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 (β2M) 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 β2M levels and the associations of serum β2M levels or dialyzer β2M clearance with mortality. In a multivariable regression model that examined 1704 patients, baseline residual kidney urea clearance and dialyzer β2M clearance were strong predictors of predialysis serum β2M 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 β2M levels (P < 0.05). In time-dependent Cox regression models, mean cumulative predialysis serum β2M levels but not dialyzer β2M clearance were associated with all-cause mortality (relative risk = 1.11 per 10-mg/L increase in β2M level; 95% confidence interval 1.05 to 1.19; P = 0.001), after adjustment for residual kidney urea clearance and number of pre-study years on dialysis. This association is supportive of the potential value of β2M as a marker to guide chronic hemodialysis therapy.


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 β2-microglobulin (β2M; 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 pre-study 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 β2M levels were systematically determined to monitor the flux intervention, we examined the determinants on serum β2M levels as well as the
relationship between serum β₂M levels and mortality. Confirmation of such a relationship would support the utility of β₂M 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) or 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 25 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 β₂M 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 β₂M of <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 β₂M 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 β₂M Kinetics

The kinetics of β₂M 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, β₂M clearance was determined at the first and fourth months and annually thereafter. The more frequent study of β₂M kinetics in the high-flux arm was necessary to ensure that dialyzer β₂M clearance was maintained according to the study protocol, in view of the changes in β₂M clearance observed with dialyzer reuse (6). Blood samples for β₂M 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 β₂M 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 1060; ICN Biomedicals, Costa Mesa, CA). The intraassay and interassay coefficients of variation were 3.6 and 5.0%, respectively.

Dialyzer clearance of β₂M was determined on the basis of the change in serum β₂M concentration during the dialysis session as described previously (2,6) using the following equation:

\[
Q_t \times [1 - \log(C_{\text{post}}/C_{\text{pre}})/\log(1 + Q_t \times TV_{\beta_2M})]
\]

where \(Q_t\) denotes the average net ultrafiltration rate calculated as the difference between the predialysis and postdialysis body weights divided by treatment time (T); \(C_{\text{post}}\) and \(C_{\text{pre}}\) denote the postdialysis and predialysis serum β₂M concentrations, respectively; and \(V_{\beta_2M}\) denotes the postdialysis volume of extracellular fluids. This calculation assumes no intradialytic generation of β₂M and no residual kidney or gastrointestinal clearances of β₂M. In addition, it does not account for postdialysis rebound of serum β₂M concentration. The Kt/V for β₂M was calculated by multiplying the dialyzer clearance of β₂M 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 β₂M Kt/V is important because dialyzer β₂M clearance alone does not account for the dialysis time and therefore the total β₂M 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 β₂M level, β₂M clearance, and Kt/V for β₂M 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 β₂M clearance and β₂M Kt/V for different dialyzer/reprocessing method combinations were based on averages of predicted values at each follow-up kinetic modeling session. The predicted β₂M clearance and β₂M 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 β₂M levels were measured.

To explore the determinants of predialysis serum β₂M levels, a multivariable regression model was used to relate a number of factors to predialysis serum β₂M 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]), 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 \( \beta_2M \) 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 \( \beta_2M \) levels in the general population. Urea clearance by the kidney was used because data on the GFR or kidney clearance of \( \beta_2M \) 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 \( \beta_2M \) 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 \( \beta_2M \) levels from 4 to 36 mo. Similar models were used to evaluate the longitudinal changes in dialyzer \( \beta_2M \) Kt/V.

The association between the risk for all-cause mortality and serum \( \beta_2M \) 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 \( \beta_2M \) 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 \( \beta_2M \) clearance or dialyzer \( \beta_2M \) 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 \( \beta_2M \) kinetics or serum \( \beta_2M \) levels to mortality. Because the cumulative mean level of dialyzer \( \beta_2M \) kinetics or serum \( \beta_2M \) 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 \( \beta_2M \) 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 \( \beta_2M \) 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 \( \beta_2M \) Kinetics and Serum \( \beta_2M \) Levels

Serum \( \beta_2M \) levels were not obtained at entry into the HEMO Study. The first available \( \beta_2M \) levels were collected at 1 mo of follow-up. The distributions of the mean dialyzer Kt/V of \( \beta_2M \) and mean predialysis serum \( \beta_2M \) levels during the entire follow-up period are presented in Figures 1 and 2, respectively.

The \( \beta_2M \) 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 \( \beta_2M \) 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 \( \beta_2M \) clearance largely paralleled those of the \( \beta_2M \) 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 \( \beta_2M \) level during follow-up for the entire cohort was 37.3 ± 11.9 and 41.5 ± 12.9 mg/L (\( n = 1813 \)).

Table 1. Baseline characteristics of the 1704 randomly assigned patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD or Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>57.8 ± 14.0 yr</td>
</tr>
<tr>
<td>Female</td>
<td>55.9%</td>
</tr>
<tr>
<td>Black</td>
<td>62.9%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>44.6%</td>
</tr>
<tr>
<td>Years on dialysis</td>
<td>3.7 ± 4.3</td>
</tr>
<tr>
<td>Postdialysis weight</td>
<td>69.3 ± 14.8 kg</td>
</tr>
<tr>
<td>Urea distribution volume</td>
<td>31.2 ± 6.6 L</td>
</tr>
<tr>
<td>Residual kidney urea clearance &gt;0</td>
<td>33.3%</td>
</tr>
<tr>
<td>Residual kidney urea clearance &gt;0.75 ml/min&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.0%</td>
</tr>
<tr>
<td>High-flux dialysis</td>
<td>60.2%</td>
</tr>
<tr>
<td>Comorbidity (ICED) score&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>80.3%</td>
</tr>
<tr>
<td>Serum albumin concentration</td>
<td>3.6 ± 0.4 g/dl</td>
</tr>
<tr>
<td>Ultrafiltration volume/postradial</td>
<td>3.0 ± 1.1 L/70 kg weight</td>
</tr>
</tbody>
</table>

<sup>a</sup>All data are presented as mean ± SD or percentages. Only patients who had \( \beta_2 \) microglobulin (\( \beta_2M \)) clearance measurement at 1 mo of follow-up were included. ICED, Index of Coexisting Disease.

<sup>b</sup>Urea distribution volume as determined by kinetic modeling.

<sup>c</sup>Normalized to 35 L urea distribution volume.

<sup>d</sup>ICED severity score (21) computed with diabetes excluded.
Determinants of Predialysis Serum $\beta_2$M Levels

Table 2 presents the association of various factors with predialysis serum $\beta_2$M 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 $\beta_2$M levels, whereas baseline age, diabetes, BMI, and residual kidney urea clearance correlated negatively ($P < 0.05$) with serum $\beta_2$M levels. In this model, in which dialyzer $\beta_2$M clearance was used as a continuous variable and randomization to the high-flux arm both were included, the former but not the latter correlated negatively with serum $\beta_2$M levels. If dialyzer $\beta_2$M clearance was excluded from the model, however, randomization to the high-flux arm correlated negatively with serum $\beta_2$M levels, with a regression coefficient of $-0.27 \pm 0.04 \text{ mg/L per year}$ ($P < 0.0001$), indicating the expected strong correlation between dialyzer $\beta_2$M clearance and flux randomization.

The baseline residual kidney urea clearance was a particularly strong predictor of serum $\beta_2$M levels at 1 mo of follow-up; the regression coefficient was $-7.21 \pm 0.69 \text{ SE} \text{ mg/L per ml/min per 35 L body urea volume}$ ($P < 0.0001$; Figure 3). Exclusion of residual kidney urea clearance from the model increased the regression coefficient of prerandomization years on dialysis from $0.30 \pm 0.08 \text{ mg/L per yr}$ ($P < 0.0001$) to $0.62 \pm 0.08 \text{ mg/L per yr}$ ($P < 0.0001$), whereas the regression coefficient of dialyzer $\beta_2$M clearance remained unchanged ($-1.94 \pm 0.30$ and $-2.01 \pm 0.31 \text{ mg/L per yr}$; $P < 0.0001$ for both).

Longitudinal Changes in Predialysis Serum $\beta_2$M Levels and Residual Kidney Urea Clearance

Mean predialysis serum $\beta_2$M 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 ($\pm \text{ SE}$) of the mean $\beta_2$M levels for the low-flux arm was $1.06 \pm 0.28 \text{ mg/L per year}$ ($P = 0.0002$); whereas that for the high-flux arm was $0.56 \pm 0.27 \text{ mg/L per year}$ ($P = 0.04$). While the mean serum $\beta_2$M 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 $\beta_2$M levels over the course of follow-up in the subgroups without and with substantial overlap in serum $\beta_2$M levels between the low-flux and high-flux arms (Figure 2). The mean predialysis serum $\beta_2$M levels over the course of follow-up in the subgroups with $\leq 3.7$ and $>3.7$ yr on dialysis prestudy were $35.3 \pm 11.2 \text{ mg/L (n = 1164)}$ and $41.7 \pm 11.8 \text{ mg/L (n = 540)}$ respectively ($P < 0.0001$, $\leq 3.7 \text{ versus } >3.7 \text{ yr}$). The mean predialysis serum $\beta_2$M levels over the course of follow-up in the subgroups without and with detectable residual kidney urea clearance were $39.3 \pm 12.1 \text{ mg/L (n = 1136)}$ and $33.4 \pm 10.1 \text{ mg/L (n = 568)}$, respectively ($P < 0.0001$, without versus with residual urea clearance).

817) for the low-flux arm and $33.5 \pm 9.1 \text{ mg/L (n = 887)}$ for the high-flux arm ($P < 0.0001$, low flux versus high flux). There was substantial overlap in serum $\beta_2$M levels between the low-flux and high-flux arms (Figure 2). The mean predialysis serum $\beta_2$M levels over the course of follow-up in the subgroups with $\leq 3.7$ and $>3.7$ yr on dialysis prestudy were $35.3 \pm 11.2 \text{ mg/L (n = 1164)}$ and $41.7 \pm 11.8 \text{ mg/L (n = 540)}$ respectively ($P < 0.0001$, $\leq 3.7 \text{ versus } >3.7 \text{ yr}$). The mean predialysis serum $\beta_2$M levels over the course of follow-up in the subgroups without and with detectable residual kidney urea clearance were $39.3 \pm 12.1 \text{ mg/L (n = 1136)}$ and $33.4 \pm 10.1 \text{ mg/L (n = 568)}$, respectively ($P < 0.0001$, without versus with residual urea clearance).

Figure 1. Distribution of mean dialysis $Kt/V$ of $\beta_2$ microglobulin ($\beta_2$M) during the entire follow-up period. The values of $\beta_2$M $Kt/V$ are derived from the dialyzer $\beta_2$M clearances using the equation described in Materials and Methods. Each panel shows the percentage of the cohort ($N = 1704$) with the designated dialysis $\beta_2$M $Kt/V$. (A) Entire cohort. (B) Low-flux arm. (C) High-flux arm.

Figure 2. Distribution of mean predialysis serum $\beta_2$M levels during the entire follow-up period. Each panel shows the percentage of the cohort ($N = 1704$) with the designated serum $\beta_2$M levels. (A) Entire cohort. (B) Low-flux arm. (C) High-flux arm.
Further analyses showed that the longitudinal increases in serum $\beta_2$M levels in either the low-flux or high-flux arm were not due to decreases in dialyzer $\beta_2$M Kt/V; the slope of change in $\beta_2$M 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 $\beta_2$M 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 $\beta_2$M levels were related to changes in

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**Table 2. Multivariable regressions** of predialysis serum $\beta_2$M level at 1 mo of follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient (mg/L)</th>
<th>SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline age (per 10 yr)</td>
<td>-1.32</td>
<td>0.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>-1.32</td>
<td>0.80</td>
<td>0.098</td>
</tr>
<tr>
<td>Race (black)</td>
<td>2.94</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline diabetes</td>
<td>-3.62</td>
<td>0.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline duration of dialysis (per year)</td>
<td>0.41</td>
<td>0.08</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline ICED score (per unit)</td>
<td>0.74</td>
<td>0.40</td>
<td>0.069</td>
</tr>
<tr>
<td>Baseline serum albumin (per g/dl)</td>
<td>-1.81</td>
<td>0.93</td>
<td>0.052</td>
</tr>
<tr>
<td>History of malignancy or AIDS at baseline</td>
<td>1.32</td>
<td>1.08</td>
<td>0.219</td>
</tr>
<tr>
<td>Baseline high-flux dialysis</td>
<td>-0.22</td>
<td>0.95</td>
<td>0.820</td>
</tr>
<tr>
<td>Baseline ultrafiltration volume/weight (per L/70 kg)</td>
<td>0.17</td>
<td>0.30</td>
<td>0.571</td>
</tr>
<tr>
<td>Baseline modeled urea V (per L)</td>
<td>0.06</td>
<td>0.06</td>
<td>0.308</td>
</tr>
<tr>
<td>Baseline body mass index (per kg/m$^2$)</td>
<td>-0.27</td>
<td>0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline residual kidney urea clearancec (per ml/min)</td>
<td>-7.21</td>
<td>0.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dialyzer $\beta_2$M clearance at 1 mo (per 10 ml/min)</td>
<td>-1.94</td>
<td>0.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Randomized to high-dose (urea Kt/V) arm</td>
<td>0.62</td>
<td>0.62</td>
<td>0.312</td>
</tr>
<tr>
<td>Randomized to high-flux arm</td>
<td>-0.97</td>
<td>1.02</td>
<td>0.339</td>
</tr>
</tbody>
</table>

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$^a$Adjusted for clinical center.

$^b$Only patients who had $\beta_2$M clearance measurement at 1 mo of follow-up were included ($n = 1704$).

$^c$Normalized to 35 L of urea distribution volume.

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**Figure 3.** Relationship between serum $\beta_2$M level and residual kidney urea clearance. Each box shows the distribution of predialysis serum $\beta_2$M 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 $\beta_2$M levels. Presented are the estimated mean predialysis serum $\beta_2$M 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 $\beta_2$M levels were different ($P < 0.001$) between low flux and high flux at all time points.
residual kidney urea clearances, the longitudinal analyses of serum β₂M 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 β₂M 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 β₂M 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 β₂M Level for All-Cause Mortality

The association of the cumulative mean predialysis serum β₂M 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 β₂M levels over time correlated with mortality (RR = 1.11 per 10-mg/L increase in β₂M 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 β₂M 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 β₂M levels (Figure 5).

The predialysis serum β₂M 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 β₂M 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 β₂M 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

<table>
<thead>
<tr>
<th>Baseline Variables</th>
<th>RR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative mean predialysis serum β₂M level over time during follow-up (per 10-mg/L increase)</td>
<td>1.11</td>
<td>1.05 to 1.19</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (per 10-yr increase)</td>
<td>1.45</td>
<td>1.35 to 1.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>0.85</td>
<td>0.71 to 1.02</td>
<td>0.075</td>
</tr>
<tr>
<td>Race (black)</td>
<td>0.77</td>
<td>0.64 to 0.91</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.36</td>
<td>1.16 to 1.60</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of dialysis (per year increase)</td>
<td>1.03</td>
<td>1.02 to 1.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ICED score (per unit increase)</td>
<td>1.34</td>
<td>1.22 to 1.47</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum albumin (per g/dl increase)</td>
<td>0.43</td>
<td>0.34 to 0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual kidney urea clearance (per ml/min)</td>
<td>0.96</td>
<td>0.80 to 1.15</td>
<td>0.681</td>
</tr>
<tr>
<td>Baseline high-flux dialysis</td>
<td>1.04</td>
<td>0.82 to 1.32</td>
<td>0.733</td>
</tr>
<tr>
<td>Ultrafiltration volume/weight (per L/70 kg)</td>
<td>1.16</td>
<td>1.08 to 1.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Modeled body urea distribution volume (per L)</td>
<td>0.99</td>
<td>0.98 to 1.01</td>
<td>0.236</td>
</tr>
<tr>
<td>Randomization to high urea Kt/V arm</td>
<td>0.95</td>
<td>0.83 to 1.10</td>
<td>0.501</td>
</tr>
</tbody>
</table>

*Statified by clinical center.

Only patients who had at least one β₂M clearance measurement during follow-up were included; n = 1813. RR, relative risk; CI, confidence interval.

All variables were baseline except for the cumulative mean predialysis serum β₂M 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).

Normalized to 35 L of urea distribution volume.
Further addition of serum albumin, high-flux or low-flux dialysis, ultrafiltration volume were included in the HEMO Study. In patients with residual urea clearance of 1 ml/min is continuous, predictions 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 β2M 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 β2M levels.

**Figure 5.** Association of all-cause mortality with cumulative mean predialysis serum β2M levels. Mean predialysis serum β2M levels over the follow-up period correlated significantly with mortality (n = 1813; P = 0.001) with an apparent plateau phase beyond β2M 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.

**Discussion**

**Predictors of Predialysis Serum β2M Levels**

The positive correlation of predialysis serum β2M 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 β2M 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 β2M 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 β2M 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 β2M levels, although the magnitude of the association was modest (0.46-mg/L increase in serum β2M level for each additional year of dialysis). Residual kidney clearance of β2M would be more relevant than residual kidney urea clearance in this analysis, but these data are difficult to obtain because β2M is largely cleared by filtration, reabsorption, and degradation in the proximal tubule rather than excreted in the urine.

Dialyzer β2M clearance was also a predictor of serum β2M 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 β2M 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 β2M level. Caution should be exercised to interpret this equivalence because the dialyzer β2M 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, β2M 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 β2M levels or dialyzer β2M kinetics with all-cause mortalitya

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort</th>
<th>≤3.7 Years on Dialysis</th>
<th>&gt;3.7 Years on Dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RRb 95% CI</td>
<td>P</td>
<td>RR 95% CI</td>
</tr>
<tr>
<td>Serum β2M level</td>
<td>1.11 1.05 to 1.19</td>
<td>0.001</td>
<td>1.13 1.04 to 1.23</td>
</tr>
<tr>
<td>β2M clearance</td>
<td>0.97 0.93 to 1.02</td>
<td>0.306</td>
<td>1.01 0.96 to 1.07</td>
</tr>
<tr>
<td>β2M Kt/V</td>
<td>0.99 0.97 to 1.01</td>
<td>0.232</td>
<td>1.01 0.98 to 1.03</td>
</tr>
</tbody>
</table>

aAnalyzed 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 β2M clearance measurement during follow-up were included; n = 1813.
bRR of all-cause mortality per 10-mg/L increase in mean cumulative predialysis serum β2M level or per 0.1-unit increase in mean cumulative dialyzer β2M clearance or per 0.1-unit increase in mean cumulative dialyzer β2M Kt/V.

β2M clearance and serum β2M level may be less apparent. Collectively, these data suggest that residual kidney clearance is an important contributor to total body β2M clearance.

Active malignancies, such as hematologic cancers (19), and AIDS (20) are known to be associated with elevated serum β2M levels in the general population. Patients with known active nonhematologic malignancy or AIDS, however, were excluded from the HEMO Study. A history of malignancy or AIDS was not associated with serum β2M levels in those without measurable baseline residual kidney function, suggests that the increase in serum β2M 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 β2M levels in patients with baseline kidney function and the absence of longitudinal changes in serum β2M levels in those without measurable baseline kidney function lend further support to this hypothesis. The lack of information on nonknight (e.g., gastrointestinal) body clearance of β2M 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 β2M levels over time, however, may be possible with greater removal of the peptide by hemodialfiltration (23) or daily long hemodialysis (24), which are more effective in removing β2M.

Prediction of Mortality by Serum β2M Levels and β2M Kinetics

In patients who were on dialysis ≤3.7 yr before the study, neither β2M clearance nor β2M Kt/V correlated with mortality. In contrast, in patients who were on dialysis >3.7 yr before the study, both β2M clearance and β2M 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 β2M 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 β2M clearance, and mortality. It should be noted that, although the method used in our study to estimate dialyzer β2M 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 β2M clearances from the patient during the entire session instead of the dialyzer performance at a single time point.

The association of serum β2M levels with clinical outcome was different from that of dialyzer β2M clearance. The risk for all-cause death increased almost linearly with increases in baseline serum β2M levels (Figure 5). Patients with β2M levels of 42.5 to 50 mg/L had RR of death that were approximately 60% greater than those with β2M levels <27.5 mg/L during follow-up. Although this interesting relationship should be explored further in future studies, it is unlikely that the association of β2M per se is sufficient to account for the enhanced mortality. Other toxic middle molecules (25–29) or independent toxic process for which β2M may serve as a surrogate could be contributory. The stronger association between serum β2M 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 β2M 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 β2M 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 β2M level for mortality independent of residual kidney urea clearance.

The higher mortality in patients with higher serum β2M 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 β2M cannot be deduced reliably from the available data. Although one might assume that the differences between the predialysis and postdialysis serum β2M levels reflect the generation rates in a given individual, this assumption is contingent on a constant predialysis serum β2M level over time (i.e., the kinetics of β2M are in steady state). Second, it assumes that there is no nonrenal, nondialyzer clearance of β2M. 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 β2M 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 β2M 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 β2M 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 β2M levels in chronic hemodialysis patients. Serum β2M 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 β2M 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 β2M as a marker for middle molecules and maximize the removal of middle molecules (30), although previous studies have largely related β2M to amyloidosis (31,32). This analysis relating serum β2M to mortality lends further justifications for these recommendations. The value of β2M as a marker to guide routine chronic hemodialysis therapy should be evaluated further.

Acknowledgments

We express our gratitude to the patients who volunteered in the HEMO Study and clinical center study coordinators who collected the data. The data contained in this article have not been published previously except in abstract form.

References


5. Leyboldt JK, Cheung AK, Agodoa L, Daugirdas JT, Greene T, Keshaviah PR; for the Hemodialysis (HEMO) Study: Hemo-

Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/