO in patients with chronic kidney disease (CKD) and their outcomes. Hemodialysis treatment, even with the use of biocompatible membranes and ultrapure dialysate, may contribute to an increase in leukocyte activation and enhanced oxidative stress.¹¹⁻¹³ Recent genetic studies reported that the presence of a functional variant of the MPO gene is associated with rate of cardiovascular disease in French-Canadian men,⁴ as well as a group of patients with CKD.² However, to our knowledge, serum MPO has not been measured systematically in large cohorts of dialysis patients. Moreover, whether serum MPO levels are linked to malnutrition-inflammation-cachexia syndrome (MICS), a strong predictor of clinical outcome and survival in patients with CKD, has not been examined. Here, we measured serum MPO levels in a large cohort of maintenance hemodialysis (MHD) patients, examined its relationship with clinical and laboratory surrogates of nutrition and inflammation, and evaluated whether serum MPO levels predict mortality risk in these patients.

METHODS

Patient Population

Subjects participating in the Nutritional and Inflammatory Evaluation in Dialysis (NIED) Study originated from a pool of approximately 1,300 MHD outpatients in 8 DaVita Inc dialysis facilities in the South Bay–Los Angeles area (see NIED Study Web site: www.NIEDStudy.org for more details, as well as previous publications¹⁴). Inclusion criteria were outpatients who had been undergoing MHD for at least 8 weeks, were 18 years or older, and signed a local institutional review board–approved consent form. Patients with an anticipated life expectancy less than 6 months (eg, because of metastatic malignancy or advanced human immunodeficiency virus disease) were excluded.

In the initial phase of the NIED Study (October 2001 to March 2002), 385 patients from 8 dialysis units signed the written consent form. Subsequently, blood samples were obtained from 367 of these individuals because 18 patients were not present in the dialysis units at the time of blood drawing. After the planned laboratory measurements were performed (discussed later), residual sera of all except 11 patients remained and were frozen for future investigations.

The medical chart of each MHD patient was reviewed thoroughly by a nephrologist (K.K.-Z.), and data pertaining to underlying kidney disease, cardiovascular history, and other comorbid conditions were extracted. A modified version of the Charlson Comorbidity Index, ie, without the age and kidney disease components, was used to assess the severity of comorbidity.^{15,16}

Control Subjects

Control samples were selected randomly from volunteers at the time of blood donation and included 289 subjects (age ≥ 21 years) without obvious history of coronary artery disease. Healthy volunteers were 54 ± 12 years old and included 80% men. The Institutional Review Board at the Cleveland Clinic Foundation (Cleveland, OH) approved the study protocol for the controls.

Near-Infrared Interactance

To measure percentage of body fat and estimate lean body mass, near-infrared interactance^{17,18} technology was used at the same time as the foregoing anthropometric measurements. A commercial near-infrared interactance sensor with a coefficient of variation of 0.5% for total body fat measurement (portable Futrex 6100; Gaithersburg, MD; www.futrex.com) was used. Near-infrared measurements were performed by placing a Futrex sensor on the nonaccess upper arm for several seconds, after entering the required data (date of birth, sex, weight, and height) for each patient. Near-infrared measurements of body fat were shown to correlate significantly with other nutritional measures in MHD patients.^{17,18}

Simultaneous Laboratory Tests

Predialysis blood samples and postdialysis serum urea nitrogen levels were obtained on a midweek day and coincided chronologically with quarterly blood tests at DaVita facilities. Single-pool Kt/V was used to represent weekly dialysis dose. All routine laboratory measurements were performed by DaVita Laboratories (Deland, FL) by using automated methods.

Serum C-reactive protein (CRP) and 2 proinflammatory cytokines, interleukin 6 (IL-6) and tumor necrosis factor α (TNF- α), were measured as indices of degree of inflammation. High-sensitivity CRP was measured by using a turbidometric immunoassay in which a serum sample is mixed with latex beads coated with antihuman CRP antibodies, forming an insoluble aggregate (WPCI, Osaka, Japan; unit, milligrams per liter; normal range, <3.0 mg/L).^{19,20} High sensitivity IL-6 and TNF- α immunoassay kits based on a solid-phase sandwich enzyme-linked immunosorbent assay using recombinant human IL-6 and TNF- α were used to measure serum proinflammatory cytokines (R&D Systems, Minneapolis, MN; units: picograms per milliliter; normal range: IL-6, <9.9 pg/mL; TNF- α , <4.7 pg/mL).²¹⁻²³ CRP and cytokines were measured in the General Clinical Research Center Laboratories of Harbor-UCLA Medical Center. Serum prealbumin was analyzed by using an antigen-antibody complex assay, and total homocysteine concentrations were determined by means of high-performance liquid chromatography at Harbor-UCLA Clinical Laboratories.

Retrospective MPO Measurement

Frozen sera were transported to Cleveland Clinic General Clinical Research Center Laboratories for MPO measurements. A clinically validated and approved sandwich-based enzyme-linked immunosorbent assay (CardioMPO; Prognostix, Cleveland, OH) was used to measure serum MPO. MPO

Fig 1. Distribution of serum MPO levels among 356 MHD patients.

assay performance showed an assay limit of quantification of 13 pmol/L, intraplate and interplate coefficients of variance of 5.5% and 6.2%, and near quantitative recovery of MPO when spiked in serum ($96.7\% \pm 2.6\%$), respectively. Laboratory technicians were blinded to patient identifiers and clinical parameters.

Statistical Methods

Conventional analysis of variance, Kruskal-Wallis test, and chi-square were used, as appropriate, to detect significant differences among tertiles of MPO. Multivariate regression analyses and analysis of covariance were performed to obtain adjusted *P* controlled for case-mix and comorbidity covariates. Pearson and Spearman correlation coefficients (*r*) were used for analyses of associations, as appropriate. To calculate relative risks for death, we obtained hazard ratios (HRs) by using Cox proportional hazard models after controlling for the mentioned covariates. Plots of log (−log [survival rate]) against log (survival time) were performed to establish the validity of the proportionality assumption. Kaplan-Meier analyses were used to assess differences in surviving proportions among tertiles of MPO. Case-mix covariates included sex (female), age, race (black versus other), and dialysis vintage (number of months on MHD treatment); comorbidity covariates included diabetes mellitus (yes/no), history of cardiovascular disease (yes/no), and Charlson Comorbidity Index; and laboratory covariates in fully adjusted multivariate models included blood hemoglobin and serum MPO, CRP, IL-6, TNF- α , and albumin concentrations. Fiducial limits are given as mean $\pm$ SD or median and interquartile range. Risk ratios include 95% confidence intervals (CIs). *P* less than 0.05 or a 95% CI that did not span 1.0 is considered statistically significant. *P* between 0.05 and 0.20 also is listed with 2 decimals for the

sake of potential type II errors. Descriptive and multivariate statistics were carried out using the statistical software Stata, version 9.0 (Stata Corp, College Station, TX).

RESULTS

The 356 MHD patients under study included 46% women, 28% blacks, and 54% patients with diabetes. They were 54.6 ± 14.6 years old and had undergone MHD for a median vintage of 26 months (interquartile range, 13 to 51 months). **Figure 1** shows the distribution of measured serum MPO among 356 MHD patients. For MHD patients at the start of the cohort, serum MPO averaged $2,005 \pm 1,877$ pmol/L (median, 1,444 pmol/L; interquartile range, 861 to 2,490 pmol/L; minimum, 27 pmol/L; maximum, 13,268 pmol/L). Control subjects had an average MPO level of $2,032 \pm 724$ pmol/L (median, 1,755 pmol/L).

To examine the relationship between different levels of serum MPO and relevant clinical and laboratory measures, serum MPO was divided into 3 equal tertiles (**Table 1**). The highest MPO tertile included more women, but this difference was not statistically significant. Diabetes proportion and age distribution were similar across the 3 groups. Near-infrared measured total body fat and body mass index (BMI) were incrementally greater across increasing MPO tertiles. Of inflammatory mark-

Table 1. Baseline Demographic, Clinical, and Laboratory Measures for 3 Tertiles of Serum MPO Levels in 356 MHD Patients

Variable	Lowest Tertile: 27-994 pmol/L (n = 118)	Middle Tertile: 997-2,116 pmol/L (n = 119)	Highest Tertile: 2,118-13,268 pmol/L (n = 119)	Analysis of Variance <i>P</i>	Multivariate Adjusted <i>P</i>
Sex (% female)	42	42	52	0.3	NA
Race (% black)	23	29	30	0.4	NA
Diabetes mellitus (%)	53	54	56	0.9	NA
All-cause death (%)	28	29	34	0.5	NA
Cardiovascular death (%)	47	45	47	0.9	NA
Age (y)	55 ± 15	56 ± 14	53 ± 14	0.21	NA
Dialysis vintage (mo)	37.5 ± 37.6	32.8 ± 28.9	39.1 ± 34.1	0.3	NA
Charlson Comorbidity Index	1.9 ± 1.4	2.0 ± 1.5	2.1 ± 1.7	0.8	NA
Near-infrared total body fat (%)	24.5 ± 10.9	26.6 ± 10.3	28.4 ± 11	0.03	0.01
BMI (kg/m ²)	25.2 ± 5.9	26.6 ± 6.1	27.6 ± 6	0.02	0.02
Kt/V (single pool)	1.58 ± 0.28	1.56 ± 0.28	1.59 ± 0.29	0.6	0.8
Serum MPO (pmol/L)	667 ± 223	1,483 ± 321	3,863 ± 2,213	<0.001	NA
CRP (mg/L)	5.9 ± 5.7	5.7 ± 5.3	8.0 ± 11.1	0.04	0.03
IL-6 (ng/L)	10.6 ± 12.8	30.4 ± 83	28.5 ± 52.6	0.01	0.04
TNF-α (ng/L)	6.9 ± 3.6	7.4 ± 3.9	10.1 ± 9	<0.001	<0.001
Albumin (g/dL)	3.85 ± 0.4	3.81 ± 0.3	3.87 ± 0.3	0.3	0.4
Prealbumin (mg/dL)	28.7 ± 8.8	27.5 ± 10.3	27.8 ± 9.6	0.6	0.6
Total cholesterol (mg/dL)	142.1 ± 47.9	143.6 ± 46.5	148.6 ± 45.4	0.5	0.24
Total homocysteine (μmol/L)	24.8 ± 10.8	24.6 ± 12.5	23.9 ± 12.5	0.8	0.5
Creatinine (mg/dL)	10.4 ± 3.2	10.8 ± 3.5	10.9 ± 3.3	0.5	0.23
Calcium (mg/dL)	9.3 ± 0.7	9.3 ± 0.6	9.3 ± 0.7	0.7	0.4
Phosphorus (mg/dL)	5.7 ± 1.5	5.8 ± 1.5	6.0 ± 1.5	0.3	0.18
Intact parathyroid hormone (pg/mL)	287 ± 256	344 ± 359	396 ± 459	0.09	0.07
Lactate dehydrogenase (U/L)	159 ± 35	166 ± 38	170 ± 48	0.14	0.09
Blood hemoglobin (g/dL)	11.9 ± 1	11.9 ± 1	11.9 ± 0.9	0.9	0.7
WBCs (× 1,000)	6.8 ± 1.7	7.2 ± 3.2	7.9 ± 2.4	0.002	<0.001
Administered erythropoietin (U/wk)	12,860 ± 7,969	15,492 ± 13,353	15,967 ± 14,276	0.14	0.19

NOTE. *P* < 0.20 are in bold type for convenient comparison. To convert serum creatinine in mg/dL to μmol/L, multiply by 88.4; albumin and hemoglobin in g/dL to g/L, multiply by 10; calcium in mg/dL to mmol/L, multiply by 0.2495; cholesterol in mg/dL to mmol/L, multiply by 0.02586; phosphorus in mg/dL to mmol/L, multiply by 0.3229.

Abbreviation: NA, not applicable.

*Multivariate adjusted regression controls for age, sex, race (black), vintage, diabetes, Charlson Comorbidity Index, and history of cardiovascular disease.

ers, serum CRP and TNF-α were high in the highest MPO tertile, and serum IL-6 was almost equally high in the 2 higher MPO tertiles. Serum parathyroid hormone and lactate dehydrogenase levels, peripheral white blood cell (WBC) count, and administered dose of erythropoietin showed an increasing trend across increasing MPO tertiles.

To assess the relationship of these markers with MPO levels within the MHD cohort, Spearman correlation coefficients were evaluated (Table 2). Associations between levels of serum MPO and the 3 inflammatory markers were mod-

erate and statistically significant. Peripheral WBC count, near-infrared measured body fat percentage, and BMI also had significant and positive associations with serum MPO levels. Scatter diagrams showing associations between serum MPO and values for serum CRP, IL-6, TNF-α, and WBCs are shown in Fig 2.

To examine the mortality predictability of MPO and compare it with levels of the 3 measured inflammatory markers, Cox proportional hazard regressions were modeled (Table 3). The a priori selected increment in each variable was approximately half of 1 SD or interquartile range. For

Table 2. Comparison of Spearman Correlation Coefficients (*r*) of Serum MPO With 3 Measures of Inflammation: Serum CRP, IL-6, and TNF- α

Variables	MPO	CRP	IL-6	TNF- α
Serum CRP (mg/L)	0.15*			
IL-6 (ng/L)	0.23*	0.39*		
TNF- α (ng/L)	0.21*	0.09	0.30*	
Sex (female)	0.08	-0.03	0.03	0.03
Race (black)	0.06	0.13†	0.05	0.01
Diabetes mellitus	0.03	0.06	0.08	-0.09
Age (y)	-0.03	0.15*	0.11†	0.13†
Dialysis vintage (mo)	0.06	0.04	0.12†	0.18*
Charlson Comorbidity Index	0.04	0.14*	0.14*	0.01
Near-infrared total body fat (%)	0.16*	0.14†	0.15*	0.02
BMI (kg/m ²)	0.17*	0.26*	0.13†	0.02
Kt/V (single pool)	0.05	-0.10	0.02	0.14†
Albumin (g/dL)	0.02	-0.22*	-0.35*	0.01
Prealbumin (mg/dL)	0.05	-0.20*	-0.43*	0.10
Total cholesterol (mg/dL)	0.06	-0.04	-0.10†	0.00
Total homocysteine (μ mol/L)	-0.07	-0.10	-0.06	0.07
Creatinine (mg/dL)	0.05	-0.12†	-0.20*	0.07
Calcium (mg/dL)	0.02	-0.07	0.09	0.09
Phosphorus (mg/dL)	0.11†	0.07	0.09	0.01
Intact parathyroid hormone (pg/mL)	0.12†	0.05	0.05	0.05
Lactate dehydrogenase (U/L)	0.08	0.04	0.17*	0.10
Blood hemoglobin (g/dL)	0.00	-0.10	-0.08	0.02
WBCs ($\times 1,000$)	0.21*	0.11†	0.18*	0.02
Administered erythropoietin (U/wk)	0.05	0.26*	0.30*	0.07

NOTE. Correlation coefficients of 0.15 or greater are in bold type for convenient comparison.

* $P < 0.01$.

† P between 0.05 and 0.01.

each 1,000-pmol/L increase in serum MPO level, there was 8% to 15% increase in risk for death during the observed 3-year interval according to the level of multivariate adjustment. In the fully adjusted model in which case-mix and comorbid variables, along with all 5 laboratory markers of inflammation and blood hemoglobin, were added, only serum MPO, CRP, and albumin levels remained predictive of mortality risk, with similar associations noted (Table 3). The ability of serum MPO level to predict mortality risk did not change with controlling for these inflammatory markers, indicating its statistical independence in this regard. Figure 3 shows Kaplan-Meier survival analysis for the 3 tertiles of MPO. Adjusted death HR for the highest compared with the lowest tertile was not statistically significant. However, when the highest MPO tertile was compared with the middle MPO tertile, death HR was 1.70 (95% CI, 1.03 to 2.78; $P = 0.04$). HR of the lowest compared with the middle MPO tertile was 1.16 (95% CI, 0.70 to 1.90; $P = 0.6$).

DISCUSSION

The present studies show that serum MPO levels in MHD patients independently predict 3-year mortality risks despite statistical adjustments for multiple alternative inflammation markers and other clinical correlates of mortality risk in the population. Interestingly, we observed that MPO levels correlated with levels of CRP and the proinflammatory cytokines IL-6 and TNF- α , but only CRP and MPO levels predicted mortality after multiple logistic regression analyses. Serum MPO level also was associated with higher body fat percentage and higher WBC count. These findings may have important clinical implications for the management of inflammation and oxidative stress in MHD patients.

Human MPO is a 140-kd hemoprotein composed of 4 subunits and is stored in azurophilic granules of neutrophils, monocytes, and certain tissue macrophages.^{2,24} It is a major component of the bactericidal armamentarium of leukocytes

Fig 2. Bivariate correlations between levels of serum MPO and (A) serum CRP, (B) serum IL-6, (C) serum TNF- α , and (D) serum intact parathyroid hormone (PTH) among 356 MHD patients (4 panels).

Table 3. Relative Risk for Death During the 3-Year Follow-Up Period in 256 MHD Patients Based on Cox Proportional Hazard Regression

Serum Measures	Unadjusted	Case-Mix Adjusted	Case-Mix & Comorbidity Adjusted	Case-Mix, Comorbidity, & Laboratory Values Adjusted
MPO (each 1,000 pmol/L $\uparrow$)	1.08 (1.00-1.16) $P = 0.05$	1.15 (1.06-1.26) $P = 0.001$	1.15 (1.05-1.26) $P = 0.002$	1.14 (1.03-1.26) $P = 0.01$
CRP (each 5 mg/L $\uparrow$)	1.13 (1.05-1.22) $P = 0.001$	1.21 (1.11-1.32) $P < 0.001$	1.24 (1.13-1.37) $P < 0.001$	1.16 (1.04-1.30) $P = 0.01$
IL-6 (each 10 ng/L $\uparrow$)	1.01 (0.99-1.03) $P = 0.20$	1.01 (0.99-1.04) $P = 0.21$	1.01 (0.98-1.03) $P = 0.4$	1.00 (0.97-1.03) $P = 0.8$
TNF- α (each 5 ng/L $\uparrow$)	0.98 (0.83-1.16) $P = 0.7$	1.06 (0.90-1.23) $P = 0.5$	1.06 (0.91-1.24) $P = 0.5$	0.94 (0.78-1.13) $P = 0.5$
Albumin (each 0.1 g/dL $\downarrow$)	1.20 (1.14-1.25) $P < 0.001$	1.18 (1.11-1.24) $P < 0.001$	1.16 (1.09-1.23) $P < 0.001$	1.15 (1.08-1.23) $P < 0.001$

NOTE. Case-mix adjustment included age, sex, race (black), and dialysis vintage; comorbidity adjustment included diabetes mellitus, history of cardiovascular disease, and Charlson Comorbidity Index; and laboratory value adjustment included serum MPO, CRP, IL-6, TNF- α , albumin, and hemoglobin values.

Fig 3. Kaplan-Meier survival analysis for tertiles of serum MPO in 356 MHD patients followed up for up to 3 years.

because of its capacity to catalyze the production of hypochlorous acid, a powerful oxidant derived from chloride ion and hydrogen peroxide.^{6,8,9,24} MPO is a catalyst for lipoprotein oxidation and hypochlorous acid production.¹ Increased MPO activity also can contribute to consumption of nitric oxide.^{9,25} MPO generates reactive oxygen species as part of its function in innate host defense mechanisms, but it may become detrimental if chronically present.^{1,6,25} Evidence suggests mechanistic links between MPO, inflammation, and cardiovascular disease in the general population.⁶

Despite the many links between elevated MPO levels and atherosclerosis,¹⁻⁵ only recently has a potential role for MPO and its oxidants in the development of vulnerable plaque been addressed. Low doses of hypochlorous acid lead to endothelial cell activation and elaboration of tissue factor messenger RNA, protein, and tissue factor pathway activity.^{7,26} Hence, MPO measurements may be useful in cardiac risk stratification,^{6,8} especially in dialysis patients who have a larger burden of atherosclerotic cardiovascular disease than the general population. However, very few studies examined the role of MPO in patients with end-stage renal disease and their outcomes. To our knowledge, our study is the

first in which serum MPO was measured systematically in a large cohort of MHD patients.

Oxidative stress is likely to contribute to morbidity and mortality in MHD patients and may be a link between MICS and poor outcome in these individuals.²⁷⁻²⁹ Some small-scale clinical trials implied possible associations between oxidative stress and adverse clinical outcomes in patients with CKD because administration of such antioxidant agents as vitamin E³⁰ or acetylcysteine^{31,32} was associated with decreased rates of cardiovascular events and improved endothelial function in MHD patients. However, it is not clear whether the improved outcomes in these studies were caused by antioxidative properties of the interventions. Although the field of oxidative stress in uremia has been studied extensively in the past several years, to our knowledge, there are only very few clinical studies in a CKD population that showed the mortality predictability of selected measures of oxidative stress, ie, oxidized low-density lipoprotein,³³ malondialdehyde,³⁴ and plasmalogen.³⁵ The foregoing studies had small sample sizes within European dialysis patients. Although circulating MPO was measured in several recent studies in a limited number of dialysis patients,^{36,37} to the best of our knowledge, the association of MPO level with levels of

nutritional and inflammatory markers or clinical outcomes has not yet been investigated.

Protein-energy malnutrition and inflammation, independently or concurrently together as MICS, are common occurrences in MHD patients.^{38,39} MICS is associated with poor clinical conditions and worse outcomes in patients with CKD.^{40,41} The confounding effect of MICS on associations between such traditional risk factors as obesity and hypercholesterolemia and clinical outcome is so strong that it even reverses these associations. Hence, a low, rather than a high, BMI or serum cholesterol level is associated with mortality in MHD patients.^{42,43} This phenomenon is known as reverse epidemiology.⁴⁴ Nevertheless, despite this known effect of MICS on poor outcome, the pathophysiological process of MICS and its potential link with oxidative stress has not been well examined in clinical studies of a CKD patient population. Our present study is an effort to fill in the gaps in our knowledge about the field of oxidative stress and inflammation in patients with CKD. It should be noted that in our study, serum MPO level was associated with a greater body fat percentage and BMI, indicating a “paradox within the paradox”⁴⁵ given the paradoxically positive association between greater body fat mass and better survival in dialysis patients.⁴⁶

A potential limitation of the present study is a selection bias during enrollment. We cannot exclude the possibility that during the recruitment in 8 dialysis units (with >1,200 MHD patients), MHD patients who were generally healthier, more health-conscious, and had better nutritional status agreed to participate (360 patients). The annual mortality rate among all patients of the study dialysis units was 15%, whereas it was only 10% among patients enrolled in the NIED Study. However, it might be argued that a selection bias with such a direction generally would lead to bias toward the null, so without this bias, our positive results may have been even stronger and the associations may have been more prominent.

There are strengths to our investigation. First, the sample size is large and includes many individuals with diabetes mellitus. Second, unlike previous cohorts, ours has been characterized extensively for markers of inflammation and nutrition. The availability of these measures al-

lowed us to show that the ability of MPO level to predict mortality risk is independent of influences from other known inflammatory markers and cytokines in this well-studied group of MHD patients. Third, patients in this cohort were selected randomly without prior knowledge of their oxidative stress or inflammation status. Finally, the same blood specimens used to measure markers of MICS and cytokines also were used for MPO measurements.

In conclusion, we find that serum MPO level has a wide range of variation in MHD patients and correlates with several surrogates of body composition and inflammation in this group of patients. Moreover, increased MPO level is associated independently with increased death risk. This suggests that measuring MPO may be an important tool for diagnosing unrecognized clinical risks in this population. Given our observed relationship between MPO level and MICS and the association between levels of detrimental cytokines and poor survival in this population, further investigation is needed to define whether MPO level or oxidative stress explains the greater mortality seen in this population.

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