A Prospective Study of Pyrogenic Reactions in Hemodialysis Patients Using Bicarbonate Dialysis Fluids Filtered to Remove Bacteria and Endotoxin

David A. Pegues, Carl W. Oettinger, Lee A. Bland, Jamie C. Oliver, Matthew J. Arduino, Sonia M. Aguero, Sigrid K. McAllister, Steven M. Gordon, Martin S. Favero, and William R. Jarvis


C.W. Oettinger, Emory University School of Medicine, Renal Division, Department of Medicine, Atlanta, GA

J.C. Oliver, Dialysis Clinic Inc., Atlanta, GA

S.M. Gordon, Emory University School of Medicine, Infectious Disease Division, Atlanta, GA


ABSTRACT

Pyrogenic reactions (PR) are a well-recognized complication of hemodialysis and have been associated with dialyzer reuse, high-flux dialysis, and bicarbonate dialysate. However, the roles of bacteria and endotoxin in dialysate for producing PR are not well defined. To determine the effect of removing most bacteria and endotoxin from the dialysate on the incidence of PR, a cohort of chronic hemodialysis patients receiving high-flux, high-efficiency, or conventional hemodialysis at three centers with bicarbonate dialysis fluids that had been filtered with a polysulfone high-flux hemodialyzer was prospectively studied. Unfiltered bicarbonate concentrate had median bacterial and endotoxin concentrations of 479,000 CFU/mL and 39,800 pg/mL, respectively. After filtration of the bicarbonate concentrate at the central proportioner, dialysate had a median 9.2 CFU/mL of bacteria and 17.8 pg/mL of endotoxin. Dialysate filtered at individual proportioning dialysis machines had a median 0.001 CFU/mL of bacteria and 0.19 pg/mL of endotoxin. Nine PR were identified among 303 patients after 28,007 hemodialysis treatments (0.3 PR/1,000 treatments). The rate of PR was similar for the three hemodialysis treatment modalities and for first-use compared with reused dialyzers. Although the PR rate in this study was lower ($P = 0.046$) than the PR rate of a previous study with unfiltered dialysis fluids (0.7 PR/1,000 treatments), it represents a difference of only 10 PR in over 28,000 treatments. It was concluded that filtration of hemodialysis fluids is efficacious in removing bacterial and endotoxin contamination and can result in a lower incidence of PR in patients receiving high-flux, high-efficiency, or conventional hemodialysis.

Key Words: Hemodialysis, high-flux dialysis, pyrogenic reaction, bicarbonate dialysate, endotoxin, filtration

Pyrogenic reactions (PR), characterized by fever, rigors, and/or hypotension, may complicate hemodialysis treatments. Potential sources of PR include bacteria and endotoxin, which contaminate dialysate or product water, and decreased biocompatibility of certain dialyzer membranes (1-9). Some studies have suggested that PR may arise from endotoxin passing directly through the more permeable membranes of high-flux dialyzers (10-12). Other studies, however, have shown that intact dialyzer membranes are impermeable to endotoxin (13-16). It also has been hypothesized that transmembrane stimulation of circulating mononuclear cells by dialysate endotoxin could produce PR by the liberation of endogenous cytokines (16).

In a previous study of PR at three dialysis centers, we found that the incidence of PR after 26,877 hemodialysis treatments was low (0.7 PR/1,000 treatments) despite high bacterial and endotoxin concentrations in the bicarbonate dialysate (mean, 19,000 CFU/mL of bacteria and 380 pg/mL of endotoxin) (17). In addition, the PR rate did not differ significantly by treatment modality (conventional, high-efficiency, or high-flux hemodialysis). Although epidemiologic investigations in the 1970s found that an increased risk of PR was associated with increased dialysate bacterial counts (1), our prospective study
showed that if dialyzers were carefully reprocessed, the use of bicarbonate dialysate containing high bacterial and endotoxin concentrations did not result in a high PR rate, even in hemodialysis patients receiving high-flux hemodialysis (17).

Since that study was completed, the proportion of high-flux hemodialysis performed at the three centers has increased almost threefold. The three centers also have installed filters, either at the dialysate central proportioner or individual proportioning dialysis machines, to remove bacteria and endotoxin from the dialysate. Because of these changes, we prospectively studied a cohort of chronic hemodialysis patients receiving high-flux, high-efficiency, or conventional hemodialysis treatments at the three centers to compare the incidence of PR when essentially bacteria and endotoxin free dialysis fluids were used with the PR rates in our previous study.

METHODS

Study Population

The study population included all patients and all hemodialysis treatments performed between April 1, 1990, and February 24, 1991, at three outpatient chronic hemodialysis centers. The study protocol was approved by the Institutional Human Subject Review Board at the Centers for Disease Control (CDC) and the Human Investigations Committee of Emory University (Atlanta, GA). All patients were dialyzed with filtered dialysis fluids three times a week for 4 wk before the study was initiated.

Case Definition

A case of a PR was defined as the onset of objective chills (visible rigors) and/or fever (oral temperature \(\geq 37.8^\circ\text{C}\)) in a patient who was afebrile and who had no recorded signs or symptoms of infection before the dialysis treatment.

Case Ascertainment

Oral temperatures were obtained for all patients immediately before dialysis, 1 h into the dialysis session, and immediately after the dialysis treatment with an IVAC\textsuperscript{5} (Model 200; TempPlus, San Diego, CA) thermometer. Thermometers were calibrated before the study and retested monthly.

Observations of clinical signs and symptoms were recorded on the dialysis treatment record by the nursing staff. Data from each dialysis treatment record were entered daily into a central computer system. Dialysis treatment records for all patients with sus-

\textsuperscript{5} Use of trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

pected PR were reviewed by a physician to ensure that the patient had no apparent signs or symptoms of infection before the dialysis treatment.

Preparation of Dialysis Fluids

Bicarbonate dialysate was used exclusively for all dialysis treatments. The dialysate was manufactured from individual ingredients at a central location every 4 days by methods previously described (18). The water treatment system at each center contained a sand filter, water softener, carbon filter, and hollow-fiber reverse osmosis unit.

Dialysis fluids were filtered to remove bacteria and endotoxin by the placement of a hollow-fiber high-flux dialyzer (F-80; Fresenius AG, Bad Homburg, Germany) in the bicarbonate concentrate or dialysate delivery circuits by previously reported methods (19). Filters from the central proportioners were reprocessed nightly with an automated dialyzer reprocessing system (Renatron\textsuperscript{6}; Minntech, Minneapolis, MN) and were replaced after 30 uses. Filters from individual patient machines were removed and reprocessed with a Renatron\textsuperscript{6} each week and replaced each month.

Dialysis Monitors and Dialyzers

Conventional dialysis treatments were performed with hollow-fiber dialyzers containing cellulose-derived (CA-110; Baxter Healthcare, Deerfield, IL) or cellulose membranes (CF-23.08; Baxter Healthcare). High-efficiency and high-flux hemodialysis was defined as treatments with hollow-fiber dialyzers with ultrafiltration coefficients of 10 to 19 mL/h/mm Hg and \(\geq 20\) mL/h/mm Hg, respectively (CA-210, CT-190, Baxter Healthcare, or F-80 [Fresenius AG]), surface areas \(\geq 1.7\text{m}^2\), blood flow rates of 400 mL/min, and dialysate flow rates of 500 mL/min in conjunction with dialysis machines equipped with an ultrafiltration controller (Model 480; Drake-Willock, Portland, OR).

Method of Dialyzer Processing for Reuse

New dialyzers were processed once before patient use. After each patient use, the dialyzers were rinsed with processed water from the centers' water treatment system and reprocessed with a Renatron\textsuperscript{6} with a peracetic acid/hydrogen peroxide–based dialyzer disinfectant (Renalin\textsuperscript{7}; Minntech). After reprocessing, the dialyzers were removed from the machine, capped, and stored at room temperature until the next use (usually within 48 h). The reuse rate for a particular type of dialyzer was defined as the ratio of the total number of hemodialysis treatments with that type of dialyzer divided by the number of new dialyzers of that type used during the study period.

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Laboratory Methods

Hemodialysis fluids (bicarbonate concentrate, bicarbonate dialysate, and processed water) were collected weekly in sterile, endotoxin-free tubes from each study center for microbiologic and endotoxin analysis. Samples of bicarbonate concentrate and dialysate were collected at points located before and after the in-line filters. The membrane filtration technique was used for all microbiologic assays (20). Membrane filters were plated onto trypticase soy agar plates and incubated at 35 to 37°C for 48 h (21). Colonies were counted with the aid of a dissecting microscope (magnification, ×15).

Endotoxin concentrations were determined via a turbidimetric Limulus amebocyte lysate assay system (LAL-5000; Associates of Cape Cod, Woods Hole, MA) (22).

Renalin® concentrations were determined by a ferroin/ceric sulfate titration method (23).

Statistical Analysis

The rate of PR was compared by center, by treatment modality, and by dialyzer reuse status by the χ² test, Fisher exact test, or χ² test for trend, where appropriate. The rate of PR was compared with the rate in patients receiving hemodialysis with filtered dialysis fluids from our previous study by using χ² or Fisher exact test, where appropriate.

RESULTS

Descriptive Epidemiology

A total of 303 patients received 28,277 hemodialysis treatments during the study period; 54% were female, 94% were black, and the mean age was 57 yr (range, 24 to 84 yr). During the study, 215 (71%) received hemodialysis throughout the entire period, 54 (18%) began treatment, and 34 (11%) stopped treatment. A total of 270 (1%) dialysis treatments were excluded from the analysis because a patient’s temperature was not recorded (201) or the temperature was ≥37.8°C at the onset of dialysis.

Nine PR occurred among nine patients for an incidence of 0.3 PR/1,000 treatments. The most frequent signs and symptoms of PR were fever (89%), rigors (22%), hypotension (22%), and nausea/vomiting (11%). Seven (78%) patients with PR had fever only; all seven of these patients were febrile only at the end of the treatment. The median time between the start of hemodialysis treatment to the onset of PR was 140 min (range, 120 to 240 min).

There was no clustering of PR by treatment shift, week, or center (Figure 1). The rate of PR did not differ significantly between centers (Center A, 0.5 PR/1,000 treatments [5 of 9,947] versus Center B, 0.2 PR/1,000 [2 of 8,349] versus Center C, 0.2 PR/1,000 [2 of 9,711]; P = 0.45). Of the 28,007 dialysis treatments performed at the three centers, 19,040 (68%) were high efficiency, 6,518 (23%) were high flux, and 2,449 (9%) were conventional. The rate of PR by treatment modality was similar (high flux, 0.3 PR/1,000 treatments [2 of 6,518] versus high efficiency, 0.3 PR/1,000 [6 of 19,040] versus conventional, 0.4 PR/1,000 [1 of 2,449]; χ² test for trend, P = 0.85).

The overall mean reuse rate of hemodialyzers during the study period was 10.8 uses per dialyzer. Mean rates of reuse were similar for cellulose acetate (CA-210 [11.1 reuses; range, 1 to 32] and CA-110 [10.8 reuses; range, 1 to 26]), cellulose triacetate (CT-190) (10.7 reuses; range, 1 to 30), polysulfone (F-80) (10.0 reuses; range, 1 to 27), and conventional cellulose membranes (CF-23.08) (8.7 reuses; range, 1 to 28). Among centers, there was no significant difference in mean reuse rates by type of dialyzer.

The PR rate with first-use dialyzers was not significantly different than the PR rate for reused dialyzers (0.4 PR/1,000 treatments [1 of 2,638] versus 0.3 PR/1,000 [8 of 25,369]; P = 0.59). In addition, there was no significant increase in the rate of PR as the number of dialyzer reuses increased (<5 reuses versus ≥5 reuses, 0.4 PR/1,000 treatments [4 of 11,264] versus 0.3 PR/1,000 [5 of 16,743]; P = 1.0).

Because fever during dialysis identified 89% of the episodes of PR, we determined the frequency of temperature recordings for all dialysis treatments during the study period. Temperatures were recorded immediately before and after hemodialysis in 99.3 and 98.7% of treatments, respectively, and at 1 h into the session in 97.8%.

Microbial and Endotoxin Surveillance of Dialysis Fluids

Bacterial and endotoxin concentrations in dialysis fluids collected weekly (wk 1 through 20) or every
other week thereafter (wk 22 to 46) did not differ significantly between centers. Therefore, the results of testing were combined. The median bacterial concentration in processed water was 58 CFU/mL (range, 2 to 341 CFU/mL), and the median endotoxin concentration was 28 pg/mL (range, 4 to 157 pg/mL) (Table 1).

**Filtration of Dialysis Fluids**

High concentrations of bacteria and endotoxin were present in the unfiltered bicarbonate concentrate and dialysate used at the three centers (Table 1). The median bacterial and endotoxin concentrations for the bicarbonate concentrate before filtration were 479,000 CFU/mL and 39,800 pg/mL, respectively. After filtration, median bacterial and endotoxin concentrations were 0.4 CFU/mL and 155 pg/mL (Table 1). All samples of dialysate made from filtered bicarbonate concentrate contained very low concentrations of bacteria and endotoxin (median, 9 CFU/mL and 18 pg/mL, respectively). Filtration of bicarbonate dialysate at the individual proportioning dialysis machines reduced the median bacterial and endotoxin concentrations in the dialysate from 30,200 to 0.001 CFU/mL and from 1,700 to 0.2 pg/mL, respectively (Table 1).

The most prevalent bacteria isolated from dialysate (both unfiltered and filtered) were *Pseudomonas* and *Xanthomonas* species. The predominant microorganisms isolated in the processed water were *Alcaligenes*, *Flavobacterium*, *Methylobacterium*, and *Pseudomonas* species.

### TABLE 1. Median microbial and endotoxin concentrations of unfiltered and filtered hemodialysis fluids

<table>
<thead>
<tr>
<th>Fluid Specimen</th>
<th>Bacteria (CFU/mL)</th>
<th>Endotoxin (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse osmosis water</td>
<td>58</td>
<td>28</td>
</tr>
<tr>
<td>(N = 82)</td>
<td>(2–341)</td>
<td>(4–157)</td>
</tr>
<tr>
<td>Bicarbonate Concentrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfiltered (N = 83)</td>
<td>479,000</td>
<td>39,800</td>
</tr>
<tr>
<td>(9,440–2,360,000)</td>
<td>(8,630–227,000)</td>
<td></td>
</tr>
<tr>
<td>Filtered (N = 83)</td>
<td>0.4</td>
<td>155</td>
</tr>
<tr>
<td>(0.0–19)</td>
<td>(8–1,720)</td>
<td></td>
</tr>
<tr>
<td>Bicarbonate Dialysate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfiltered (N = 55)</td>
<td>30,200</td>
<td>1,700</td>
</tr>
<tr>
<td>(556–60,000)</td>
<td>(426–10,500)</td>
<td></td>
</tr>
<tr>
<td>Filtered at central</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>proportioner (N = 110)</td>
<td>(4–433)</td>
<td>(4–57)</td>
</tr>
<tr>
<td>Filtered at dialysis</td>
<td>0.001</td>
<td>0.2</td>
</tr>
<tr>
<td>machine (N = 55)</td>
<td>(0.0–0.6)</td>
<td>(0.0–3)</td>
</tr>
</tbody>
</table>

The concentration of Renalin® used during a reprocessing cycle was determined for each of the automated reprocessing machines at the three centers; the mean concentration was 3.9% (range, 3.4 to 4.4).

We also compared the incidence of PR in patients receiving hemodialysis with filtered (this study) versus unfiltered dialysis fluids (previous study) for each of the three treatment modalities (Table 2). The overall incidence of PR with the use of filtered compared with unfiltered dialysis fluids was significantly lower (0.3 PR/1,000 [9 of 28,007] versus 0.7 PR/1,000 [19 of 26,877]; *P* = 0.046), although there was no significant difference in PR rate by individual treatment modality between the two studies.

**DISCUSSION**

The use of bicarbonate dialysate in chronic hemodialysis centers in the United States has increased dramatically from 22% in 1986 to 72% in 1989 (24). However, bicarbonate concentrate, unlike acetate concentrate, can support the prolific growth and endotoxin production of certain types of gram-negative bacteria (25). In this study, bicarbonate concentrate was the primary source of bacterial and endotoxin contamination of the dialysate. Concern has been expressed that the use of bicarbonate dialysate contaminated with bacteria and endotoxin could cause an increase in adverse patient reactions, especially with the use of more permeable high-flux dialyzer membranes (4). In the 1989 surveillance of dialysis-associated diseases by the CDC, the use of bicarbonate dialysate and high-flux dialysis was associated with a higher risk of PR (24).

Bacterial and endotoxin contamination of bicarbonate hemodialysis fluids was substantially re-
duced by filtration. Bacteria and endotoxin that were occasionally detected in the bicarbonate dialysate, whether prepared from a central proportioner or at individual proportioning dialysis machines, were probably due to bacterial colonization in the dialysate lines after the filter. Although filtration did not result in sterile dialysate, the bacterial and endotoxin concentrations were reduced >99.9% compared with unfiltered bicarbonate concentrate or dialysate. The processed water used to reprocess dialyzers at all three centers during this study consistently met the Association for Advancement of Medical Instrumentation (AAMI) bacterial and endotoxin standards of <200 CFU/mL and <1 ng/mL, respectively, for water used to reprocess dialyzers (26). The use of filtered bicarbonate dialysis fluids, in conjunction with a carefully controlled dialyzer reprocessing program, was associated with a very low incidence of PR (0.3/1,000 treatments). Most of the PR that did occur were mild reactions and did not require medical intervention. Seven (78%) of the nine patients who met the case definition for PR developed fever only; two patients (22%) developed rigors and accompanying hypotension.

Although the filtration of dialysis fluids was associated with a twofold reduction in the incidence of PR compared with the incidence in our first study (when unfiltered dialysis fluids were used), this difference should be interpreted with caution. The reduction in the incidence of PR represented a difference of only 10 PR in over 28,000 treatments. Although the overall incidence of PR was reduced significantly (P = 0.046), there was no statistically significant reduction in PR by type of treatment modalities (Table 2). Also, although a uniform and sensitive definition for PR was used and a high proportion of patient treatment records had all three recorded temperatures during the two study periods, marked changes in the type of dialysis treatments had occurred at the three centers between study 1 and study 2. The number of high-flux and high-efficiency treatments increased from 9 to 23% and from 49 to 68%, respectively, whereas the number of conventional treatments fell from 49 to 9%.

Earlier studies have suggested that endotoxin in the dialysate may pass through the membranes of high-flux dialyzers or may induce transmembrane stimulation of circulating monocytes and thereby cause a PR (10–12). However, no significant differences in the incidence of PR were found between high-flux, high-efficiency, or conventional dialysis in this study with filtered dialysis fluids compared with our previous study when highly contaminated dialysis fluids were used. This would support previous studies that indicate that endotoxins do not pass readily through dialyzer membranes, even the more permeable high-flux membranes (13–16). These findings differ from results obtained from the national surveillance by the CDC of dialysis-associated diseases, which reported an association between PR in patients and high-flux dialysis (24). This difference likely reflects less rigorous data collection inherent in passive surveillance systems. Although the definition for PR used for the 1989 survey by the CDC (i.e., onset of rigors or temperature >100°F during dialysis) was similar to the definition used in this study, the survey relied on passive self-reporting of PR and did not estimate the rate of PR at a given center, only whether one or more PR had occurred at a center.

In this study, the filtration of bicarbonate dialysis fluids resulted in bacterial and endotoxin concentrations in the bicarbonate dialysate that approached undetectable levels. However, nine patients during the 11-month study period met our case definition for PR. This finding suggests that additional factors associated with hemodialysis treatment and dialyzer reuse may contribute to the incidence of PR. The overall PR rate of 0.3 PR/1,000 treatments may represent a lower limit for the PR rate with bicarbonate dialysate.

In conclusion, we found that polysulfone high-flux membrane dialyzers used to filter bicarbonate dialysis fluids markedly reduced bacterial and endotoxin contamination. The use of filtered dialysate was associated with a further reduction in an already low incidence of PR in patients receiving high-flux, high-efficiency, or conventional hemodialysis in centers that carefully reprocessed hemodialyzers and strictly adhered to AAMI standards for water used in dialyzer reprocessing.

REFERENCES


