Serum β-2 Microglobulin Levels Predict Mortality in Dialysis Patients: Results of the HEMO Study

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In the randomized Hemodialysis (HEMO) Study, chronic high-flux dialysis, as defined by higher β-2 microglobulin (β_2M) clearance, compared with low-flux dialysis did not significantly alter all-cause mortality in the entire cohort but was associated with lower mortality in long-term dialysis patients. This analysis examined the determinants of serum β_2M levels and the associations of serum β_2M levels or dialyzer β_2M clearance with mortality. In a multivariable regression model that examined 1704 patients, baseline residual kidney urea clearance and dialyzer β_2M clearance were strong predictors of predialysis serum β_2M levels at 1 mo of follow-up, with regression coefficients of −7.21 (±0.69 SE) mg/L per ml/min per 35 L urea volume ($P < 0.0001$) and −1.94 (±0.30) mg/L per ml/min ($P < 0.0001$),respectively. In addition, black race and baseline years on dialysis correlated positively whereas age, diabetes, serum albumin, and body mass index correlated negatively with serum β_2M levels ($P < 0.05$). In time-dependent Cox regression models, mean cumulative predialysis serum β_2M levels but not dialyzer β_2M clearance were associated with all-cause mortality (relative risk = 1.11 per 10-mg/L increase in β_2M level; 95% confidence interval 1.05 to 1.19; $P = 0.001$), after adjustment for residual kidney urea clearance and number of prestudy years on dialysis. This association is supportive of the potential value of β_2M as a marker to guide chronic hemodialysis therapy.

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The Hemodialysis (HEMO) Study was a randomized, prospective, clinical trial that was designed to examine the impact of two treatment parameters, membrane flux and dialysis dose, on clinical outcomes of chronic dialysis patients (1). Membrane flux was classified on the basis of the clearance of the middle molecule β_2-microglobulin (β_2M; molecular weight 11,800), whereas dialysis dose was determined by the Kt/V of urea (molecular weight 60).

The primary analysis of the HEMO Study did not show a statistically significant effect of higher dialyzer flux (relative risk [RR] 0.92; 95% confidence interval [CI] 0.81 to 1.06) or higher urea Kt/V (RR 0.96; 95% CI 0.84 to 1.10) on all-cause mortality (1). In the subgroup of patients who had been on dialysis for >3.7 yr (the mean duration of the cohort) before entering the HEMO Study, however, high flux was associated with a 32% decrease in the RR (0.68; 95% CI 0.53 to 0.86) of all-cause mortality, although the beneficial effect of high flux diminished when the total number of dialysis years (instead of only prestudy dialysis years) was taken into account (1,2). In contrast, in the subgroup of patients who had been on dialysis for ≤3.7 yr before the study, high flux was not associated with a difference in all-cause mortality (RR 1.05; 95% CI 0.89 to 1.24). This secondary analysis suggests that high-flux dialysis is beneficial to some chronic hemodialysis patients.

In this prospective study in which serum β_2M levels were systemically determined to monitor the flux intervention, we examined the determinants on serum β_2M levels as well as the

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relationship between serum β_2M levels and mortality. Confirmation of such a relationship would support the utility of β_2M as a marker for middle molecules in uremia and a potential guide for adequacy of chronic hemodialysis therapy.

Materials and Methods

HEMO Study Design

The HEMO Study was a prospective, randomized, multicenter clinical trial with a 2 × 2 factorial design and equal allocation to each treatment arm (1). A total of 1846 patients were randomly assigned to either low-flux or high-flux membrane dialyzers and to either a standard dose of dialysis targeting an equilibrated dose (eKt/V of urea) of 1.05 or a high dose targeting an eKt/V of urea of 1.45. Among the eligibility criteria were (*1*) a minimum of 3 mo on hemodialysis and (*2*) residual kidney urea clearance of <1.5 ml/min per 35 L of urea distribution volume (2,3) to minimize the contribution from native kidneys and hence maximize the relative effect of dialysis on total body solute clearances.

Dialyzers and Dialysis Procedure

The dialyzers and dialysis procedures that were used during the HEMO Study and the reuse techniques and the β_2M clearances associated with these dialyzer-reprocessing technique combinations were described previously (1–7). All dialyzers used had *in vitro* urea mass transfer-area coefficients >500 ml/min at a dialysate flow rate of 500 ml/min. Low-flux dialyzers had a mean clearance of β_2M <10 ml/min during clinical dialysis. Among the eight low-flux dialyzers in the study, F8 (Fresenius Medical Care–North America, Lexington, MA) and CA210 (Baxter Healthcare Corp., McGaw Park, IL) accounted for 46 and 43% of the sessions, respectively. The criteria for high-flux dialyzers were an *in vitro* or extracorporeal ultrafiltration coefficient of ≥14 ml/h per mmHg and a β_2M clearance >20 ml/min averaged over the lifespan of the dialyzer during clinical dialysis. Among the 17 high-flux dialyzers used, F80 (Fresenius) and CT190 (Baxter) accounted for 43 and 48% of the sessions, respectively. Two dialyzers (one high-flux and one low-flux) connected in series were used to achieve the prescribed urea Kt/V in 2.5% of all follow-up sessions among patients who were randomly assigned to the high-dose goal.

The blood flow rate, dialysate flow rate, and treatment time were tailored to individual patients to achieve the target urea eKt/V. The achieved urea eKt/V was 1.16 ± 0.08 and 1.53 ± 0.09, whereas the spKt/V was 1.32 ± 0.09 and 1.71 ± 0.11 in the standard-dose arm and the high-dose arm, respectively. Other aspects of the dialysis treatment, including the dry weight prescription and dialysate composition, were prescribed by the primary nephrologists according to routine clinical practice and general guidelines provided by the HEMO Study protocol. The duration of dialysis could be adjusted to achieve the fluid removal goal, as long as the other parameters were also adjusted to achieve the target urea Kt/V. All dialysis machines used were governed by volumetric control, and all dialysates were bicarbonate based. Standards for the quality of the dialysate water and the dialysates proposed by the Association for the Advancement of Medical Instrumentation were followed; however, ultrapure dialysate was not used.

Sample Collection and Dialyzer β_2M Kinetics

The kinetics of β_2M during hemodialysis was determined at the first and second months and then every other month during the follow-up phase for patients who were randomly assigned to the high-flux arm. For the low-flux arm, β_2M clearance was determined at the first and fourth months and annually thereafter. The more frequent study of β_2M kinetics in the high-flux arm was necessary to ensure that dialyzer β_2M clearance was maintained according to the study protocol, in view of the changes in β_2M clearance observed with dialyzer reuse (6). Blood samples for β_2M were collected from the vascular access immediately before dialysis and 20 s after dialysis from the arterial blood tubing after the dialyzer blood flow rate had been reduced to <80 ml/min. All blood samples were centrifuged, and the serum samples were shipped to a central laboratory (Spectra East, Rockleigh, NJ) for assay. The concentrations of β_2M were measured using a solid-phase competitive RIA with reagents supplied by Abbott Laboratories (Abbott Park, IL), and radioactivity was determined by a Micromedic Apex Automatic Counter (model 10600; ICN Biomedicals, Costa Mesa, CA). The intra-assay and interassay coefficients of variation were 3.6 and 5.0%, respectively.

Dialyzer clearance of β_2M was determined on the basis of the change in serum β_2M concentration during the dialysis session as described previously (2,6) using the following equation:

$$Q_f \times [1 - \log(C_{post}/C_{pre})/\log(1 + Q_f \times T/V_{\beta 2M})]$$

where Q_f denotes the average net ultrafiltration rate calculated as the difference between the predialysis and postdialysis body weights divided by treatment time (T); C_{post} and C_{pre} denote the postdialysis and predialysis serum β_2M concentrations, respectively; and $V_{\beta 2M}$ denotes the postdialysis volume of extracellular fluids. This calculation assumes no intradialytic generation of β_2M and no residual kidney or gastrointestinal clearances of β_2M. In addition, it does not account for postdialysis rebound of serum β_2M concentration. The Kt/V for β_2M was calculated by multiplying the dialyzer clearance of β_2M by the treatment time and dividing by the postdialysis volume of extracellular fluid volume, which was calculated as one third of the urea distribution volume estimated by urea kinetics (6). The determination of β_2M Kt/V is important because dialyzer β_2M clearance alone does not account for the dialysis time and therefore the total β_2M removed during the session.

Follow-Up and Outcomes

The planned duration of follow-up in the HEMO Study ranged from 0.8 to 6.6 yr (mean 4.48 yr), depending on the date of randomization for the individual patients. Because of deaths and kidney transplantation, however, the mean actual follow-up duration was only 2.84 yr. The primary outcome variable of the study was all-cause mortality, with the survival times censored at the time of kidney transplantation or at the end of the study. Vital statistics were captured in 100% of the randomly assigned patients.

Statistical Analyses

Unless specified otherwise, mean follow-up predialysis serum β_2M level, β_2M clearance, and Kt/V for β_2M were determined for each patient by averaging all available follow-up values. For avoiding confounding from different reuse limits for different dialyzer/reprocessing method combinations, summaries of β_2M clearance and β_2M Kt/V for different dialyzer/reprocessing method combinations were based on averages of predicted values at each follow-up kinetic modeling session. The predicted β_2M clearance and β_2M Kt/V were obtained by a multiple regression analysis of the observed values on the type of dialyzer, reuse number, and type of reprocessing method, based on those sessions in which the serum β_2M levels were measured.

To explore the determinants of predialysis serum β_2M levels, a multivariable regression model was used to relate a number of factors to predialysis serum β_2M levels obtained at 1 mo after randomization. For this regression model and the presentation of baseline patient characteristics, only patients who had undergone the kinetic modeling session at 1 mo (n = 1704) were included. The model included, as

independent variables, the seven prespecified baseline covariates used in the primary analysis, which were age, gender, race, diabetes, years on dialysis, serum albumin level, and comorbidity (Index of Coexisting Disease severity or [ICED] score [8]) (1,9), membrane flux (classified as low flux or high flux) of the dialyzer used, and ultrafiltration volume (expressed as a percentage of the postdialysis weight and used as an indicator of predialysis hemodilution) before randomization, history of malignancy or AIDS, baseline modeled urea distribution volume (10) (representing total body fluid), baseline body mass index (BMI), baseline 44-h residual kidney urea clearance, dialyzer β_2M clearance determined at the first month of follow-up (reflecting the clearance since randomization), dose randomization, and flux randomization. Malignancy and AIDS were included in the model because these disorders were known to increase serum β_2M levels in the general population. Urea clearance by the kidney was used because data on the GFR or kidney clearance of β_2M were not available in the HEMO Study. Adjustment for clinical center was also performed in the model. Another regression model in which the baseline residual kidney urea clearance was excluded to examine the significance of years on dialysis was used.

The mean changes in predialysis serum β_2M levels over follow-up time were evaluated by randomized flux group using a longitudinal mixed-effects model that adjusted for informative censoring as a result of death and other causes of early dropout (11). These changes are expressed as the slope of the changes in β_2M levels from 4 to 36 mo. Similar models were used to evaluate the longitudinal changes in dialyzer β_2M Kt/V.

The association between the risk for all-cause mortality and serum β_2M levels was investigated using time-dependent Cox regression model (12) in which the relative mortality risk at a given time point was related to the cumulative mean of the serum β_2M levels throughout follow-up before that time point. Similar time-dependent Cox models were performed to relate all-cause mortality with the cumulative mean of predicted dialyzer β_2M clearance or dialyzer β_2M Kt/V. For these Cox regression models, all randomly assigned patients who had undergone any kinetic modeling sessions during follow-up (n = 1813) were included. The seven prespecified baseline factors, residual kidney urea clearance, dialyzer flux, ultrafiltration volume normalized by body weight, and kinetically modeled urea distribution volume, all obtained at baseline, were treated as covariates in these analyses. The cohort was divided further into two subgroups on the basis of the mean prestudy years on dialysis (3.7 yr), and similar Cox regression analyses were performed relating dialyzer β_2M kinetics or serum β_2M levels to mortality. Because the cumulative mean level of dialyzer β_2M kinetics or serum β_2M levels also may be confounded by follow-up levels instead of baseline levels of serum albumin, ICED, and residual kidney urea clearance, time-dependent Cox regression models that included the follow-up values of these three variables were also analyzed.

The association between the risk for all-cause mortality and residual kidney urea clearance was performed using several Cox models in which residual kidney urea clearance was analyzed as either a continuous or a categorical independent variable. These models were adjusted for various combinations of case-mix factors (age, gender, race, diabetes, and duration of dialysis), baseline ICED score, serum albumin, high-flux or low-flux dialysis, ultrafiltration volume and body urea distribution volume, and follow-up predialysis serum β_2M levels.

Results

Patient Characteristics

Although 1846 individuals were randomly assigned in the entire HEMO Study cohort, the patients who were included in this analysis were restricted to those who had β_2M kinetic modeling performed at 1 mo of follow-up (n = 1704). The baseline characteristics of this subpopulation are presented in Table 1. A total of 55.9% were female, and 62.9% were black, with a mean age of 57.8 ± 14.0 yr. The average duration of dialysis was 3.7 yr, and 60.2% of the patients were treated with high-flux dialyzers before entry to the study. Only 33.3% of the cohort had measurable residual urine output. The mean ultrafiltration volume was 3.0 ± 1.1 L/70 kg body wt.

Distribution of Dialyzer β_2M Kinetics and Serum β_2M Levels

Serum β_2M levels were not obtained at entry into the HEMO Study. The first available β_2M levels were collected at 1 mo of follow-up. The distributions of the mean dialyzer Kt/V of β_2M and mean predialysis serum β_2M levels during the entire follow-up period are presented in Figures 1 and 2, respectively.

The β_2M Kt/V values during follow-up had a relatively narrow distribution in the low-flux arm, with a mean of 0.07 ± 0.14 (Figure 1, middle). Because of the differences in membrane materials and reprocessing procedures, the Kt/V of β_2M in the high-flux arm had a larger variation and a mean of 0.66 ± 0.23 ($P < 0.0001$, mean of high flux *versus* low flux; Figure 1, bottom). The distributions of dialysis β_2M clearance largely paralleled those of the β_2M Kt/V (data not shown). The mean values were 3.4 ± 7.2 ml/min for the low-flux arm and 33.7 ± 11.4 ml/min for the high-flux arm ($P < 0.0001$, low flux *versus* high flux).

The mean predialysis serum β_2M level during follow-up for the entire cohort was 37.3 ± 11.9 and 41.5 ± 12.9 mg/L (n =

Table 1. Baseline characteristics of the 1704 randomly assigned patients[a]

Age	57.8 ± 14.0 yr
Female	55.9%
Black	62.9%
Diabetes	44.6%
Years on dialysis	3.7 ± 4.3
Postdialysis weight	69.3 ± 14.8 kg
Urea distribution volume[b]	31.2 ± 6.6 L
Residual kidney urea clearance >0	33.3%
Residual kidney urea clearance >0.75 ml/min[c]	14.0%
High-flux dialysis	60.2%
Comorbidity (ICED) score[d]	2.0 ± 0.8
Cardiac disease	80.3%
Serum albumin concentration	3.6 ± 0.4 g/dl
Ultrafiltration volume/postdialysis weight	3.0 ± 1.1 L/70 kg

[a]All data are presented as mean ± SD or percentages. Only patients who had β-2 microglobulin (β_2M) clearance measurement at 1 mo of follow-up were included. ICED, Index of Coexisting Disease.
[b]Urea distribution volume as determined by kinetic modeling.
[c]Normalized to 35 L urea distribution volume.
[d]ICED severity score (21) computed with diabetes excluded.

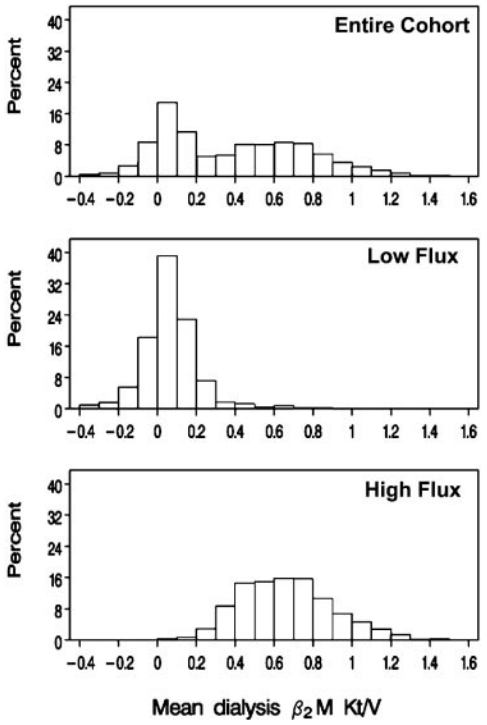

Figure 1. Distribution of mean dialysis Kt/V of β-2 microglobulin (β_2M) during the entire follow-up period. The values of β_2M Kt/V are derived from the dialyzer β_2M clearances using the equation described in Materials and Methods. Each panel shows the percentage of the cohort ($N = 1704$) with the designated dialysis β_2M Kt/V. (A) Entire cohort. (B) Low-flux arm. (C) High-flux arm.

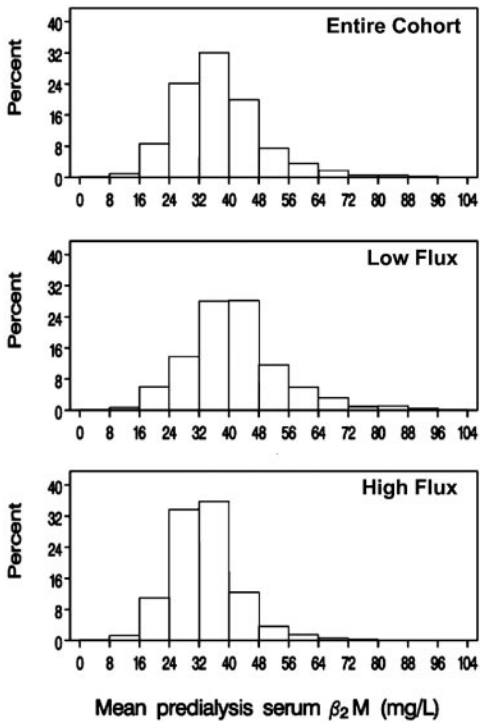

Figure 2. Distribution of mean predialysis serum β_2M levels during the entire follow-up period. Each panel shows the percentage of the cohort ($N = 1704$) with the designated serum β_2M levels. (A) Entire cohort. (B) Low-flux arm. (C) High-flux arm.

817) for the low-flux arm and 33.5 ± 9.1 mg/L ($n = 887$) for the high-flux arm ($P < 0.0001$, low flux *versus* high flux). There was substantial overlap in serum β_2M levels between the low-flux and high-flux arms (Figure 2). The mean predialysis serum β_2M levels over the course of follow-up in the subgroups with ≤3.7 and >3.7 yr on dialysis prestudy were 35.3 ± 11.2 mg/L ($n = 1164$) and 41.7 ± 11.8 mg/L ($n = 540$) respectively ($P < 0.0001$, ≤ 3.7 *versus* > 3.7 yr). The mean predialysis serum β_2M levels over the course of follow-up in the subgroups without and with detectable residual kidney urea clearance were 39.3 ± 12.1 mg/L ($n = 1136$) and 33.4 ± 10.1 mg/L ($n = 568$), respectively ($P < 0.0001$, without *versus* with residual urea clearance).

Determinants of Predialysis Serum β_2M Levels

Table 2 presents the association of various factors with predialysis serum β_2M levels at 1 mo of follow-up in a multivariable regression model. Black race and baseline duration of dialysis correlated positively ($P < 0.05$) with serum β_2M levels, whereas baseline age, diabetes, BMI, and residual kidney urea clearance correlated negatively ($P < 0.05$) with serum β_2M levels. In this model, in which dialyzer β_2M clearance at 1 mo and randomization to the high-flux arm both were included, the former but not the latter correlated negatively with serum β_2M levels. If dialyzer β_2M clearance was excluded from the model, however, randomization to the high-flux arm correlated negatively with serum β_2M levels, with a regression coefficient of −6.23 ± 0.62 mg/L ($P < 0.0001$), indicating the expected strong correlation between dialyzer β_2M clearance and flux randomization.

The baseline residual kidney urea clearance was a particularly strong predictor of serum β_2M levels at 1 mo of follow-up; the regression coefficient was −7.21 (±0.69 SE) mg/L per ml/min per 35 L body urea volume ($P < 0.0001$; Figure 3). Exclusion of residual kidney urea clearance from the model increased the regression coefficient of prerandomization years on dialysis from 0.41 ± 0.08 mg/L per yr ($P < 0.0001$) to 0.62 ± 0.08 mg/L per yr ($P < 0.0001$), whereas the regression coefficient of dialyzer β_2M clearance remained unchanged (−1.94 ± 0.30 and −2.01 ± 0.31 mg/L per yr; $P < 0.0001$ for both).

Longitudinal Changes in Predialysis Serum β_2M Levels and Residual Kidney Urea Clearance

Mean predialysis serum β_2M levels continued to increase during follow-up in both the low-flux and high-flux arm (Figure 4). Between 4 mo and 6 mo after randomization, the slope (± SE) of the mean β_2M levels for the low-flux arm was 1.06 ± 0.28 mg/L per year ($P = 0.0002$); while that for the high-flux arm was 0.55 ± 0.27 mg/L per year ($P = 0.04$). While the mean serum β_2M levels were consistently higher ($P < 0.001$) in the low-flux arm than in the high-flux arm at all time points after randomization, the mean slopes starting at 4 mo did not differ significantly ($P = 0.105$) between the low-flux and high-flux arms, suggesting that most of the differences in serum β_2M

Table 2. Multivariable regressions[a] of predialysis serum β_2M level at 1 mo of follow-up[b]

Variable	Regression Coefficient (mg/L)	SE	*P*
Baseline age (per 10 yr)	−1.32	0.26	<0.0001
Gender (female)	−1.32	0.80	0.098
Race (black)	2.94	0.77	<0.0001
Baseline diabetes	−3.62	0.70	<0.0001
Baseline duration of dialysis (per year)	0.41	0.08	<0.0001
Baseline ICED score (per unit)	0.74	0.40	0.069
Baseline serum albumin (per g/dl)	−1.81	0.93	0.052
History of malignancy or AIDS at baseline	1.32	1.08	0.219
Baseline high-flux dialysis	−0.22	0.95	0.820
Baseline ultrafiltration volume/weight (per L/70 kg)	0.17	0.30	0.571
Baseline modeled urea V (per L)	0.06	0.06	0.308
Baseline body mass index (per kg/m^2)	−0.27	0.07	<0.0001
Baseline residual kidney urea clearance[c] (per ml/min)	−7.21	0.69	<0.0001
Dialyzer β_2M clearance at 1 mo (per 10 ml/min)	−1.94	0.30	<0.0001
Randomized to high-dose (urea Kt/V) arm	0.62	0.62	0.312
Randomized to high-flux arm	−0.97	1.02	0.339

[a]Adjusted for clinical center.
[b]Only patients who had β_2M clearance measurement at 1 mo of follow-up were included (n = 1704).
[c]Normalized to 35 L of urea distribution volume.

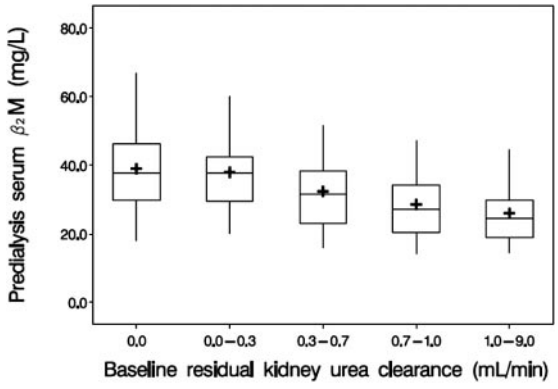

Figure 3. Relationship between serum β_2M level and residual kidney urea clearance. Each box shows the distribution of predialysis serum β_2M levels at 1 mo of follow-up for the range of baseline residual kidney urea clearances (adjusted to 35 L of body distribution volume of urea) indicated at the bottom of the box. The mean is shown by the plus sign, the median by the middle horizontal line, and the 25th and 75th percentiles by the bottom and top of the box, respectively.

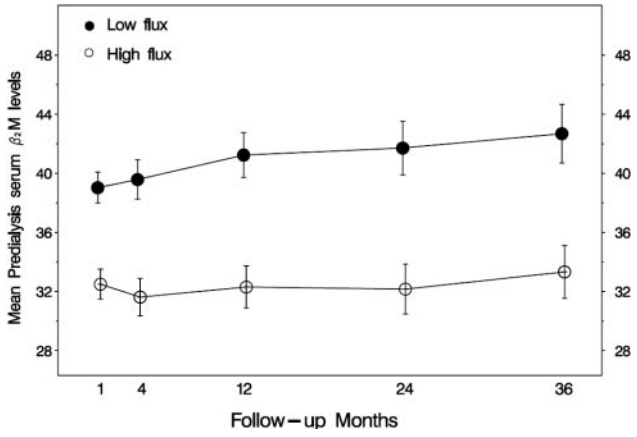

Figure 4. Longitudinal changes in predialysis serum β_2M levels. Presented are the estimated mean predialysis serum β_2M levels with 95% confidence intervals for the low-flux (●) and high-flux (○) arms during follow-up, using a longitudinal mixed-effects model that adjusted for seven baseline covariates (age, gender, race, diabetes, years on dialysis, serum albumin level, and Index of Coexisting Disease severity [ICED] score) and informative censoring. The serum β_2M levels were different ($P < 0.001$) between low flux and high flux at all time points.

levels between the two arms occurred during the first 4 mo. Further analyses showed that the longitudinal increases in serum β_2M levels in either the low-flux or high-flux arm were not due to decreases in dialyzer β_2M Kt/V; the slope of change in β_2M Kt/V from 4 mo to 36 mo were statistically insignificant ($P > 0.4$) for the high-flux arm, low-flux arm or the entire cohort.

Changes in residual kidney urea clearance were also examined longitudinally to see if residual clearances declined with time as serum β_2M levels increased. Of the 1704 patients in this cohort, 903 had residual urea clearance reported at both 1 mo and 24 mo. Among these 903 patients, 583 patients had no residual urea clearance during this time interval. In the remaining 320 patients, residual clearances declined in 244, increased in 73, and were unchanged in 3 patients. To examine further if the increasing serum β_2M levels were related to changes in

residual kidney urea clearances, the longitudinal analyses of serum β_2M levels were repeated according to the presence or absence of residual clearance at baseline. For those without residual kidney clearance at baseline, the mean (± SE) slope of increase in serum β_2M levels from 4 mo to 36 mo was not statistically significant in either the low-flux (0.55 ± 0.35 mg/L per yr; $P = 0.11$) or high-flux (0.20 ± 0.34 mg/L per yr; $P = 0.56$) arm. For those who had residual kidney clearance at baseline, serum β_2M levels increased significantly with time, with slopes of 2.02 ± 0.47 mg/L per year ($P < 0.001$) and 1.11 ± 0.44 mg/L per year ($P = 0.011$) from 4 mo to 36 mo in the low-flux and high-flux arms respectively. More rigorous modeling the rate of decline in residual kidney function is prohibited because the majority of patients had no measurable residual kidney clearance at baseline.

Predictive Value of Predialysis Serum β_2M Level for All-Cause Mortality

The association of the cumulative mean predialysis serum β_2M levels over time during follow-up with all-cause mortality was assessed in time-dependent Cox regression models. As previously reported, age, diabetes, prestudy years on dialysis, and comorbidity (ICED) score correlated with mortality, whereas black race and serum albumin correlated negatively with mortality. The negative association between female gender and mortality was statistically insignificant.

Mean cumulative predialysis serum β_2M levels over time correlated with mortality (RR = 1.11 per 10-mg/L increase in β_2M level; 95% CI 1.05 to 1.19; $P = 0.001$; Table 3). This association was apparent despite the inclusion of residual kidney urea clearance in the model; *i.e.*, serum β_2M level predicted mortality after adjustment for the effect of residual kidney function. This relationship, however, did not seem to be truly linear but plateau at high serum β_2M levels (Figure 5).

The predialysis serum β_2M levels predicted mortality in patients who had been on dialysis ≤3.7 yr but not in patients who were on dialysis for longer durations (Table 4). Similar analysis was performed in the subgroups defined by residual kidney function, using Cox regression that included multiple factors in the model. The predialysis serum β_2M levels marginally predicted mortality in patients who were anuric at baseline (RR = 1.07; 95% CI 1.00 to 1.16; $P = 0.059$). The predictive value was stronger in the subgroup with detectable residual kidney function (RR = 1.31; 95% CI 1.15 to 1.50; $P < 0.0001$).

When the follow-up values instead of baseline values of serum albumin, ICED score, and residual kidney urea clearance were used as covariates in the time-dependent Cox models, similar results were obtained (RR = 1.09 [95% CI 1.02 to 1.16; $P = 0.011$], 1.12 [95% CI 1.02 to 1.22; $P = 0.014$], and 1.05 [95% CI 0.94 to 1.16; $P = 0.408$] for the entire cohort, subgroup with ≤3.7 prestudy years on dialysis, and subgroup with >3.7 prestudy years on dialysis, respectively).

Predictive Value of Residual Kidney Function for All-Cause Mortality

Because serum β_2M levels were highly correlated with residual kidney urea clearance (Table 2) and previous studies have implicated a strong effect of residual kidney function in clinical outcomes in chronic peritoneal dialysis patients (13,14), further analysis was performed to examine the predictive value of residual kidney urea clearance for all-cause mortality. When the Cox model was adjusted only for the randomized interven-

Table 3. Time-dependent Cox regressions analysis[a] of all-cause mortality[b]

Baseline[c] Variables	RR	95% CI	*P*
Cumulative mean predialysis serum β_2M level over time during follow-up (per 10-mg/L increase)	1.11	1.05 to 1.19	0.001
Age (per 10-yr increase)	1.45	1.35 to 1.55	<0.0001
Gender (female)	0.85	0.71 to 1.02	0.075
Race (black)	0.77	0.64 to 0.91	0.003
Diabetes	1.36	1.16 to 1.60	<0.0001
Duration of dialysis (per year increase)	1.03	1.02 to 1.05	<0.0001
ICED score (per unit increase)	1.34	1.22 to 1.47	<0.0001
Serum albumin (per g/dl increase)	0.43	0.34 to 0.54	<0.0001
Residual kidney urea clearance[d] (per ml/min)	0.96	0.80 to 1.15	0.681
Baseline high-flux dialysis	1.04	0.82 to 1.32	0.733
Ultrafiltration volume/weight (per L/70 kg)	1.16	1.08 to 1.24	<0.0001
Modeled body urea distribution volume (per L)	0.99	0.98 to 1.01	0.236
Randomization to high urea Kt/V arm	0.95	0.83 to 1.10	0.501

[a]Stratified by clinical center.
[b]Only patients who had at least one β_2M clearance measurement during follow-up were included; $n = 1813$. RR, relative risk; CI, confidence interval.
[c]All variables were baseline except for the cumulative mean predialysis serum β_2M level over time (which was a time-dependent variable calculated as the mean over all preceding and the current sessions; presented in the first row of the table) and randomization to high urea Kt/V arm during follow-up (presented in the last row of the table).
[d]Normalized to 35 L of urea distribution volume.

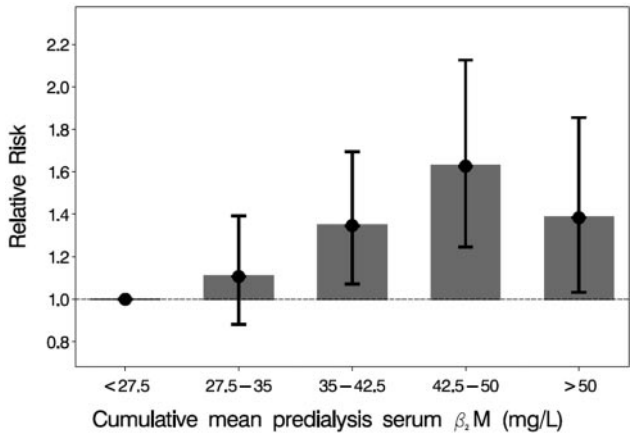

Figure 5. Association of all-cause mortality with cumulative mean predialysis serum β_2M levels. Mean predialysis serum β_2M levels over the follow-up period correlated significantly with mortality (n = 1813; P = 0.001) with an apparent plateau phase beyond β_2M levels of 42.5 to 50 mg/L. The statistical analysis was performed using time-dependent Cox regression, adjusted for age, gender, race, diabetes, years on dialysis, serum albumin level, ICED score, residual kidney urea clearance, dialyzer flux, ultrafiltration volume normalized by body weight, and kinetically modeled urea distribution volume, all obtained at baseline, and stratified by clinical center.

tions and clinical centers, the association between baseline residual kidney function mortality was marginal (RR = 0.86 for each ml/min per 35 L increase in urea clearance; 95% CI 0.73 to 1.02; P = 0.084). When case-mix factors (age, gender, race, diabetes, and duration of dialysis) were added to the model, the association became statistically significant (RR = 0.81; 95% CI 0.68 to 0.97; P = 0.023). When baseline comorbidity (ICED) score, serum albumin, high-flux or low-flux dialysis, ultrafiltration volume, and body urea distribution volume were further added to the model, however, the association again was statistically insignificant (RR = 0.89; 95% CI 0.75 to 1.06; P = 0.201). Further addition of serum β_2M levels to the model yielded the analysis presented in Table 3, in which residual kidney urea clearance was not significantly associated with all-cause mortality (RR = 0.96; 95% CI 0.80 to 1.15; P = 0.681).

When these Cox regression analyses in which residual kidney urea clearance was treated as a categorical variable (presence *versus* absence) instead of a continuous variable were repeated, similar results were obtained (data not shown). Collectively, these results strongly suggest that the association of residual kidney urea clearance with mortality could be explained by the association with other factors, including serum β_2M levels.

Predictive Values of Dialyzer β_2M Kinetics for All-Cause Mortality

The association of the mean cumulative dialyzer β_2M clearance or β_2M Kt/V during follow-up with all-cause mortality was also assessed in time-dependent Cox regression models with the β_2M clearances or Kt/V treated as a time-dependent variable, adjusting for baseline values of all variables presented in Table 3. In the entire cohort, neither dialyzer β_2M clearance nor dialyzer β_2M Kt/V was independently associated with mortality (Table 4). In patients who were on dialysis ≤3.7 yr before the study, β_2M clearance or β_2M Kt/V also did not correlate with mortality. In contrast, in patients who were on dialysis >3.7 yr before the study, both β_2M clearance and β_2M Kt/V correlated negatively with mortality. The P value for the difference in the effects of β_2M clearance and β_2M Kt/V on mortality between the two duration subgroups (test for interaction) was 0.01 and 0.002, respectively. When the most recent measurements of potential confounding variables (serum albumin, ICED score, and residual kidney urea clearance) were used instead of baseline values as covariates in the models, similar results were obtained (data not shown).

Discussion

Predictors of Predialysis Serum β_2M Levels

The positive correlation of predialysis serum β_2M levels with years on dialysis (Table 2) agrees with that previously reported by other investigators (15,16) and supports the hypothesis that middle molecules gradually accumulate in chronic kidney failure and the observations that the prevalence of amyloidosis increases with years on dialysis (16,17). Residual kidney function is known to be an important determinant of serum β_2M levels (16,18), because the kidneys are the primary routes for the elimination of this protein. Indeed, our data show that baseline residual kidney urea clearance was a strong predictor of serum β_2M levels, independent of years on dialysis (Table 2). Each increment of 1 ml/min in residual urea clearance, adjusted for body fluid volume, was associated with a decrease in serum β_2M level of 7.21 mg/L. Despite including residual kidney urea clearance in the statistical model, years on dialysis remained an independent predictor of serum β_2M levels, although the magnitude of the association was modest (0.46-mg/L increase in serum β_2M level for each additional year of dialysis). Residual kidney clearance of β_2M would be more relevant than residual kidney urea clearance in this analysis, but these data are difficult to obtain because β_2M is largely cleared by filtration, reabsorption, and degradation in the proximal tubule rather than excreted in the urine.

Dialyzer β_2M clearance was also a predictor of serum β_2M levels, although the magnitude of the association was modest compared with residual kidney function. As shown in Table 2, a 37-ml/min increase in dialyzer β_2M clearance would be equivalent to a 1.0-ml/min increase in residual kidney urea clearance, yielding a 7.2-mg/L decrease in predialysis serum β_2M level. Caution should be exercised to interpret this equivalence because the dialyzer β_2M clearance of 37 ml/min is only intermittent, totaling only 10 to 15 h per week, whereas the residual kidney urea clearance of 1 ml/min is continuous, totaling 168 h per week. Furthermore, urea is reabsorbed in the renal tubule; therefore, β_2M clearance by the kidney may actually be greater than urea clearance by the kidney. These notions suggest that the importance of residual kidney function is exaggerated by this comparison. However, only patients with residual urea clearance <1.5 ml/min per 35 L urea distribution volume were included in the HEMO Study. In patients with substantially greater residual kidney function, for example, the incident dialysis patients, the relationship between dialyzer

Table 4. Association of serum β_2M levels or dialyzer β_2M kinetics with all-cause mortality[a]

	Entire cohort			≤3.7 Years on Dialysis			>3.7 Years on Dialysis		
	RR[b]	95% CI	*P*	RR	95% CI	*P*	RR	95% CI	*P*
Serum β_2M level	1.11	1.05 to 1.19	0.001	1.13	1.04 to 1.23	0.005	1.08	0.97 to 1.20	0.142
β_2M clearance	0.97	0.93 to 1.02	0.206	1.01	0.96 to 1.07	0.630	0.89	0.82 to 0.96	0.004
β_2M Kt/V	0.99	0.97 to 1.01	0.232	1.01	0.98 to 1.03	0.481	0.94	0.90 to 0.97	0.001

[a]Analyzed by time-dependent Cox regression models, adjusting for baseline values of all variables presented in Table 3 and stratified by clinical center. Only patients who had at least one β_2M clearance measurement during follow-up were included; $n = 1813$.

[b]RR of all-cause mortality per 10-mg/L increase in mean cumulative predialysis serum β_2M level or per 10-ml/min increase in mean cumulative dialyzer β_2M clearance or per 0.1-unit increase in mean cumulative dialyzer β_2M Kt/V.

β_2M clearance and serum β_2M level may be less apparent. Collectively, these data suggest that residual kidney clearance is an important contributor to total body β_2M clearance.

Active malignancies, such as hematologic cancers (19), and AIDS (20) are known to be associated with elevated serum β_2M levels in the general population. Patients with known active nondermatologic malignancy or AIDS, however, were excluded from the HEMO Study. A history of malignancy or AIDS was not associated with serum β_2M levels in this analysis (Table 2). Perhaps unexpected were the statistically strong positive associations of black race and the negative association of age, diabetes, and BMI with serum β_2M levels. The mechanisms underlying these associations are uncertain. In a cohort of 237 patients who were on chronic hemodialysis or peritoneal dialysis, Canaud *et al.* (15) also observed that serum β_2M levels correlated negatively with age and residual urine volume but bore no relationship to gender. In that study, the relationships between serum β_2M levels and the other variables were not adjusted for potential confounders.

There are suggestions in the literature that microinflammation in the dialysis circuit may enhance the release of β_2M. Although the exact source of the increased β_2M is unclear, switching from conventional dialysate to ultrapure dialysate has been reported to be associated with a decrease in serum β_2M levels (21). Because ultrapure dialysate was not used routinely in the HEMO Study participating centers and dialysate endotoxin levels were not available, the potential contribution of dialysate contamination to serum β_2M levels cannot be evaluated in our study.

Longitudinal Changes in Serum β_2M Levels

The mean predialysis serum β_2M levels during follow-up were 6 mg/L higher in the low-flux arm than in the high-flux arm. This finding is in agreement with those reported by Koda *et al.* (22) and McCarthy *et al.* (16). The longitudinal trend of predialysis serum β_2M levels was analyzed further. In the HEMO Study, baseline serum β_2M levels were not available. The mean levels in the high-flux and low-flux arms, however, were presumably equivalent at baseline, because the patients were randomly assigned to the two arms and all of the demographic characteristics and baseline laboratory values examined were similar (1). The analysis showed a clear separation in serum β_2M levels between the two flux arms as early as 1 mo after randomization (Figure 4). Nonetheless, serum β_2M levels continued to increase regardless of flux assignment, suggesting that the dialytic removal could not keep pace with the generation of the peptide. The absence of changes in dialyzer β_2M Kt/V, in conjunction with a decrease in kidney function in most patients in the subgroup with measurable baseline residual kidney function, suggests that the increase in serum β_2M levels over time was attributed at least in part to the loss of residual kidney function during follow-up. The significant longitudinal increase in serum β_2M levels in patients with baseline kidney function and the absence of longitudinal changes in serum β_2M levels in those without measurable baseline kidney function lend further support to this hypothesis. The lack of information on nonkidney (*e.g.*, gastrointestinal) body clearance of β_2M precludes more definitive conclusions. Although the slope of increase in the low-flux arm was twice that of the high-flux arm, the difference was not statistically significant. A decrease in serum β_2M levels over time, however, may be possible with greater removal of the peptide by hemodiafiltration (23) or daily long hemodialysis (24), which are more effective in removing β_2M.

Prediction of Mortality by Serum β_2M Levels and β_2M Kinetics

In patients who were on dialysis ≤3.7 yr before the study, neither β_2M clearance nor β_2M Kt/V correlated with mortality. In contrast, in patients who were on dialysis >3.7 yr before the study, both β_2M clearance and β_2M Kt/V correlated negatively with mortality. Because patients who were on dialysis for a longer period of time had lower residual kidney function than those who were on dialysis for a shorter period of time (2), these observations highlight the importance of residual kidney function. The effect of dialyzer clearance of β_2M on outcome was not apparent until the residual kidney function became minimal. These observational data seem to be in accordance with the results of the randomized trial that showed that the beneficial effect of high-flux dialysis was present only in the subgroup of patients who were on dialysis >3.7 yr (2). However, there was only a trend toward decreased mortality in patients with normalized baseline residual kidney urea clearance ≤0.24 ml/min per 35 L (RR = 0.90; 95% CI 0.77 to 1.05 for high flux), and there was no interaction between the level of baseline residual kidney function and the flux intervention (2). These data illustrate the complex relationship among years on

dialysis, residual kidney function, dialyzer β_2M clearance, and mortality. It should be noted that, although the method used in our study to estimate dialyzer β_2M clearances has been well described (2,6), these clearances were not direct measurements using dialyzer afferent and efferent plasma concentrations. Nonetheless, this method provides an estimate of β_2M clearances from the patient during the entire session instead of the dialyzer performance at a single time point.

The association of serum β_2M levels with clinical outcome was different from that of dialyzer β_2M clearance. The risk for all-cause death increased almost linearly with increases in baseline serum β_2M levels (Figure 5). Patients with β_2M levels of 42.5 to 50 mg/L had RR of death that were approximately 60% greater than those with β_2M levels <27.5 mg/L during follow-up. Although this interesting relationship should be explored further in future studies, it is unlikely that the accumulation of β_2M *per se* is sufficient to account for the enhanced mortality. Other toxic middle molecules (25–29) or independent toxic process for which β_2M may serve as a surrogate could be contributory. The stronger association between serum β_2M level and mortality in the subgroup with detectable residual kidney function, compared with the anuric subpopulation, suggests that residual kidney function might be an important determinant of clinical outcome. The correlation between serum β_2M levels and mortality, however, was apparent in the entire cohort despite the inclusion of kidney urea clearance in the statistical model (Table 3). However, residual kidney urea clearance was not associated with mortality in models that included serum β_2M level and other factors, suggesting that the effect of residual kidney function was mediated by these factors. Nonetheless, these data collectively support the predictive value of serum β_2M level for mortality independent of residual kidney urea clearance.

The higher mortality in patients with higher serum β_2M levels may be due to higher generation of this peptide and/or other middle molecules that have similar body or extracorporeal kinetics. The generation rate of β_2M cannot be deduced reliably from the available data. Although one might assume that the differences between the predialysis and postdialysis serum β_2M levels reflect the generation rates in a given individual, this assumption is contingent on a constant predialysis serum β_2M level over time (*i.e.*, the kinetics of β_2M are in steady state). Second, it assumes that there is no nonrenal, nondialyzer clearance of β_2M. Neither of these assumptions is valid.

Limitations

There are several limitations to our study. First, although the data were collected prospectively and systematically, the association of serum β_2M levels with clinical outcome was not in the original analysis plan of the HEMO Study. Second, various modalities, such as high-flux hemodialysis, hemofiltration, sorbents, and native kidney, have different solute clearance profiles. Therefore, the body accumulation and serum concentrations of other toxic molecules and the associated clinical outcome may be different among these modalities, even if the serum β_2M levels are similar. Caution is necessary to extrapolate our results to these other modalities. Third, the range of residual kidney function in the HEMO Study was small, because all patients with adjusted residual urea clearance 1.5 ml/min were excluded. The extent to which serum β_2M level and residual kidney urea clearance independently predict clinical outcomes in patients with higher residual kidney urea clearances cannot be determined from these data.

Conclusions

In addition to residual kidney function and dialyzer clearance, the duration of ESRD, body composition, and other demographic factors were independent determinants of serum β_2M levels in chronic hemodialysis patients. Serum β_2M has been proposed to be a surrogate for other uremic middle molecules that are more effectively removed by high-flux than low-flux dialysis. Our study showed that the mean predialysis serum β_2M level over time was predictive of all-cause mortality, independent of the chronicity of dialysis and residual kidney function. The European Best Practice Guidelines have recommended the use of β_2M as a marker for middle molecules and maximize the removal of middle molecules (30), although previous studies have largely related β_2M to amyloidosis (31,32). This analysis relating serum β_2M to mortality lends further justifications for these recommendations. The value of β_2M as a marker to guide routine chronic hemodialysis therapy should be evaluated further.

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