Efficient Removal of Immunoglobulin Free Light Chains by Hemodialysis for Multiple Myeloma: *In Vitro* and *In Vivo* Studies


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Of patients with newly diagnosed multiple myeloma, approximately 10% have dialysis-dependent acute renal failure, with cast nephropathy, caused by monoclonal free light chains (FLC). Of these, 80 to 90% require long-term renal replacement therapy. Early treatment by plasma exchange reduces serum FLC concentrations, but randomized, controlled trials have shown no evidence of renal recovery. This outcome can be explained by the low efficiency of the procedure. A model of FLC production, distribution, and metabolism in patients with myeloma indicated that plasma exchange might remove only 25% of the total amount during a 3-wk period. For increasing FLC removal, extended hemodialysis with a protein-leaking dialyzer was used. *In vitro* studies indicated that the Gambro HCO 1100 dialyzer was the most efficient of seven tested. Model calculations suggested that it might remove 90% of FLC during 3 wk. This dialyzer then was evaluated in eight patients with myeloma and renal failure. Serum FLC reduced by 35 to 70% within 2 hr, but reduction rates slowed as extravascular re-equilibration occurred. FLC concentrations rebounded on successive days unless chemotherapy was effective. Five additional patients with acute renal failure that was caused by cast nephropathy then were treated aggressively, and three became dialysis independent. A total of 1.7 kg of FLC was removed from one patient during 6 wk. Extended hemodialysis with the Gambro HCO 1100 dialyzer allowed continuous, safe removal of FLC in large amounts. Proof of clinical value now will require larger studies.


Renal failure is a major cause of morbidity and mortality in patients with multiple myeloma. At initial presentation, up to 50% of patients have renal impairment and 12 to 20% have acute renal failure; 10% become dialysis dependent (1–4). They represent 2% of the dialysis population (5) and there are approximately 5000 new patients, worldwide, each year (6). The major renal lesion is cast formation (cast nephropathy) in the distal tubules, which evolves to interstitial fibrosis (7,8). In these patients, large amounts of free light chains (FLC) readily pass through the glomerular fenestrations and overwhelm the absorptive capacity of the proximal tubules. On entering the distal tubules, they co-precipitate with Tamm-Horsfall protein to form waxy casts that both block the flow of urine and cause interstitial inflammation (7,9,10).

Studies have analyzed renal recovery rates after FLC removal by plasma exchange in multiple myeloma. This is a logical approach, but results have been disappointing. Although an early report was optimistic (11), the largest and most recent controlled trial (97 patients) showed no clinical benefit (12). A subsequent editorial in the *Journal of the American Society of Nephrology* listed the shortcomings of this study, including the failure to monitor either serum or urine FLC concentrations (13). It was noted, “This resembles antihypertensive treatment without measuring BP.” Clearly, the efficiency of plasma exchange for serum FLC removal could not be judged.

Winearls (14), in 1995, considered that plasma exchange was unlikely to remove sufficient FLC for clinical benefit. Because FLC are relatively small protein molecules (κ 25 kD and λ 50 kD), they are present in similar concentrations in serum, the extravascular compartment, and tissue edema fluid (15). Thus, the intravascular compartment may contain only 15 to 20% of the total amount. A series of 3.5-L plasma exchanges that removed only 65% of intravascular FLC on each occasion might have little overall impact, particularly if production were not reduced at the same time by chemotherapy.

An alternative approach is to remove FLC by hemodialysis (16). Although this is not possible with routine dialyzers (because of their small pores), a new generation of protein-leaking dialyzers, with very large pores, could be useful (17). By using
extended dialysis, large amounts of FLC might be removed without the attendant clotting and deproteination problems that limit the extended use of plasma exchange. The aims of this study were to (1) make *in vitro* and *in vivo* assessments of several protein-leaking hemodialyzers, (2) develop a theoretical model for FLC removal, and (3) identify a clinical strategy for reduction of FLC in patients with multiple myeloma, with a view towards facilitating renal recovery.

**Materials and Methods**

This study was approved by the Solihull and South Birmingham Research Ethics Committees and the Research and Development Department of the University Hospitals Birmingham NHS Foundation Trust. All patients gave informed, oral and written consent.

**Study Design and Participants**

The study comprised (1) an initial *in vitro* and *in vivo* assessment of dialyzers for clearance of FLC, (2) development of a compartmental model for FLC removal on the basis of observed dialysis results, and (3) use of the model and the most efficient dialyzer to determine the optimal strategy for removal of FLC from patients with renal failure complicating multiple myeloma. The patients investigated were attending or referred to the nephrology department at the Queen Elizabeth Hospital.

**In Vitro Assessment of FLC Removal by Isolated Ultrafiltration**

Seven dialyzers were assessed for filtration efficiency (Table 1). Human serum, obtained from the Blood Transfusion Service, was spiked with 1000 mg of both monoclonal κ and λ FLC. Each dialyzer was placed in a simple circuit and primed with 1 L of normal saline. One liter of serum then was recirculated through the dialyzers at 400 ml/min, with transmembrane pressures of between 300 and 400 mmHg. The procedure was stopped when production of ultrafiltrate (UF) fluid ceased. The dialyzers finally were flushed with fresh saline to remove fluid that contained any residual protein. The quantities of FLC in the filtered serum, UF, and flushed fluid were calculated from the FLC concentrations and measured volumes. Serum FLC reductions were calculated by subtraction of the final FLC concentrations from the initial values. The percentage of the original 1000 mg of each FLC, present in the UF at the end of the experiment, was calculated to determine the ability of the membrane to filter FLC. These assessments were repeated three times for each dialyzer, and the mean values were determined.

**In Vitro Assessment of FLC Removal by Hemodialysis**

The two dialyzers that filtered most FLC, the Toray BK-F 2.1 (Toray Industries Inc, Tokyo, Japan) and the Gambro HCO (high cutoff) 1100 (Gambro Dialysatoren GmbH, Hechingen, Germany), were assessed *in vitro* for dialysis efficiency. Each dialyzer was connected to a Gambro AK 90 hemodialysis machine and primed with 1 L of normal saline. One liter of human serum that contained 1000 mg of both κ and λ FLC then was dialyzed for 4 h. Serum flow rates were set at 300 ml/min, dialysate flow rates were set at 500 ml/min, and transmembrane pressures were set at 0 to 10 mmHg. Ultrafiltration rates of 0.05 and 0.25 L/h were used arbitrarily for the Gambro HCO 1100 and the Toray BK-F, respectively. Serum volumes were maintained at 1 L by an infusion of normal saline. After 2 h, the serum was spiked with 24 ml of saline that contained an additional 1000 mg of both κ and λ FLC to assess dialyzer blockage. Serum and dialysate fluids were sampled at short intervals for the FLC measurements (12 to 17 samples for each experimental part). Clearance values for κ and λ were calculated as follows (18):  

\[
\text{Clearance (ml/min)} = \frac{\text{dialysate concentration of FLC} \times \text{dialysate flow rate}}{\text{inlet serum concentration of FLC}}
\]

Mean dialysate concentrations of FLC and clearance rates were calculated from both pre- and postspike samples, for both dialyzers and differences assessed.

**In Vivo Assessment of FLC Removal in Patients with Multiple Myeloma**

During the study period, 13 patients with dialysis-dependent renal failure (estimated GFR <15 ml/min per 1.73 m²) and multiple myeloma presented to the Nephrology Department. The first three patients underwent dialysis on one or more of the following dialyzers to determine their individual efficiency for FLC clearance: B. Braun Hi-PeS 18, (B. Braun Medical Ltd, Sheffield, UK) Toray BK-F 2.1, and Gambro HCO 1100. Subsequent patients underwent dialysis only on the Gambro HCO 1100 because of its superior FLC clearance rates (Tables 2 and 3). Patients 4 and 5 had routine dialysis for 4 h thrice weekly. Extended

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**Table 1. Efficiency of dialyzers for *in vitro* removal of FLC by isolated ultrafiltration**

<table>
<thead>
<tr>
<th>Class</th>
<th>Make</th>
<th>Model</th>
<th>Membrane Material</th>
<th>Surface Area (m²)</th>
<th>Molecular Cutoff in Blood (kD)</th>
<th>Mean Reduction in FLC (%)</th>
<th>Mean FLC Concentration in UF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>κ</td>
<td>λ</td>
<td>κ</td>
</tr>
<tr>
<td>High flux</td>
<td>B. Braun</td>
<td>Hi-PeS 18</td>
<td>PES</td>
<td>1.8</td>
<td>10</td>
<td>54</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Asahi</td>
<td>APS-1050</td>
<td>PS</td>
<td>2.1</td>
<td>10b</td>
<td>71</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Nikkiso</td>
<td>FLX 8GWS</td>
<td>PEPA</td>
<td>1.8</td>
<td>10b</td>
<td>68</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Idemsa</td>
<td>200 MHP</td>
<td>PES</td>
<td>2.0</td>
<td>10b</td>
<td>67</td>
<td>59</td>
</tr>
<tr>
<td>Super flux</td>
<td>Toray</td>
<td>BK-F 2.1</td>
<td>PMMA</td>
<td>2.1</td>
<td>20c</td>
<td>88</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Toray</td>
<td>BG 2.1</td>
<td>PMMA</td>
<td>2.1</td>
<td>20c</td>
<td>71</td>
<td>41</td>
</tr>
<tr>
<td>High cutoff</td>
<td>Gambro</td>
<td>HCO 1100</td>
<td>PAES</td>
<td>1.1</td>
<td>45c</td>
<td>76</td>
<td>94</td>
</tr>
</tbody>
</table>

FLC, free light chains; PAES, polyarylethersulfone; PEPA, polyester polymer alloy; PES, polyethersulfone; PMMA, polymethyl methacrylate; PS, polysulfone; UF, ultrafiltrate.

a Obtained from manufacturer.

b This is an approximate size because manufacturers’ data were not available.
hemodialysis with the Gambro HCO dialyzer was evaluated in patients 6 through 8. Daily extended hemodialysis on the Gambro HCO dialyzer was evaluated in patients 9 through 13, who presented with cast nephropathy.

Serum and dialysate concentrations of FLC were measured at short intervals during the dialysis sessions. Percentage serum reductions in FLC, mean dialysate concentrations (mg/L), dialysate FLC content per hour of dialysis (g/h), and clearance rates (ml/min) were calculated. These results were compared for each membrane for the first three patients.

**Evaluation of FLC removal by hemodialysis**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Myeloma Type</th>
<th>Presentation FLC Concentration (mg/L)</th>
<th>Chemotherapy Regimen</th>
<th>Renal Diagnosis</th>
<th>Adverse Events</th>
<th>Supportive Therapy</th>
<th>Clinical Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61</td>
<td>New IgA</td>
<td>17 000</td>
<td>CThal Dex</td>
<td>ARF, no biopsy</td>
<td>C. diff.</td>
<td>Nil</td>
<td>Renal recovery</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>Relapsing IgA</td>
<td>12 000</td>
<td>CThal Dex</td>
<td>ARF, cast nephropathy</td>
<td>Bone fractures</td>
<td>Nil</td>
<td>ESRF</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>New FLC k</td>
<td>69 000</td>
<td>CThal Dex</td>
<td>ARF, cast nephropathy</td>
<td>AL amyloidosis</td>
<td>Albumin</td>
<td>ESRF</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>New IgA</td>
<td>5 140</td>
<td>Cyc and Dex</td>
<td>ARF, no biopsy</td>
<td>Septicemia</td>
<td>Albumin</td>
<td>Died of MRSAspinaemia</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>New IgG</td>
<td>7 340</td>
<td>CThal Dex</td>
<td>ARF, no biopsy</td>
<td>Nil</td>
<td>Nil</td>
<td>Dialysis dependent</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>New FLC λ</td>
<td>15 900</td>
<td>CThal Dex</td>
<td>CKD, severe interstitial fibrosis</td>
<td>Nil</td>
<td>Albumin</td>
<td>ESRF</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>Relapsing FLC λ</td>
<td>7 950</td>
<td>Idarubicin, Dex, Cyc</td>
<td>ARF, no biopsy</td>
<td>C. diff. and septicemia</td>
<td>Albumin, NHlg</td>
<td>Died from neutropenic sepsis</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>New IgG</td>
<td>656</td>
<td>Dex</td>
<td>ARF, ATN</td>
<td>Nil</td>
<td>Nil</td>
<td>Renal recovery</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
<td>MGUS → IgGx</td>
<td>10 300</td>
<td>Thal Dex</td>
<td>ARF, cast nephropathy</td>
<td>Nil</td>
<td>Nil</td>
<td>Albumin</td>
</tr>
<tr>
<td>10</td>
<td>51</td>
<td>New IgA</td>
<td>42 000</td>
<td>VAD</td>
<td>ARF, cast nephropathy</td>
<td>Nil</td>
<td>Nil</td>
<td>Albumin, NHlg, prophylactic antibiotics, GCSF</td>
</tr>
<tr>
<td>11</td>
<td>61</td>
<td>New IgA</td>
<td>13 500</td>
<td>Thal Dex</td>
<td>ARF, cast nephropathy</td>
<td>Nil</td>
<td>Albumin, NHlg</td>
<td>Renal recovery eGFR at 4 mo = 29</td>
</tr>
<tr>
<td>12</td>
<td>68</td>
<td>New IgA</td>
<td>1 220</td>
<td>Thal Dex</td>
<td>ARF, cast nephropathy</td>
<td>C. diff, lobar pneumonia and ACS</td>
<td>Albumin, NHlg</td>
<td>Ongoing treatment</td>
</tr>
<tr>
<td>13</td>
<td>81</td>
<td>New IgA</td>
<td>1 10</td>
<td>Dex, Cyc</td>
<td>ARF, cast nephropathy</td>
<td>Nil</td>
<td>Albumin, NHlg</td>
<td>Renal recovery eGFR at 3 mo = 36</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndrome; ARF, acute renal failure; ATN, acute tubular necrosis; C. diff., *Clostridium difficile*; CKD, chronic kidney disease; CThalDex, cyclophosphamide, thalidomide, and dexamethasone; Thal Dex, thalidomide and dexamethasone; Cyc, cyclophosphamide; Dex, dexamethasone; ESRF, end-stage renal failure (eGFR <10 ml/min per 1.73 m²); eGFR, estimated GFR (by Cockroft-Gault equation in ml/min per 1.73 m²); GCSF, granulocyte colony-stimulating factor; MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance evolved to MM; MRSAspinaemia, methicillin-resistant *Staphylococcus aureus*; NHlg, normal human Ig; VAD, vincristine, Adriamycin (doxorubicin), dexamethasone.

### Laboratory Measurements of FLC

Serum and dialysate κ and λ FLC concentrations were measured by nephelometry, on a Dade-Behring BNII Analyser, using a particle-enhanced, high-specificity, homogeneous immunoassay (FREELITE; The Binding Site, Birmingham, UK) (19). Normal serum reference ranges used were 7.3 mg/L (range 3.3 to 19.4) for κ and 12.7 mg/L (range 5.7 to 26.3) for λ with an assay sensitivity of <1 mg/L (20).

### Mathematical Model of FLC Removal in Patients with Multiple Myeloma

A two-compartment mathematical model of FLC production, distribution, and removal in multiple myeloma was constructed to compare the efficiencies of plasma exchange and hemodialysis (Figure 1) (21). This was similar in structure to models for dialysis removal of urea and β2-microglobulin (22,23). It consisted of intravascular and extravascu-
Free light chain (FLC) compartmental model. Parameters were as follows: $P(t)$, FLC production rate (23 mg/min); $k_w$, elimination rate as a result of renal function (0 mg/min); $k_{ir}$, elimination rate as a result of dialysis ($1.5 \times 10^{-2}/\text{min}$); $k_{12}$, rate constant of FLC flow between intra- and extravascular compartments ($2.15 \times 10^{-2}/\text{min}$); $k_{21}$, rate constant of FLC flow between extravascular and intravascular compartments ($4.3 \times 10^{-3}/\text{min}$); $k_{10}$, elimination rate as a result of the reticuloendothelial metabolism ($1.6 \times 10^{-4}/\text{min}$). Intravascular compartment volume 2.5 L; extravascular compartment volume 12 L.

Table 3. Summary of FLC removal by hemodialysis in patients with MM\textsuperscript{a}

<table>
<thead>
<tr>
<th>Patient</th>
<th>FLC</th>
<th>Dialyzer Make</th>
<th>No. of Dialysis Sessions (Dialysers)\textsuperscript{d}</th>
<th>Mean (Range) Length of Dialysis Sessions (h)</th>
<th>Mean Predialysis Serum Concentration (mg/L)</th>
<th>Mean % Reduction in Serum Concentrations</th>
<th>Mean (Range) Dialysate Concentration (mg/L)</th>
<th>Mean Dialysate Content (mg/h)</th>
<th>Mean Clearances Rates (ml/min)</th>
<th>Sustained % Reduction Achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>λ</td>
<td>Toray BK-F 2.1</td>
<td>7</td>
<td>3.6 (2 to 4)</td>
<td>11,580</td>
<td>3.2</td>
<td>6.9 (0.8 to 20.3)</td>
<td>200</td>
<td>0.29</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>λ</td>
<td>B. Braun Hi-Pes 18</td>
<td>2</td>
<td>3.75 (3.5 to 4)</td>
<td>1795</td>
<td>5.6\textsuperscript{b}</td>
<td>5.3 (2.7 to 9.5)</td>
<td>169\textsuperscript{b}</td>
<td>1.5\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toray BK-F 2.1</td>
<td>3</td>
<td>4</td>
<td>2990</td>
<td>24.2\textsuperscript{b}</td>
<td>2\textsuperscript{a} (0.5 to 3.5)</td>
<td>60\textsuperscript{b}</td>
<td>0.5\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gambro HCO 1100</td>
<td>2</td>
<td>4</td>
<td>9155</td>
<td>58.5</td>
<td>265.6 (88 to 648)</td>
<td>7800</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>κ</td>
<td>Toray BK-F 2.1</td>
<td>4</td>
<td>3.6 (3 to 4)</td>
<td>8002</td>
<td>22.5\textsuperscript{b}</td>
<td>11.1\textsuperscript{b} (6 to 30.4)</td>
<td>720\textsuperscript{b}</td>
<td>1.6\textsuperscript{b}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gambro HCO 1100</td>
<td>6</td>
<td>4</td>
<td>2880</td>
<td>44.5</td>
<td>163 (120 to 219)</td>
<td>4900</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>λ</td>
<td>Gambro HCO 1100</td>
<td>6</td>
<td>2.9 (2 to 4)</td>
<td>3361</td>
<td>23.6</td>
<td>101 (36 to 241)</td>
<td>3200</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>κ</td>
<td>Gambro HCO 1100</td>
<td>3</td>
<td>3.3 (2 to 4)</td>
<td>536</td>
<td>57.9</td>
<td>7.1 (4.2 to 9.9)</td>
<td>200</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>λ</td>
<td>Gambro HCO 1100</td>
<td>10</td>
<td>4.6 (4 to 6)</td>
<td>10,548</td>
<td>58.9</td>
<td>219 (65 to 843)</td>
<td>6600</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>λ</td>
<td>Gambro HCO 1100</td>
<td>11</td>
<td>6.9 (2 to 11)</td>
<td>4651</td>
<td>57.8</td>
<td>137 (28.6 to 411)</td>
<td>2600</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>λ</td>
<td>Gambro HCO 1100</td>
<td>3</td>
<td>10.7 (10 to 12)</td>
<td>494</td>
<td>53.8</td>
<td>18 (8 to 37.3)</td>
<td>300</td>
<td>15.9</td>
<td></td>
</tr>
</tbody>
</table>

Extended daily hemodialysis for cast nephropathy

9 \kappa Gambro HCO 1100 13 | 4.8 (2 to 8) | 445 | 45 | 18.1 (1.6 to 56) | 370 | 17.1 | 95\textsuperscript{c} |

10 \kappa Gambro HCO 1100 (1) 12 | 7.25 (1 to 10) | 22,408 | 36 | 439 (15 to 1610) | 9700 | 9.2 | 50 |

(2) 6 | 6.5 (6 to 8) | 17,610 | 57 | 514 (187 to 1370) | 15,700 | 25.6 |

(3) 1 | 8 | 18,800 | 75 | 515 (151 to 1810) | 11,600 | 31.5 |

11 \kappa Gambro HCO 1100 (1) 2 | 6 (6 to 12) | 12,850 | 35.1 | 307 (200 to 414) | 5600 | 11.6 | 85\textsuperscript{c} |

(2) 20 | 6.3 (3 to 10) | 6887 | 81 | 193 (53 to 409) | 5700 | 25.5 |

12 \kappa Gambro HCO 1100 (1) 2 | 9 (6 to 12) | 1004 | 66.4 | 34 (21 to 47) | 600 | 28.5 |

(2) 46 | 6.3 (4 to 10) | 1157 | 80.4 | 46 (21 to 91) | 1300 | 42.9 |

13 \kappa Gambro HCO 1100 (1) 2 | 5 (4 to 6) | 1357 | 38 | 28.9 (9 to 48) | 800 | 15.5 | 80\textsuperscript{c} |

(2) 12 | 6.3 (6 to 10) | 397 | 74 | 137 (6 to 26) | 250 | 8 |

\textsuperscript{a}NA, not applicable.

\textsuperscript{b}Significantly less than the Gambro HCO 1100 result for this patient ($P < 0.02$).

\textsuperscript{c}Percentage of FLC reduction achieved when patients became dialysis independent.

\textsuperscript{d}Number of dialysers in series.

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In Vitro Assessment of FLC Removal by Hemodialysis

The results for FLC removal by \textit{in vitro} hemodialysis using the Toray BK-F 2.1 and the Gambro HCO 1100 dialyzers are shown in Table 5. Significantly higher FLC dialysate concentrations and greater serum reductions were achieved using the Gambro HCO dialyzer. Clearance rates of both FLC were 60-

Serum FLC removal. Simulations then were conducted to compare six and 10 plasma exchange treatments (over 12 d) with five different hemodialysis protocols. Chemotherapeutic tumor killing rates of 0, 2, 5, and 10% per day and 100% on the first day were used (Table 4).

Statistical Analyses

\textit{In vitro} and \textit{in vivo} studies of FLC removal by hemodialysis were compared using \textit{t} test (two tailed, type 2) for significant differences, $P < 0.05$ was considered statistically significant.

Results

In Vitro Assessment of FLC Removal by Isolated Ultrafiltration

The efficiencies of the various dialyzers for removal of FLC are shown in Table 1. All dialyzers caused substantial reductions of FLC concentrations in the circulated serum. Varying amounts of FLC were identified in UF, and it was assumed that the amounts missing were bound to the membranes. The Gambro HCO 1100 was the most efficient dialyzer; only small amounts of FLC bound to the membranes.

In Vitro Assessment of FLC Removal by Hemodialysis

Data from a patient with multiple myeloma were analyzed using the model within the software package Facsimile (25) to generate rates of
fold higher using the Gambro dialyzer compared with the Toray dialyzer.

In Vivo Use of Dialyzers for FLC Removal in Patients with Multiple Myeloma

The clinical details of patients who were studied for FLC removal are summarized in Table 2. All were in dialysis-dependent renal failure. FLC removal by hemodialysis was evaluated for three different dialyzers in the first three patients. Details of the dialysis periods and the amounts of FLC removed are shown in Table 3. For example, in patient 2, use of the Gambro HCO 1100 resulted in greater reductions in serum FLC concentrations (58.5%) than either the B. Braun Hi-Pes 18 (5.6%; \(P < 0.002\)) or the Toray BK-F 2.1 (24.2%; \(P < 0.001\)). The mean dialysate concentrations of FLC were many times higher during the dialysis sessions using the Gambro HCO 1100 (266 versus 5 mg/L using the B. Braun Hi-Pes 18 \(P < 0.02\) and 2 mg/L using the Toray BK-F 2.1 \(P < 0.004\)). Later patients (4 through 13) were treated only with the Gambro HCO 1100 dialyzer.

Evaluation of FLC Removal by Extended Hemodialysis on the Gambro HCO 1100

Extended hemodialysis (>4 h) on the Gambro HCO 1100 was evaluated for FLC removal in patients 6 through 13 (Table 3). The procedure was well tolerated with no cardiovascular complications. During sessions, there was a mean serum albumin reduction of 3.9 g/L \(P < 0.03\) that was replaced routinely with 20% albumin solution. Calcium and magnesium were replaced as required. Measurements indicated that there was no IgG leakage into the dialysate fluid.

The amounts of FLC in the dialysate fluids correlated with predialysis serum concentrations \(R = 0.74, P < 0.0001\). Figure 2 shows serum and dialysate FLC concentrations during a 6-h session for patient 6. When the dialyzer was replaced, there was a transient increase in FLC removal. Figures 3 through 5 show the daily pre- and postdialysis serum FLC concentrations and the amounts of FLC removed in the dialysate fluid (per 10-d periods) for patients 9 through 11, together with details of chemotherapy.

There was a significant correlation between percentage serum FLC reduction and the time on hemodialysis for all patients \(R = 0.53, P < 0.001\). Under similar conditions, mean clearance rates of FLC varied little between patients and correlated with dialysate flow rates \(R = 0.58, P < 0.0001\). The clearance was 10.8 ml/min at flow rates of 300 ml/min (range 5.2 to 22.6) and 19.3 ml/min (range 7.2 to 39.8) at 500 ml/min. Dialyzer surface area also was related to FLC clearance rates. For example, patient 10 was dialyzed on separate occasions on the Toray dialyzer.

### Table 4. Model calculations of the efficiency of therapeutic removal of FLC

<table>
<thead>
<tr>
<th>Method of FLC Removal</th>
<th>Percentage of FLC Removed by Intervention (and Time (D), to Reduce from 10 to 0.5 g/L) with Different Chemotherapeutic Tumor Killing Rates (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100% NA (14) (^1), 10% NA (30) (^2), 5% NA (52), 2% NA (121), 0% NA (10 g/L) (^b) (^h)</td>
</tr>
<tr>
<td>PE (\times 6) in 10 d</td>
<td>29 (10) 24 (29) (^3), 17 (52), 9 (121), 3 (10 g/L) (^b) (^h)</td>
</tr>
<tr>
<td>PE (\times 10) in 10 d</td>
<td>40 (8) 34 (29), 25 (52), 13 (121), 4 (10 g/L) (^b) (^h)</td>
</tr>
<tr>
<td>HD 4 h (\times 3)/wk</td>
<td>60 (7) 54 (19) (^4), 53 (31), 51 (73), 50 (3.6 g/L) (^b)</td>
</tr>
<tr>
<td>HD 4 h/d</td>
<td>76 (4) 73 (13) (^5), 72 (23), 71 (55), 70 (1.9 g/L) (^b) (^7)</td>
</tr>
<tr>
<td>HD 8 h alternate days</td>
<td>79 (4) 73 (13), 72 (19), 70 (47), 69 (1.5 g/L) (^b) (^7)</td>
</tr>
<tr>
<td>HD 8 h/d</td>
<td>87 (3) 85 (7), 84 (14), 83 (29), 82 (1.0 g/L) (^h)</td>
</tr>
<tr>
<td>HD 12 h/d</td>
<td>91 (2) 89 (5) (^8), 89 (8), 88 (16), 88 (0.7 g/L) (^b)</td>
</tr>
<tr>
<td>HD 18 h/d</td>
<td>93 (2) 93 (3), 93 (4), 92 (8), 91 (0.6 g/L) (^b)</td>
</tr>
</tbody>
</table>

\(^a\)Numbers are the additional percentage of FLC removed by intervention compared with normal metabolism. Numbers in parentheses are the time in days for FLC concentrations to reduce by 95% (from 10 to 0.5 g/L). For superscript numbers 1 through 8, the simulations are shown in Figure 7. HD, hemodialysis; PE, plasma exchange.

\(^b\)Serum FLC concentrations at day 150 for simulations in which reductions to 0.5 g/L did not occur.

\(^c\)Percentage tumor kill rates per day.

### Table 5. Efficiency of dialyzers for in vitro removal of FLC during 4 h of hemodialysis

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Test samples</th>
<th>Prespike (k)</th>
<th>Prespike (\lambda)</th>
<th>Postspike (k)</th>
<th>Postspike (\lambda)</th>
<th>Mean Values (k)</th>
<th>Mean Values (\lambda)</th>
<th>Clearance Rates (ml/min) (k)</th>
<th>Clearance Rates (ml/min) (\lambda)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toray BK-F 2.1</td>
<td>Serum % removed</td>
<td>77.9</td>
<td>75</td>
<td>88.8</td>
<td>84.3</td>
<td>81.5</td>
<td>78</td>
<td>0.59 (0.02 to 1.6)</td>
<td>0.47 (0.02 to 1.4)</td>
</tr>
<tr>
<td></td>
<td>Dialysate concentration (^b)</td>
<td>0.63</td>
<td>0.87</td>
<td>2</td>
<td>2.2</td>
<td>1.3</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gambro HCO 1100</td>
<td>Serum % removed</td>
<td>95</td>
<td>93</td>
<td>96</td>
<td>95</td>
<td>95.5</td>
<td>94</td>
<td>35.1 (^1) (7.5 to 50.9)</td>
<td>32.2 (^2) (19.1 to 45.9)</td>
</tr>
<tr>
<td></td>
<td>Dialysate concentration (^b)</td>
<td>50</td>
<td>88</td>
<td>75</td>
<td>93</td>
<td>62.5</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Mean results were determined for pre- and postspike data.

\(^b\)FLC concentrations in dialysate fluid in mg/L.

\(^c\)Gambro dialyzer significantly more efficient \((P < 0.02)\).
one, two, or three dialyzers, in series, with progressive increases in FLC clearance rates (Table 3 and Figure 6). The albumin loss in the dialysate increased significantly with each additional dialyzer (one: 0.16 g/L; two: 0.44 g/L; three: 0.58 g/L). In this patient, measurement of dialysate κ FLC concentrations during a 6-wk period indicated removal of 1.7 kg. Daily measurements of removal by hemodialysis and urine excretion plus estimated internal metabolism indicated a production rate of 150 to 200 g/d.

Therapeutic Extended Daily Hemodialysis on the Gambro HCO 1100 for Patients with Cast Nephropathy

During the study period, five unselected new patients presented with multiple myeloma and cast nephropathy (patients 9 through 13). All were dialysis dependent and were given dexamethasone-based induction chemotherapy. They were treated with an extended dialysis schedule of between 13 and 48 dialysis sessions, ranging from 2 to 12 h. Patients initially underwent dialysis on one dialyzer, for one or two sessions, and then two dialyzers in series. In the first week, they underwent dialysis on a daily basis and subsequently on alternate
days. In all patients, extended hemodialysis resulted in large serum FLC reductions that were accounted for in the dialysate fluids (Table 3).

Three of the five patients became dialysis independent. Two patients (10 and 12) developed infections that prevented further use of chemotherapy. Although dialysis removed significant quantities of FLC, concentrations rebounded within 1 to 2 days and patients remained dialysis dependent. By contrast, the three patients who became dialysis independent (9, 11, and 13) responded well to chemotherapy, as evidenced by long-term reductions in serum FLC concentrations (Figures 3 and 5).

Simulation Model for FLC Removal

The results of the simulation studies are shown in Table 4 and Figure 7. With complete tumor killing on day 1 (simulation 1), serum FLC were >500 mg/L for 2 wk (assuming no therapeutic FLC removal). With a chemotherapeutic tumor kill rate of 10% per day and no dialysis, serum FLC concentrations remained >500 mg/L on day 30 (simulation 2). Plasma exchange (simulation 3) was less effective in reducing serum FLC than hemodialysis for 4 h three times per week using the Gambro HCO 1100 dialyzer (simulation 4), and neither method was rapid. Extended daily dialysis (for 12 h) reduced FLC concentrations to 5% of the starting concentrations in 5 d (simulation 6) compared with 29 d for plasma exchange (simulation 3). Analysis of the FLC load on the kidneys over 3 wk (area under the curves) showed that for simulation 3, 76% remained using plasma exchange and 11% remained after 5 d of 12 h/d hemodialysis (simulation 6), a 6.5-fold reduction. When chemotherapeutic killing rates were <10% per day, plasma exchange became progressively less effective than extended hemodialysis (Table 4). Ineffective chemotherapy, even with extended dialysis, did not normalize serum FLC concentrations (simulation 7).

Discussion

The first aim of the study was to determine whether FLC could be cleared effectively by hemodialysis. Results from the initial, in vitro, ultrafiltration experiments suggested that several different dialyzers might be useful. For dialyzers with up to a 20-kD cutoff, protein recovery data indicated that membrane binding was the main clearance mechanism (Table 1). Subsequent in vitro and in vivo hemodialysis results demonstrated that the Gambro HCO 1100 dialyzer, with cutoff of 45 kD, was much more efficient than all others. Typically, serum FLC clearance rates of 10 to 40 ml/min were achieved, although filtration of both \(\kappa\) and \(\lambda\) molecules slowed with time. When dialyzers were replaced, FLC clearance increased slightly (Figure 2).

The amounts of serum FLC that were removed by hemodialysis were influenced by the initial serum FLC concentrations, time periods of dialysis, dialysis flow rates, and dialyzer surface area. The largest amounts removed were from patient 10, who had 42 g/L of serum \(\kappa\) FLC at clinical presentation. During a 6-week period, comprising 18 sessions of up to 10 h each, >1.7 kg of FLC was removed. For later dialysis sessions on this patient, two Gambro HCO 1100 dialyzers were connected in series. This added a convective element in addition to increas-
ing the surface area, and the resulting FLC removal more than doubled. This occurred not only in the initial hour as the blood pool was reduced but also during the following hours, when the extravascular reservoir was partially cleared. After 4 to 5 h, serum FLC reductions slowed as the tumor production rate gradually was approached. As an alternative and perhaps more practical option, a single 2-m² dialyzer could be used.

Although these studies did not assess specifically serum FLC removal by ultrafiltration, it probably would be effective. Figure 6 suggests that maximum clearance rates from the extravascular compartment were being approached with the use of two membranes in series, and there would have been a convective element. Further minor increases in FLC removal rates could be achieved by adjusting the blood or dialysis fluid flow rates. An additional factor that would cause variations in clearance rates between patients would be the degree of FLC polymerization, but this was not assessed (26).

Overall, the extended dialysis was well tolerated with no adverse effects. Previous studies showed the safe use of the Gambro HCO 1100 dialyzer in an intensive care setting (27,28). As predicted, we noted substantial albumin loss that required replacement on a regular basis (20 to 40 g per dialysis session and given as 20% human albumin solution). Such leakage is inevitable with a dialyzer that has a molecular cutoff of similar size to albumin (65 kD). Its use was not associated with hemodynamic or other adverse effects. Prophylactic antibiotics were given before invasive procedures and normal human Ig were used when serum IgG concentrations were <5 g/L. Patients with multiple myeloma usually are immunocompromised, so prevention of infections was important. Overall, our findings indicated that the Gambro HCO 1100 dialyzer was effective and safe when used for removal of huge amounts of monoclonal FLC.

The second aim of the study was to develop a theoretical model of FLC clearance for understanding of various treatment strategies. Using known variables for the model and patient data, we were able, on an iterative basis, to model FLC removal in vivo. This allowed calculation of possible FLC production rates, rates of movement between the extra- and intravascular compartments, and the effectiveness of hemodialysis to be compared with plasma exchange. When the model was interrogated for different treatment strategies, simulations indicated that 4 h of dialysis on alternate days (using the Gambro HCO 1100) compared favorably with recommended plasma exchange protocols (Figure 7 and Table 4) (12). The model indicated that 8 to 12 h of daily dialysis would reduce FLC to low serum concentrations within a few days, provided that chemotherapy was successful. We used a range of tumor killing rates in the model to include several clinical possibilities (Table 4). The 10 and 0% killing rates produced results that were in accordance with observed FLC responses for patient 9 (Figures 3 and 7) and for patient 10, respectively (Figures 4 and 7). There are no published reports of tumor killing rates for comparison, because multiple FLC measurements have not been made at this early stage of treatment. With less efficient tumor killing, the continuing FLC production rendered hemodialysis progressively more effective than plasma exchange (Table 4). More extensive plasma exchange regimens could have been evaluated, but five to seven procedures of 50 ml/kg over 10 to 12 d normally is recommended (12).

The accuracy and the reproducibility of the model requires further assessment, which is ongoing. However, it will always be a challenge to model renal FLC metabolism because it changes during renal recovery. Overall, it seemed that the model allowed reasonable comparison of different treatment regimens.

The third aim of the study was to identify a clinical strategy for reducing serum concentrations of FLC in multiple myeloma. Five consecutive patients with dialysis-dependent acute renal failure and biopsy-proven cast nephropathy were treated with extended daily hemodialysis, and three became dialysis independent. This compares with published figures of 15 to 20% in patients with biopsy-proven cast nephropathy (14,29). In these three patients, a reduction in serum FLC concentrations of 80 to 95% was associated with recovery of renal function. However, the toxicity of individual monoclonal FLC, the extent of underlying renal damage, and other clinical factors vary enormously, so more or less FLC removal may be appropriate in other patients. It is of note that the plasma exchange procedures that were assessed in the model (Table 4) and used in clinical practice (30) did not reduce serum FLC concentrations by even 30%.

The effectiveness of chemotherapy when treating these patients was of considerable importance. For example, in patient 9 (Figure 3), serum FLC reduced toward normal concentrations within 3 wk. Chemotherapy was effective, large amounts of FLC were removed, and renal function recovered. During the second course of dexamethasone, FLC concentrations reduced between dialysis periods. This probably was due to their metabolism and excretion by the kidneys and indicated recovering function. In patient 10 (Figure 4), chemotherapy was ineffective and then had to be stopped because of infections. Serum FLC concentrations were reduced temporarily by dialysis but rebounded within 1 to 2 d, and there was no renal recovery (Figure 4). It will be important to identify fast-acting and effective drug regimens that can be modified rapidly if FLC concentrations do not fall quickly. Combinations of bortezomib, doxorubicin, and dexamethasone or of cyclophosphamide, thalidomide, and dexamethasone are highly successful and have better response rates than vincristine, Adriamycin (doxorubicin), and dexamethasone (31).

It is possible that removal of FLC by hemodialysis can protect the kidneys from continuing damage for several weeks. Occasional reports have described late renal function recovery from cast nephropathy. For instance, two patients became dialysis independent after autologous bone marrow transplantation that was many months after their initial clinical presentation with acute renal failure (32). Serum FLC measurements were not reported, but we suggest that the use of high-dosage melphalan had stopped monoclonal FLC production. For renal recovery, however, effective tumor treatment to reduce FLC production is essential, in addition to any removal by hemodialysis. We have not removed FLC from patients who had less
severe renal failure and did not require dialysis. Such patients also might benefit from this treatment.

For all patients, daily monitoring with serum FLC tests was helpful. The results made it possible to judge the ongoing effectiveness of the dialyzers and the chemotherapy. Such daily assessments are different from the typical management pace in myeloma. Treatment outcomes normally are assessed over weeks or months, largely from observations of the slow changes that are seen in serum IgG concentrations (half-life of 3 wk). FLC have serum half-lives from 2 to 3 h (2 to 3 d in renal failure), so clinical responses can be seen and acted on much more quickly (33,34).

Our results allow some interpretation of the plasma exchange study by Clark et al. (12), referred to earlier. Although there are no published results of serum FLC concentrations in relation to plasma exchange, a report in press (30) confirms model simulations that only 25 to 30% of the total amount typically is removed during a treatment period (Figure 7 and Table 4). Therefore, switching off FLC production by chemotherapy may have been the main determinant of renal recovery. Fewer than 40% of patients would have had a very good response to vincristine, Adriamycin (doxorubicin), and dexamethasone during the first few weeks of treatment (31). Their observed renal recovery rates of approximately 40% (in both plasma exchange and control groups) may reflect only such chemotherapy responses. Other causes of renal failure, such as acute tubular necrosis (as seen in one of our patients), also would be present, but renal biopsies were not performed. Without histologic clarification and frequent measurements of serum FLC, interpretation of trials that assess renal recovery in patients with myeloma kidney will prove difficult (13).

Conclusion

Our studies have demonstrated that daily, extended hemodialysis using the Gambro HCO 1100 dialyzer could remove continuously large quantities of FLC. Modeling and clinical data suggested that this was more effective than plasma exchange procedures. This is supported by early evidence of clinical efficacy, as judged by satisfactory renal recovery in three of five patients with cast nephropathy. Studies in more patients now are required with consideration given to optimal chemotherapy and infection control. It then might be appropriate to undertake controlled trials of hemodialysis using the Gambro HCO dialyzers to determine overall clinical utility.

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Disclosures

None.

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