Effects of Hemodialyzer Reuse on Clearances of Urea and \( \beta_2 \)-Microglobulin

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Abstract. Although dialyzer reuse in chronic hemodialysis patients is commonly practiced in the United States, performance of reused dialyzers has not been extensively and critically evaluated. The present study analyzes data extracted from a multicenter clinical trial (the HEMO Study) and examines the effect of reuse on urea and \( \beta_2 \)-microglobulin (\( \beta_2 \)M) clearance by low-flux and high-flux dialyzers reprocessed with various germicides. The dialyzers evaluated contained either modified cellulosic or polysulfone membranes, whereas the germicides examined included peroxyacetic acid/acetic acid/hydrogen peroxide combination (Renalin®), bleach in conjunction with formaldehyde, glutaraldehyde or Renalin, and heated citric acid. Cleance of \( \beta_2 \)M decreased, remained unchanged, or increased substantially with reuse, depending on both the membrane material and the reprocessing technique. In contrast, urea clearance decreased only slightly (approximately 1 to 2% per 10 reuses), albeit statistically significantly with reuse, regardless of the porosity of the membrane and reprocessing method. Inasmuch as patient survival in the chronic hemodialysis population is influenced by clearances of small solutes and middle molecules, precise knowledge of the membrane material and reprocessing technique is important for the prescription of hemodialysis in centers practicing reuse.

High-flux hemodialyzers and reuse of dialyzers have been widely used for decades, yet the effects of these practices on solute clearances have not been fully evaluated. Although the beneficial effect of increasing urea clearance on clinical outcome has been established, at least up to a single-pool \( \text{Kt/V} \) value of 1.2 (1), there are also accumulating data suggesting that the removal of middle molecules (using vitamin B\(_{12} \) as marker) influences patient survival (2,3). Thus, maintenance of the clearance of both small and large solutes for reused dialyzers is important.

Reuse can affect dialyzer performance in at least two different ways. The first is the result of deposition of blood elements inside the lumen of the blood compartment and onto the dialyzer membrane. The second is the result of the reprocessing procedure. At present, the popular germicides used in reprocessing in the United States are Renalin (made up of peroxyacetic acid, acetic acid, and hydrogen peroxide, Minntech, Minneapolis, MN), formaldehyde, and glutaraldehyde (4). To enhance the aesthetic appearance of the dialyzer during reuse, sodium hypochlorite (bleach) is often used in conjunction with formaldehyde or glutaraldehyde to remove residual blood proteins. More recently, heated citric acid has also been introduced to clean and disinfect dialyzers for reuse. Because the chemical composition and mechanical structure are vastly different among various types of dialysis membranes, their interactions with the blood elements and reprocessing agents are likely to differ as well.

The HEMO Study is a prospective randomized multicenter trial sponsored by the U.S. National Institutes of Health designed to examine the effects of urea \( \text{Kt/V} \) and the type of dialysis membrane on clinical outcome of chronic hemodialysis patients (5). Various models of dialyzers and reprocessing methods are used among the 15 clinical centers (more than 45 dialysis units) in the trial. Using this large database, we have prospectively examined the effects of various combinations of dialyzers and reprocessing agents on the clearance of urea and \( \beta_2 \)-microglobulin (\( \beta_2 \)M). The data show that the effects of reuse on \( \beta_2 \)M are far more drastic than those on urea clearance. Furthermore, the effects vary greatly depending on the dialysis membrane material and reprocessing reagents. These observations confirm and extend our fundamental understanding of alterations in dialyzer performance during reuse.

Materials and Methods

Dialysis Prescriptions

The basic design of the HEMO Study has been described (5); therefore, only the parts of the protocol that are germane to the current
Hemodialyzers

Urea $K_{b}$A values for all dialyzer models were tested in vitro in a single laboratory at the University of Utah, and the results of these tests have been reported previously (7). Each dialyzer used in the HEMO Study had an in vitro urea $K_{b}$A value $\geq 500$ mL/min at a dialysate flow rate of 500 mL/min. Before acceptance into the HEMO Study, all dialyzer models were also tested for dialysate flow rate of 500 mL/min. Each dialyzer used in the study was defined as one that satisfied both of the following criteria: (1) standard urea $K_{b}$V and low-flux membrane; (2) standard urea $K_{b}$V and high-flux membrane; (3) high urea $K_{b}$V and low-flux membrane; and (4) high urea $K_{b}$V and high-flux membrane. The two standard urea $K_{b}$V groups had a dialysate treatment target equilibrated $K_{b}$V ($eK_{b}$V) of 1.05, whereas the two high urea $K_{b}$V groups have a target $eK_{b}$V of 1.45. $eK_{b}$V is a double-pool value that accounts for the postdialysis rebound of plasma urea concentration and is approximately 0.2 lower than the single-pool value under usual clinical conditions in the United States (6).

Restrictions on Number of Reuses

The maximum number of times that a dialyzer could be reused in the HEMO Study was originally set at 20. Individual dialysis units could also restrict reuse to less than 20 times. For example, the two centers that used heated citric acid limited their dialyzers to 12 and 15 reuses, respectively. Monitoring of data during the course of the study revealed a significant reduction in the $\beta_{M}$ clearance of certain high-flux dialyzers when they were reprocessed with Renalin without bleach (see below). To maintain a clear separation in $\beta_{M}$ clearances between the low-flux arm and the high-flux arm of the study, the maximum allowable number of reuses for dialysis units using Renalin without bleach was reduced to 10 in February 1997, and reduced further to 6 in October 1997.

In dialysis units where Renalin was used in conjunction with bleach, the maximum allowed number of reuses remained at 20.

Data Collection

Data for this report were collected after randomization of the patients into the follow-up phase of the HEMO Study. Only data from March 1995 (initial enrollment of patients into the full-scale phase of the HEMO Study) through February 1998 were included. Before June 1997, predialysis and postdialysis $\beta_{M}$ concentrations were obtained for determination of $\beta_{M}$ clearances during kinetic modeling sessions at months 1, 2, 3, 4, and 6 after randomization into the treatment groups, and every 6 mo thereafter. Beginning in June 1997, the frequency of the $\beta_{M}$ measurements was increased to monthly 1, 2,

Table 1. Reprocessing techniques used in the study

<table>
<thead>
<tr>
<th>Renalin® (per oxyacetic acid, acetic acid, and hydrogen peroxide; 2% Renalin [0.08% per oxyacetic acid]) for cleaning cycle, 3.25 to 3.50% Renalin [0.13%–0.14% per oxyacetic acid] for fill cycle, Minntech) with automated machine (Renatron®, Minntech, or DRS-4, Fresenius)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach (0.1 to 1.0% for $\leq 5$ min) followed by formaldehyde (0.75 to 4.0%) with automated machine (DRS-4)</td>
</tr>
<tr>
<td>Bleach (0.1 to 1.0% for $\leq 5$ min) followed by glutaraldehyde (Diacide®, Gulfstream Medical, Inc., 0.8%) with automated machine (DRS-4)</td>
</tr>
<tr>
<td>Bleach (0.26% for approximately 2 min) followed by Renalin (2.5% Renalin [0.10% per oxyacetic acid]) manually</td>
</tr>
<tr>
<td>Heated citric acid (1.5% at 95°C for 24 h) with automated machine (DRS-4)</td>
</tr>
</tbody>
</table>
and every 2 mo thereafter in the high-flux arm (both high and standard urea eKt/V) to allow more rapid understanding of dialyzer performance with various reprocessing techniques. At the same time, the frequency of $\beta_2M$ measurements in the low-flux arm (both standard and high urea eKt/V) was reduced to months 1, 12, and every 12 mo thereafter. This report includes all available $\beta_2M$ clearance determinations for the specified dialyzers. Analyses of $\beta_2M$ clearance for the high-flux F80 dialyzers were separated into those obtained before June 15, 1997, and those obtained thereafter (June 15 through February 1998) because of the changes in the dialyzers by the manufacturer. The numbers of $\beta_2M$ clearance measurements used in the analyses of specific dialyzer-reuse combinations are provided in Table 3.

Predialysis and postdialysis blood urea nitrogen (BUN) concentrations were measured at monthly kinetic modeling sessions. In this report, analyses of urea clearance were restricted to these modeling sessions performed in patients with functional arteriovenous (native or polytetrafluoroethylene) vascular accesses. Exclusions from these analyses of urea clearances were patients with access recirculation >15% determined using the slow-flow method (assessed once before randomization and once at the 4-mo follow-up visit), patients using temporary catheters, patients with extremity amputation(s), sessions with more than 15 min of interruption (which accounted for 2.3% of modeling sessions), and sessions with highly deviant predialysis or postdialysis BUN concentrations (postdialysis BUN <10% or >60% of the predialysis value, or predialysis BUN >200 mg/dl or <10 mg/dl, accounting for a total of 1.9% of modeling sessions).

Predialysis and postdialysis body weights, location of the vascular access, dialyzer model, dialyzer blood flow rate, type of delivery system, dialysate flow rate, ultrafiltration rate, and total ultrafiltration volume were noted or calculated for each session. Blood samples were collected immediately predialysis and 20 s postdialysis after the dialyzer blood flow rate had been reduced to ≤80 ml/min.

**Assays**

Blood samples were immediately centrifuged and all serum and plasma samples were assayed for urea nitrogen and $\beta_2M$ concentrations at a central laboratory (LifeChem, Rockleigh, NJ). Urea was measured by an autoanalyzer (Hitachi 747 to 200, Boehringer Mannheim, Indianapolis, IN) using a kinetic urease method. The intra-assay and interassay variations were 1.7 and 3.0%, respectively. $\beta_2M$ concentrations were determined using a solid-phase competitive RIA with high specificity from the extracellular compartment. (4) Postdialysis rebound of plasma $\beta_2M$ concentration was ignored. Clearance estimated by this equation yields an average value over the entire treatment and includes removal by diffusive, convective, and absorptive mechanisms.

**Calculation of Effective and Predicted Urea Clearance**

The patient’s predicted urea clearance was estimated from the in vitro mass transfer area coefficient ($K_oA$), blood flow rate, and dialysate flow rate using a standard formula (7), with appropriate adjustments for blood water concentration of urea (12,13) and ultrafiltration rate. The $K_oA$ values used in these calculations were determined in vitro for individual dialyzer models in a single laboratory as reported previously (7) and described above.

To determine effective urea clearance, the patient’s anthropometric volume (14) was adjusted for two pool effects to obtain an estimate of the patient’s single-pool volume using previously described methods (15). The adjusted anthropometric volume was then used as an input parameter in a two-BUN method (16), along with the predialysis and postdialysis BUN values to compute the effective clearance.

The ratio of the effective to the predicted clearance was calculated and used as the dependent variable in multivariate regression analyses to assess the effect of reuse on effective urea clearance.

**Statistical Analyses**

All data are presented as mean ± SEM unless specified otherwise. The relationship between $\beta_2M$ clearance and reuse number was described for specific dialyzer-reuse combinations by box-plots (17), and by regression of $\beta_2M$ clearance on reuse number using a mixed effects model with a compound symmetry error structure to account for multiple measurements in the same patients (18). For each dialyzer-reuse combination, linearity of the relationship between $\beta_2M$ clearance and reuse number was assessed by testing the significance of a quadratic term in the regressions. Mixed-effects regression models were also used to test for the presence of a relationship between ultrafiltration rate or total ultrafiltration volume in the session and reuse number within each dialyzer-reuse combination.

The time-weighted mean $\beta_2M$ clearance over a given number ($k$) of reuse was estimated for each dialyzer-reuse combination by first computing the mean $\beta_2M$ clearance for that dialyzer-reuse combination at each reuse number, and then averaging the values of all uses up to reuse number $k$. To relate effective urea clearance to reuse number, the ratio of the effective to the predicted urea clearance was regressed on reuse number while controlling for the vascular access location, type of dialyzer, dialyzer blood flow rate, type of delivery system, dialysate flow rate, and race of the patient in a multivariate regression analysis. This regression was also carried out using a mixed-effects model with a compound symmetry error structure.

**Results**

**Characteristics of Patients and Hemodialysis Sessions**

The characteristics of the studied patients and treatment sessions are listed in Table 2. The data are presented separately for the low-flux and high-flux groups because the effect of reuse on $\beta_2M$ clearance was far more substantial than that on urea clearance (see below). Body weights, volumes of distribution of urea, blood flow rates, dialysate flow rates, treatment session durations, and ultrafiltration rates were all similar between the high-flux and low-flux groups. By design, mean ± SD $\beta_2M$ clearance over all reuse sessions for patients in the
Table 2. Characteristics of patients and hemodialysis sessions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low Flux Arm (n = 591 patients)</th>
<th>High Flux Arm (n = 598 patients)</th>
<th>Total (n = 1189 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>69.0 ± 15.0 (35.9 to 132.9)</td>
<td>69.2 ± 14.5 (37.1 to 160.5)</td>
<td>69.1 ± 14.7 (35.9 to 160.5)</td>
</tr>
<tr>
<td>Volume of distribution of urea (L)</td>
<td>33.7 ± 7.0 (16.3 to 56.3)</td>
<td>34.4 ± 6.8 (19.2 to 58.9)</td>
<td>34.0 ± 6.9 (16.3 to 58.9)</td>
</tr>
<tr>
<td>Blood flow rate (ml/min)</td>
<td>375 ± 72 (179 to 503)</td>
<td>373 ± 69 (200 to 500)</td>
<td>374 ± 71 (179 to 503)</td>
</tr>
<tr>
<td>Dialysate flow rate (ml/min)</td>
<td>674 ± 120 (497 to 800)</td>
<td>674 ± 119 (471 to 808)</td>
<td>674 ± 119 (471 to 808)</td>
</tr>
<tr>
<td>Treatment duration (min)</td>
<td>204 ± 29 (149 to 284)</td>
<td>202 ± 27 (147 to 273)</td>
<td>203 ± 28 (147 to 284)</td>
</tr>
<tr>
<td>Ultrafiltration rate (ml/min)</td>
<td>14.4 ± 4.9 (−0.11 to 32.9)</td>
<td>14.4 ± 4.8 (2.5 to 33.6)</td>
<td>14.4 ± 4.8 (−11.1 to 33.6)</td>
</tr>
<tr>
<td>Urea clearance (ml/min)</td>
<td>249 ± 31 (139 to 312)</td>
<td>256 ± 32 (171 to 329)</td>
<td>253 ± 32 (139 to 329)</td>
</tr>
<tr>
<td>β₂M clearance (ml/min)</td>
<td>3.1 ± 8.1 (−29.1 to 47.2)</td>
<td>34.6 ± 14.3 (1.8 to 86.1)</td>
<td>18.9 ± 19.5 (−29.1 to 86.1)</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SD (range). Dialysis characteristics are presented as mean over all dialysis sessions. β₂M, β₂-microglobulin.

high-flux group (34.6 ± 14.3 ml/min) was approximately 11-fold higher than that in the low-flux group (3.1 ± 8.1 ml/min).

β₂M Clearance by High-Flux and Low-Flux Dialyzers during First Use

There was no substantial difference between β₂M clearance during first use by dialyzers that had been processed and those that had not been processed before use (data not shown). Mean clearances of β₂M for all low-flux dialyzer models during first use were well below 10 ml/min. For example, mean ± SD β₂M clearance by CA210 (n = 309) and F8 (n = 279) were 3.1 ± 11.0 and 1.7 ± 11.3 ml/min, respectively. Mean clearances of β₂M by high-flux dialyzers during first use were more variable, ranging from 14.2 ± 13.4 ml/min (n = 66) for F80B that were used early in the study to 42.3 ± 12.5 ml/min (n = 336) for CT190 dialyzers (Figure 1). The F80A and F80B dialyzers provided by the manufacturer changed during early 1997 and mean β₂M clearances by these dialyzers during first use have increased. It should be noted that the variations of β₂M clearances by individual dialyzers within any given low-flux or high-flux model were large, with SD ranging from 9.3 to 16.7 ml/min.

Effect of Renalin without Bleach on β₂M Clearance

Reuse using Renalin as germicide had a great effect on β₂M clearance by CT190 dialyzers. As can be seen in Figure 2, mean β₂M clearance declined rapidly through the first four reuses and continued to decline at a slower rate up to the tenth through 14th reuses. The mean ± SEM total decrease in β₂M clearance between first use and the tenth through 14th reuses was 67.2 ± 2.7%. The clearance did not appear to decrease further when reuse was extended to 15 to 20 times. Since the β₂M clearance during a given session decreased with increasing reuse number, so did the time-averaged clearance through the life of the dialyzers (Table 3). Similar results were observed in all six clinical centers that used this dialyzer-reprocessing combination (data not shown).

Reprocessing with Renalin produced a slight decline in β₂M clearance by the F80A dialyzers (the change was −0.64 ± 0.26 ml/min per reuse; P = 0.013 before June 15, 1997, and −0.31 ± 0.31 ml/min per reuse; P = 0.32 after June 15, 1997) (Figure 3).

Effect of Bleach on β₂M Clearance

Bleach was always used in conjunction with formaldehyde, Renalin, or Diacide® for reprocessing of dialyzers in the HEMO Study.

F80B Dialyzers. For F80B dialyzers, the rate of increase of β₂M clearance with reuse differed significantly (P < 0.001) among the three germicides (formaldehyde, Renalin, and Diacide) used in conjunction with bleach. Bleach in conjunction with formaldehyde had the greatest effect on F80B dialyzers. β₂M clearance by F80B dialyzers increased markedly through 20 reuses (Figure 4A). Accordingly, the time-averaged clear-
Figure 2. Effect of reprocessing using Renalin on $\beta_2$M clearance by CT190 (cellulose triacetate high-flux) dialyzers. The data were obtained from the full study period. The number of observations for each range of reuse numbers is presented above the x-axis. The mean ± SEM $\beta_2$M clearance declined by 67.2 ± 2.7% between first use and 10th to 14th reuses. For explanation of box-plots, see the legend to Figure 1.

ance increased by more than twofold through 20 reuses (Table 3). The mean ± SEM rate of increase was greater during the initial seven reuses (3.32 ± 0.40 ml/min per reuse; $P < 0.001$) than during the seventh through 20th reuses (1.40 ± 0.26 ml/min per reuse; $P < 0.001$).

The effectiveness of bleach in increasing $\beta_2$M clearance of F80B dialyzers was lower when it was used in conjunction with Renalin (1.88 ± 0.26 ml/min per reuse) (Figure 4B) instead of formaldehyde. When Diacide was used instead of formaldehyde or Renalin, the effect of bleach was even less (Figure 4C). $\beta_2$M clearance of F80B dialyzers reprocessed using Diacide with bleach increased only by 0.84 ± 0.25 ml/min per reuse ($P < 0.001$) before June 15, 1997, and by 0.55 ± 0.18 ml/min per reuse ($P = 0.004$) after June 15, 1997.

CT190 Dialyzers. When bleach was used in conjunction with either formaldehyde or Diacide to reprocess CT190 dialyzers, modest increases in $\beta_2$M clearance were observed (0.29 ± 0.11 ml/min per reuse; $P = 0.011$) (Figure 5). The rate of increase in $\beta_2$M clearance did not differ significantly between the two aldehydes (0.32 ± 0.16 ml/min per reuse, $P = 0.045$ for formaldehyde with bleach, and 0.22 ± 0.17 ml/min per reuse, $P = 0.20$ for Diacide with bleach).

Low-Flux Dialyzers. When low-flux dialyzers were reprocessed using any procedure that included bleach, there was a small but statistically significant increase in $\beta_2$M clearance during reuse (0.25 ± 0.07 ml/min per reuse; $P < 0.001$) (Figure 6). The large increase in $\beta_2$M clearance observed with F80B dialyzers reprocessed with bleach and formaldehyde (see above) raised the possibility that low-flux dialyzers (F8) made from the same polymer (polysulfone) might exhibit similar behavior. When F8 dialyzers (0.19 ± 0.07 ml/min per reuse; $P = 0.010$) and low-flux dialyzers made from other materials (0.52 ± 0.18 ml/min per reuse; $P = 0.004$) were analyzed separately and compared, the rates of increase in $\beta_2$M clearance with reuse tended to be greater for the nonpolysulfone dialyzers, but the difference was not statistically significant ($P = 0.09$).

The greater effect of formaldehyde than Diacide in conjunction with bleach on F80B dialyzers suggests that this difference between the two aldehydes might also be present in low-flux dialyzers. Further analysis showed that the rate of increase in $\beta_2$M clearance with reuse of low-flux dialyzers did not differ significantly among formaldehyde, Diacide, and Renalin (all in conjunction with bleach) ($P = 0.075$), but tended to be greater with formaldehyde (0.33 ± 0.09 ml/min per reuse; $P < 0.001$) than with Diacide (−0.06 ± 0.15 ml/min per reuse; $P = 0.71$) or Renalin (0.13 ± 0.14; $P = 0.37$).

Effect of Heated Citric Acid on $\beta_2$M Clearance

Polysulfone high-flux (F80A) dialyzers were also susceptible to the effect of heated citric acid. The clearance of $\beta_2$M increased in an apparently linear manner up to seven reuses at a rate of 2.31 ± 0.45 ml/min per reuse ($P < 0.001$) (Figure 7). The effect of heated citric acid appeared to have reached a plateau phase after seven reuses. The mean increase in clearance was 36 ± 8% between first use and fifth through ninth reuses. The time-averaged $\beta_2$M clearance increased accordingly with increasing reuse numbers (Table 3). In contrast, heated citric acid had no effect on $\beta_2$M clearance by F8 dialyzers (0.00 ± 0.27 ml/min per reuse; $P = 0.99$).

Relationship between Reuse and Ultrafiltration Volumes

Transmembrane convective transport may contribute significantly to the dialyzer clearance of middle molecules, such as $\beta_2$M. Since convective solute transport across a given membrane is dependent on ultrafiltration, the correlation between reuse numbers and ultrafiltration volumes was examined to determine whether the changes in $\beta_2$M clearances during reuse were due to coincidental variabilities in ultrafiltration volumes. Regression analyses, however, showed no correlation between these two variables for any combination of high-flux or low-flux dialyzers and reprocessing methods (data not shown).

Effect of Reuse on Urea Clearances

The effects of reuse on urea clearances were, in general, more modest than those on $\beta_2$M clearances, although both high-flux and low-flux dialyzers were affected (Table 4). Urea clearance by high-flux dialyzers decreased by 1.9 ± 0.3% per 10 reuses ($P < 0.001$) with no significant differences among various types of dialyzers and reprocessing methods studied ($P = 0.096$). For example, in contrast to their opposing effects on $\beta_2$M clearances, Renalin (−2.9 ± 0.4% per 10 reuses for CT190 and −2.1 ± 1.0% per 10 reuses for F80A) and bleach-containing methods (−1.6 ± 0.8% per 10 reuses for CT190 and −1.4 ± 0.5% per 10 reuses for F80B) had similar deleterious effects on urea clearances of high-flux dialyzers.

Urea clearances by low-flux dialyzers also deteriorated with reuse (−1.0 ± 0.3% per 10 reuses; $P < 0.001$), but the decrease was slightly lower than that of high-flux dialyzers.
Table 3. Reuse-averaged $\beta_2$M clearances of high-flux dialyzers through different numbers of reuses with various reprocessing methods

<table>
<thead>
<tr>
<th>Reprocessing Technique</th>
<th>Dialyzer</th>
<th>No. of Sessions/ Patients</th>
<th>Reuse-averaged $\beta_2$M clearances$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>New$^b$</td>
</tr>
<tr>
<td>Renalin$^c$</td>
<td>CT190$^d$</td>
<td>1315/203</td>
<td>42.4</td>
</tr>
<tr>
<td>Renalin</td>
<td>F80A$^e$</td>
<td>151/43</td>
<td>41.1</td>
</tr>
<tr>
<td>Bleach + formaldehyde</td>
<td>CT190$^d$</td>
<td>457/107</td>
<td>42.4</td>
</tr>
<tr>
<td>Diacide$^c$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleach + formaldehyde</td>
<td>F80B$^e$</td>
<td>303/94</td>
<td>21.3</td>
</tr>
<tr>
<td>Bleach + Renalin</td>
<td>F80B$^e$</td>
<td>100/27</td>
<td>21.3</td>
</tr>
<tr>
<td>Bleach + Diacide</td>
<td>F80B$^e$</td>
<td>96/33</td>
<td>21.3</td>
</tr>
<tr>
<td>Heated citric acid</td>
<td>F80A$^e$</td>
<td>226/58</td>
<td>41.1</td>
</tr>
</tbody>
</table>

$^a$ Clearances averaged over all sessions up to the specified number of reuses.

$^b$ Number of clearance measurements/number of patients providing data for each dialyzer-reuse combination.

$^c$ $\beta_2$M clearances averaged across all first-use measurements, regardless of subsequent method of reuse.

$^d$ Data from full study.

$^e$ Data since June 15, 1997.

$^f$ NA, not available because units employing Renalin without bleach were restricted to 10 or fewer reuses after February 1997.

Effect of Reuse on Clinical Outcome

The effect of dialyzer reuse on patient mortality has been controversial, but two commonly cited studies have reported that reuse in the United States was associated with higher mortality rates (20,21). There are a number of reasons that could potentially explain these associations, some of which have been analyzed in more detail (e.g., free-standing versus hospital-based, type of germicides used), whereas others have not been well addressed (e.g., dose of dialysis). Dose of dialysis, as determined by $Kt/V$ of urea, has been established to be a determinant of clinical outcome in chronic hemodialysis patients (1). Preliminary data suggest that middle molecule removal may also influence patient survival (2,3). To the extent that patient mortality may be affected by reuse of dialyzers, the effects of dialyzer reuse on small and middle molecule clearance should be better understood.

Discussion

Current Practice in the United States

The survey results from a 1995 study, which was recently published by the Center for Disease Control and Prevention, showed that 50% of the dialysis units in the United States treated some patients with high-flux dialyzers (4). High-flux dialyzers were defined in that report as ones that had ultrafiltration coefficients $\geq 20$ ml/h per mmHg; $\beta_2$M clearances were not part of the criteria. The report also showed that 77% of all units and 76% of all patients reused dialyzers. Renalin was the most commonly used germicide, accounting for 54% of all units reusing dialyzers. Formaldehyde and glutaraldehyde were used by 38 and 7% of the units, respectively, whereas 1.4% of the units that reused dialyzers disinfected with heat. It should be noted that peroxyacetic acid (a constituent of Renalin) in conjunction with bleach is not recommended for reprocessing dialyzers because the combination may produce hydrochloric acid vapors (19). Therefore, the bleach must be thoroughly rinsed off before the application of Renalin, or other germicides.
solute clearance, using urea and \( \beta_2 \text{M} \) as markers, respectively. The large number of enrolled patients, together with frequent assessments of urea and \( \beta_2 \text{M} \) kinetics according to strict protocols, provides a rich data source for the effect of reuse on dialyzer performance. The standard (1.05 target) and high (1.45 target) urea eKt/V groups also provide a wide spectrum of urea clearances for the examination of reuse effects.

There are limitations in these data. First, only a limited number of combinations of dialyzer and reprocessing techniques were studied. Second, blood flow rates, dialysate flow rates, and vascular access locations were not standardized, although these variables were accounted for in the statistical analyses of urea clearance. Patients with vascular access recirculation \( >15\% \) were excluded for analyses of urea clearance in this report. Although data from patients with recirculation \( >15\% \) were not analyzed further for the effect of recirculation on urea \( \text{Kt/V} \) or \( \beta_2 \text{M} \) clearance, there was no systematic bias in data collection during different reuse numbers in this large data set.

The mean \( \pm \) SEM of the intercept of the regression of \( \beta_2 \text{M} \) clearance versus reuse number is 40.9 \( \pm \) 0.8 ml/min, and the slope is 0.29 \( \pm \) 0.11 ml/min per reuse \( (P = 0.011) \). For explanation of box-plots, see the legend to Figure 1.

![Figure 4](image)

Figure 4. (A) Effect of reprocessing using formaldehyde with bleach on \( \beta_2 \text{M} \) clearance by F80B (polysulfone high-flux) dialyzers. The data were obtained since June 15, 1997. The regression of \( \beta_2 \text{M} \) clearance versus reuse numbers is significantly nonlinear \( (P = 0.002) \). The intercept is 24.3 \( \pm \) 2.0 ml/min. The slope for 0 to 7th reuses is 3.32 \( \pm \) 0.40 ml/min per reuse \( (P < 0.001) \), whereas the slope for 7th to 20th reuse is lower at 1.40 \( \pm \) 0.26 ml/min per reuse \( (P < 0.001) \). The mean \( \pm \) SEM \( \beta_2 \text{M} \) clearance increased by 150 \( \pm \) 39\% between first use and 15th to 20th reuses. (B) Effect of reprocessing using Renalin with bleach on \( \beta_2 \text{M} \) clearance by F80B (polysulfone high-flux) dialyzers. The data were obtained since June 15, 1997. The mean \( \pm \) SEM of the intercept of the regression of \( \beta_2 \text{M} \) clearance versus reuse number is 24.1 \( \pm \) 2.6 ml/min, and the slope is 1.88 \( \pm \) 0.26 ml/min per reuse \( (P = 0.001) \). (C) Effect of reprocessing using Diacide (glutaraldehyde) with bleach on \( \beta_2 \text{M} \) clearance by F80B (polysulfone high-flux) dialyzers. The data were obtained since June 1997. The mean \( \pm \) SEM intercept of the regression of \( \beta_2 \text{M} \) clearance versus reuse number is 18.9 \( \pm \) 1.6 ml/min, and the slope is 0.55 \( \pm \) 0.18 ml/min per reuse \( (P = 0.004) \). For explanation of box-plots, see the legend to Figure 1.

![Figure 5](image)

Figure 5. Effect of reprocessing using formaldehyde or Diacide (glutaraldehyde) with bleach on \( \beta_2 \text{M} \) clearance by CT190 (cellulose triacetate high-flux) dialyzers. The data were obtained from the full study period. The mean \( \pm \) SEM of the intercept of the regression of \( \beta_2 \text{M} \) clearance versus reuse number is 24.1 \( \pm \) 2.6 ml/min, and the slope is 0.29 \( \pm \) 0.11 ml/min per reuse \( (P = 0.011) \). For explanation of box-plots, see the legend to Figure 1.
Effect of Reuse on Urea Clearance

Gotch reported approximately 20 years ago that urea clearances were maintained at more than 90% of their original values when reused dialyzers retained 80% of their original total cell volume (22). Since then, this observation has formed the basis of the performance criterion for accepting dialyzers for reuse. Although this criterion was initially established only for low-flux cellulose membrane hollow-fiber dialyzers with relatively low blood flow rates, subsequent studies have suggested that it is applicable to modern conditions as well. For example, Canaud et al. found no decrease in the predialysis to postdialysis urea reduction ratio (URR) at the tenth use after reprocessing with peroxycetic acid compared with first use of polycrylonitrile membrane dialyzers (Filtral 16, Hospital) operating at blood flow rates of 300 to 400 ml/min. Their data, however, were limited to only two patients using 14 dialyzers each (23). Ouseph et al. showed that both high-flux cellulose dialyzers (AM-UP-75WET, Asahi, Tokyo; n = 8) and high-flux polysulfone (F80B; n = 5) dialyzers maintained their urea Kt/V at 12th and 15th use, respectively, after reprocessing with Renalin, as long as total cell volume remained more than 80% of the original value (24).

In contrast, several other studies have suggested that urea clearances can deteriorate with dialyzer reuse. The marked (49%) decrease in urea clearances after 15 uses reported by Delmez et al. was attributed to nonuniform dialysate flow in reused dialyzers and appeared to be associated only with a specific lot of low-flux cuprammonium (Clirans TAF, Terumo, Tokyo, Japan) dialyzers (25). Garred et al. found that when 102 high-flux polysulfone dialyzers (F60 and HF80, Fresenius) were reprocessed with peroxycetic acid compared with first use of Renalin or glutaraldehyde. The number of patients in the two latter categories was small. Dialyzer models were not specified.

Figure 6. Effect of reprocessing using formaldehyde, Diacide (glutaraldehyde), or Renalin with bleach on β₂M clearance by all low-flux dialyzers. The data were obtained from the full study period. The mean ± SEM of the intercept of the regression of β₂M clearance versus reuse numbers is 2.5 ± 0.6 ml/min, and the slope is 0.25 ± 0.07 ml/min per reuse (P < 0.001). For explanation of box-plots, see the legend to Figure 1.

Figure 7. Effect of reprocessing using heated citric acid on β₂M clearance by F80A (polysulfone high-flux) dialyzers. The data were obtained since June 1997. The regression of β₂M clearance versus reuse numbers is significantly nonlinear (P = 0.012). The mean ± SEM of the intercept is 43.6 ± 2.1 ml/min. The slope for 0 to 7th reuse is 2.31 ± 0.45 ml/min per reuse (P < 0.001), whereas the slope for 7th to 14th reuse is 0.01 ± 0.69 ml/min per reuse (P = 0.98). The mean β₂M clearance increased by 36 ± 8% between first use and 5th to 9th reuse. For explanation of box-plots, see the legend to Figure 1.

set. Third, although the heparin dosage likely remained relatively stable over time for a given individual patient, heparin dosage was not standardized in the HEMO Study. Fourth, individual dialysis units in the HEMO Study were allowed to impose a maximum number of reuses for their dialyzers, as long as that number did not exceed the maximum number set by the HEMO Study for that particular dialyzer-reprocessing technique combination. For these reasons, reliable data on the maximum reusability of specific dialyzer models could not be obtained.
cessing technique. The large sample size in this study allowed for the detection of even relatively small changes. Reuse was limited to a maximum of 20 times (and less for some dialyzer-reprocessing technique combinations) in the HEMO Study. Although the magnitude of decrease in urea clearance was small (approximately 1 to 2% per 10 reuses, Table 4), the relationship between the decrease and reuse number appeared to be linear. If this relationship holds true beyond 20 reuses, the decrease in urea Kt/V could be substantial for dialyzers that are reused many times and may potentially contribute to the reuse among the five high-flux categories. Possibly, the deposited C3 fragments and other plasma proteins that are not removed by peroxyacetic acid inhibit further binding of C3 molecules for complement activation. Reprocessing using Renalin has only a small effect on the β2M clearance of F80A dialyzers (Figure 3), suggesting that either the polysulfone membrane is more resistant to fouling by plasma proteins such that its transport and/or adsorptive capacities are largely preserved, or that these protein–membrane interactions are more susceptible to disruption by Renalin.

Table 4. Effect of reuse on urea clearance

<table>
<thead>
<tr>
<th>Reprocessing Technique</th>
<th>Dialyzer(s)</th>
<th>No. of Modeled Dialyses with Reuse &gt;0</th>
<th>Δ Urea Clearance per 10 Reuses (mean ± SEM)a</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All techniques combined</td>
<td>All high-flux dialyzers combinedb</td>
<td>6288</td>
<td>−1.9 ± 0.3%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All techniques combined</td>
<td>All low-flux dialyzers combined</td>
<td>5281</td>
<td>−1.0 ± 0.3%c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renalin®</td>
<td>High-flux CT190</td>
<td>2281</td>
<td>−2.9 ± 0.4%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renalin</td>
<td>High-flux F80A</td>
<td>493</td>
<td>−2.1 ± 1.0%</td>
<td>0.044</td>
</tr>
<tr>
<td>Renalin</td>
<td>All low-flux dialyzers combined</td>
<td>2141</td>
<td>−1.1 ± 0.4%</td>
<td>0.013</td>
</tr>
<tr>
<td>Bleach</td>
<td>High-flux CT190</td>
<td>557</td>
<td>−1.6 ± 0.8%d</td>
<td>0.043</td>
</tr>
<tr>
<td>Bleach</td>
<td>High-flux F80B</td>
<td>1238</td>
<td>−1.4 ± 0.5%</td>
<td>0.007</td>
</tr>
<tr>
<td>Bleach</td>
<td>All low-flux dialyzers combined</td>
<td>2120</td>
<td>−1.2 ± 0.4%f</td>
<td>0.005</td>
</tr>
<tr>
<td>Heated citric acid</td>
<td>High-flux F80A</td>
<td>727</td>
<td>−1.1 ± 0.9%</td>
<td>0.21</td>
</tr>
</tbody>
</table>

a Coefficients estimated using multiple regression controlling for type of dialyzer, blood flow, dialysate flow, access location, delivery system, and race.
b P = 0.096 for overall comparison of the slopes of urea clearance versus reuse among the five high-flux categories.
c P = 0.015; all low-flux combined versus all high-flux combined (all reprocessing techniques combined).
d P = 0.12, CT190-bleach versus CT190-Renalin.
e P = 0.57, F80-bleach versus F80-Renalin.
f P = 0.86, all low-flux combined with bleach versus all low-flux combined with Renalin.

Inclusion of bleach in the reprocessing procedure produces a very different effect. When bleach was used in conjunction with formaldehyde, β2M clearance by F80B dialyzers is enhanced substantially (Figure 4A), consistent with previous reports of increases in ultrafiltration coefficients (33), β2M clearance (9,27,33,34), and albumin losses (8–10) using the F80-bleach combination. The mechanism by which bleach produces these effects is unclear. The polysulfone dialysis membranes manufactured by Fresenius are made from copolymers of polysulfone and polyvinylpyrrolidone (PVP). It has been postulated that bleach removes PVP from the copolymers, thereby increasing the porosity of these membranes. It should be noted, however, that the evidence supporting this hypothesis was derived under conditions that were vastly different from those used in clinical practice. In those experiments, in vitro exposure of F60 dialyzers to 4% (compared with ≤1% clinically) of bleach for 5 d (compared with ≤5 min clinically) resulted in a loss of 38% in PVP content of the membrane (35).
When bleach was used in conjunction with Diacide instead of formaldehyde, the increases in \( \beta_2 \)M clearance by F80B dialyzers during reuse were markedly diminished (Figure 4C). The reason for this difference between the two aldehydes is also unclear. If the efficacy of bleach to increase membrane porosity is independent of formaldehyde, it would be reasonable to postulate that the glutaraldehyde applied to the dialyzers after bleach during the reprocessing procedure somehow reverses the effect of bleach. Both formaldehyde and glutaraldehyde cross-link proteins. In contrast to formaldehyde, however, the reaction produced by glutaraldehyde is rapid and irreversible (36). Theoretically, glutaraldehyde would be more effective in cross-linking any residual plasma proteins and peptides that are left after the bleach cycle. Aggregation of the cross-linked proteins in the microstructure of the membrane would therefore decrease its effective pore sizes. An alternative but less likely possibility is that glutaraldehyde may, in fact, cross-link plasma proteins to the dialysis membrane or between the membrane polymers themselves. Experimental evidence for this postulate is unavailable.

In contrast, \( \beta_2 \)M clearance by CT190 dialyzers only increased mildly after reprocessing with bleach (Figure 5), possibly because cellulose triacetate membranes are relatively resistant to oxidative damage by hypochlorite. Clearance of \( \beta_2 \)M by low-flux polysulfone dialyzers (F8) did not change after exposure to bleach, possibly because the size of the membrane pores was initially too small, such that even the damage by bleach did not enlarge the pores sufficiently to enhance the passage of small proteins. The ineffectiveness of low-flux polysulfone dialyzers to remove \( \beta_2 \)M also suggests that the mechanism of removal of this protein by polysulfone membranes is primarily transport across the membrane rather than adsorption onto the membrane surface.

Previous data regarding the effect of heated citric acid on dialyzer clearance of proteins are very limited. One previous study showed that \textit{in vitro} clearance of cytochrome C (a protein with molecular weight similar to that of \( \beta_2 \)M) by F80 dialyzers reprocessed with heated citric acid remained unchanged after 11 reuses (37). In contrast, \( \beta_2 \)M clearance by F80A dialyzers in the present study increased by approximately 36% after the dialyzers had been reprocessed with heated citric acid for five to nine times (Figure 7). This difference in results between the previous and present studies is potentially due to differences in the solute marker used (cytochrome C versus \( \beta_2 \)M), the testing condition (\textit{in vitro} versus \textit{clinical}), sample size, or changes in the intrinsic properties of F80 dialyzers.

Increases in \( \beta_2 \)M clearance during reuse of high-flux polysulfone membranes reprocessed with either bleach or heated citric acid could potentially be associated with increases in albumin loss through the membrane, as reported with polysulfone membranes used several years ago (8–10). Study of albumin loss with various combinations of dialyzers and reprocessing techniques is ongoing in the HEMO Study. Preliminary results suggest that the albumin loss from F80B dialyzers currently used in the HEMO Study and reprocessed with bleach is substantially lower than that reported previously.

### Pore Theory

The observations in the present study can be explained on the basis of a theory that encompasses two populations of pore sizes on the dialysis membranes. Regardless of the reprocessing technique, reuse of dialyzers often leads to a decrease in effective surface area, thus the number of small pores. This can occur, for example, as a result of clogging of fibers by cells and proteins even though the residual total cell volume remains above 80% of the original value. The loss of small pores then leads to a decrease in urea clearance. In support of this hypothesis, regional loss of flow in the blood compartment of reused dialyzers, suggesting the obstruction of fibers, has been recently demonstrated by a magnetic resonance velocity imaging technique (38). The structure of the residual membrane surface area may also be altered as the result of reuse, the nature and extent of which depend on the reprocessing technique and the membrane material. The size of the large pores may diminish as a result of protein layering, thus decreasing \( \beta_2 \)M transport, as in the case of cellulose triacetate membrane reprocessed with Renalin. Alternatively, the pores may enlarge, thus enhancing \( \beta_2 \)M transport, as in the case of polysulfone membrane treated with either heated citric acid or bleach in conjunction with formaldehyde.

### Conclusion

To the extent that enhanced removal of small solutes and perhaps middle molecules improves patient outcome, detailed knowledge of the effects of dialyzer reuse on performance is important. The present study illustrates that such knowledge cannot be attained without precise information about the dialyzer membrane structure and the reprocessing technique.

### Acknowledgments

The study coordinators of the 15 HEMO clinical centers were helpful in data collection. Guofen Yan assisted in data analyses, and Brooks Rogers provided valuable advice regarding reuse. Helpful discussions with Jay Radovich are appreciated.

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