Pyrogenic Reactions in Patients Receiving Conventional, High-Efficiency, or High-Flux Hemodialysis Treatments with Bicarbonate Dialysate Containing High Concentrations of Bacteria and Endotoxin

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ABSTRACT

High-efficiency (HE) and high-flux (HF) hemodialysis are becoming increasingly popular methods for treating patients with chronic renal failure because they reduce the time required for dialysis treatment. HF and HE dialyzers require bicarbonate dialysate, often prepared from concentrates that can support bacterial growth with endotoxin production. There is a concern that endotoxins or bacteria may cross or interact at the membranes of these dialyzers, triggering the release of endogenous pyrogens (cytokines) by peripheral blood mononuclear cells to cause pyrogenic reactions (PR). To determine the incidence of PR and to examine the association between PR and levels of bacteria and endotoxin in dialysate, a cohort of patients receiving conventional, HE, or HF hemodialysis with bicarbonate dialysate, identified among 18 patients in 26,877 hemodialysis treatments (0.7 PR/1,000 treatments). There was no significant difference in PR rates by treatment modality: conventional, 0.5 per 1,000 (7 PR/13,123 treatments) versus HE, 0.9 per 1,000 (9 PR/11,345) versus HF, 1.2 per 1,000 (3 PR/2,409) (P = 0.21; X² test).

Throughout the study period, bacterial counts for dialysate at each center significantly exceeded the Association for the Advancement of Medical Instrumentation’s (AAMI) microbiologic standards for dialysate of <2,000 CFU/mL (mean, 19,000 CFU/mL), but water used in the reuse of dialyzers tested <200 CFU/mL. The following was concluded: (1) the incidence of PR was low for all three types of hemodialysis treatments, despite high bacterial and endotoxin concentrations in bicarbonate dialysate, suggesting that the membranes of reprocessed HF dialyzers were as effective a barrier to bacteria and endotoxin as were the membranes in the other types of reprocessed dialyzers; (2) a plasma Limulus amebocyte lysate endotoxin test to detect PR had limited value with positive and negative predictive values of 67 and 56%, respectively; and (3) prevention of PR in centers that reuse dialyzers should emphasize adherence to AAMI microbiologic standards for water used in reuse (<200 CFU/mL) and should require a membrane integrity check of all dialyzers before each use.

Key Words: Hemodialysis, pyrogenic reaction, endotoxin

High-efficiency (HE) and high-flux (HF) hemodialysis both use dialyzers with large surface area or highly permeable membranes. They are being used increasingly for treating chronic renal failure primarily because the time required for dialysis treatment can be shortened significantly compared with that required for conventional hemodialysis. However, because time-shortened dialysis treatments require bicarbonate dialysate, usually prepared from a concentrate that can support bacterial growth with production of endotoxin, there is concern that bacteria or endotoxin in the dialysate could pass through
the permeable membranes of HF dialyzers into the patient's bloodstream, producing bacteremia and/or pyrogenic reactions (PR) (1–3). Febrile reactions or PR, characterized by chills, fever, and hypotension, are sometimes observed in patients during hemodialysis treatments (4–6), yet we could find no published epidemiologic studies to determine whether current standards for the levels of bacterial contamination in dialysate prevent PR in patients receiving hemodialysis treatments.

In 1988, approximately 58% of 1,586 hemodialysis centers in the United States used bicarbonate dialysate, up from 22% of 1,329 centers in 1986 (7). Increased use of bicarbonate dialysate is explained in part by the popularity of time-shortened hemodialysis; in 1988, 18% of hemodialysis centers used HF dialysis for the treatment of approximately 8,000 patients with chronic renal failure (8). As more centers practice time-shortened dialysis treatments, the potential for bacterial and endotoxin contamination of dialysis fluids will increase. We prospectively studied a cohort of chronic hemodialysis patients receiving conventional, HE, or HF dialysis treatments with bicarbonate dialysate to determine the following: (1) the incidence of PR among patients receiving hemodialysis; (2) the association between the risk of PR among patients receiving hemodialysis with the reuse of hemodialyzers and the levels of bacteria and endotoxin in bicarbonate dialysate; and (3) the usefulness of a plasma Limulus amebocyte lysate (LAL) test to identify endotoxin PR in patients receiving hemodialysis.

METHODS

Study Population

The study included all hemodialysis treatments performed in three outpatient hemodialysis centers located in Atlanta, Georgia, between September 25, 1988, and August 1, 1989. 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 provided informed consent before participating in the study.

Case Definition

We defined a PR case as the onset of objective chills (visible rigors) and/or fever (oral temperature ≥37.8°C) in a patient who was afebrile and who had no signs or symptoms of infection before the dialysis treatment.

Case Ascertainment

Active surveillance for PR and other adverse events was performed by the nursing staff. Oral temperatu
Method for Determining Dialyzer Reuse Rates

The reuse rate for a particular type of dialyzer was defined as the ratio of the total number of hemodialysis treatments with the dialyzer divided by the number of new dialyzers of this type used during the study period.

Case-Control Study

We conducted a case-control study of patients in all three centers with and without PR to determine the relationship between PR and dialysate endotoxin and bacterial concentrations. For study patients experiencing a PR, 5 mL of whole blood was obtained through the injection port of the arterial blood tubing at the time of the PR. Approximately 1 mL of blood was collected in a pyrogen-free, heparinized tube and was refrigerated at 4°C until tested for endotoxin; approximately 4 mL was placed directly into blood culture media bottles (DIFCO Laboratories, Detroit, MI). Approximately 5 mL of dialysate fluid was also collected in a sterile, pyrogen-free plastic tube from a disposable Luer-lock port on the venous (postdialyzer) side of the dialysis machine and was refrigerated at 4°C until processed for bacterial and endotoxin concentrations.

At the time of onset of the PR in a case patient, one control patient was selected from the pool of available control patients by the staff of the hemodialysis center according to the following criteria: (1) the control patient was receiving hemodialysis at the same time as the case patient and was afibrile without signs or symptoms of infection; (2) the control patient was receiving the same method of hemodialysis (i.e., HE, HF, or conventional) as the case patient; and (3) the control patient began the dialysis treatment at the time closest to when the treatment of the case patient began. Blood and dialysate samples were collected from the control patients at the same time and in the same manner as described for case patients.

Microbiologic Sampling of Dialysis Fluids

To provide background levels of endotoxin and bacteria in dialysis fluids, weekly samplings of processed water, dialysate, and liquid bicarbonate concentrate were performed at each center throughout the study period. Bicarbonate concentrate was sampled on the final day of use (the fourth day of storage). Samples of processed water were collected in the dialyzer reprocessing area, and samples of bicarbonate dialysate were collected from dialysis machines at point-of-use. All dialysis fluids were collected in pyrogen-free, sterile plastic tubes.

Laboratory Methods

Endotoxin concentrations were determined by the Luminus amebocyte lysate turbidimetric assay (LAL-5000; Associates of Cape Cod, Cape Cod, MA) with Pyrotell GT lysate (Associates of Cape Cod) at the CDC. The blood culture bottles were incubated for 14 days at 35 to 37°C. Dialysis fluid samples were assayed by the membrane filtration (0.45 μm pore size) technique (10). For bicarbonate concentrate and dialysate samples, membrane filters were placed on Trypticase soy agar (BBL, Microbiology Systems, Cockeysville, MD); water sample filters were placed on R2A agar (DIFCO Laboratories). All filters were incubated at 30°C for 72 h. Colonies were counted at 24, 48, and 72 h. Identification of bacterial colonies was done by standard methods (10).

Data Management

Data from the hemodialysis record of each study patient were transcribed from worksheets to a computerized database by custom-developed software of Dialysis Clinics Inc. (Medical Information System) and Digital (VAX11/780; Digital Equipment Corp.)
Bedford, MA) computer equipment. The charge nurse reviewed the dialysis record at the end of each treatment before the record was transcribed onto a computer database. Throughout the study period, investigators reviewed printouts of weekly summaries of patient treatments at each center for temperature recordings, symptoms during treatment, and other complications.

Statistical Analysis

Proportions were compared by using the \( \chi^2 \) test. The significance of difference between means was assessed by \( t \) test.

RESULTS

Descriptive Epidemiology

A total of 315 patients received 27,087 hemodialysis treatments during the study period; 50% of the patients were female, 94% were black, and the median age was 58 yr (range, 22 to 86 yr). During the study, 230 (73%) patients received hemodialysis treatments throughout the entire period; 56 patients (18%) began treatment, and 29 patients (9%) stopped treatment. A total of 138 (0.5%) dialysis treatments were excluded from the analysis because of a temperature recording of 37.8\(^\circ\)C at the onset of dialysis. In addition, the treatment sessions of a patient with ESRD secondary to intravenous drug abuse were excluded from the analysis because of an unexplained febrile illness occurring throughout the study period.

The patient died before the end of the study; consent for testing serum for antibodies to human immunodeficiency virus or for a postmortem examination was not obtained.

A total of 19 PR occurred among 18 patients during 26,877 dialysis treatments for an incidence rate of 0.7 PR/1,000 treatments. The most frequent signs and symptoms of PR were fever (80%), rigors (25%), hypotension (20%), subjective chills (20%), and nausea/vomiting (10%). There were no deaths as a result of PR. The mean time between the start of hemodialysis treatment and the onset of PR was 120 min (range, 60 to 180 min).

There was no clustering of PR, and there was never more than a single PR on any day (Figure 1). The rate of PR did not differ significantly between the three centers (Center A, 0.7/1,000 [6 PR/8,348 treatments]; versus Center B, 1/1,000 [7 PR/7,200 treatments]; versus Center C, 0.5/1,000 [6 PR/11,329 treatments]; \( P = 0.6 \)). The distribution of treatments by modalities was as follows: conventional, 13,123 (49%); HE, 11,345 (42%); and HF, 2,409 (9%). The rates of PR in the three types of hemodialysis were not significantly different: conventional, 0.5/1,000 (7 PR/13,123 treatments); versus HE, 0.9/1,000 (9 PR/11,345 treatments); versus HF, 1.2/1,000 [3 PR/2,409 treatments]; \( P = 0.21 \). There was also no significant difference in the incidence rate of PR if treatments were stratified into two groups: conventional versus HE and HF (\( P = 0.2 \)).

The overall mean reuse rate of hemodialyzers during the study period was 8.8 uses per dialyzer. The dialyzers with the highest mean rates of reuse were HE (CA-210; 12, range, 1 to 28), HF (F-80; 11.1, range, 1 to 26), cellulocic (CF) membranes (7.4, range, 1 to 27), and cellulose-acetate (CA-90 or CA-110) membranes (6.6, range, 1 to 26). Among centers, there were no significant differences in mean reuse rates by type of dialyzer.

Patients receiving treatment by reused dialyzers showed no increased risk of PR. The PR rate with "first use" dialyzer treatments was not significantly different from treatments with reused dialyzers: 1.6/1,000 (5 PR/3,038 treatments) versus 0.6/1,000 (14 PR/23,839 treatments), respectively (\( P = 0.06 \)). We also found no increased risk of PR as the numbers of reuses of dialyzers increased (<5 reuses, 0.9/1,000 [11 PR/12,691 treatments] versus ≥5 reuses 0.6/1,000 [8 PR/14,186 treatments]; \( P = 0.4 \)); <10 reuses, 0.9/1,000 [17 PR/19,397 treatments] versus ≥10 reuses, 0.4/1,000 [2 PR/7,480 treatments]; \( P = 0.09 \)).

Because fever during dialysis identified 75% of the episodes of PR, we determined the frequency of temperature recordings for all dialysis treatments during the study period. Over 99% of the treatments had a temperature recorded immediately before and after
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hemodialysis; 98% of all treatments had a 1-h temperature recorded. The most frequent signs and symptoms of patients not related to PR were cramping, 4,339 (16%); hypotension, 4,084 (15%); headache, 1,383 (5%); and back pain, 63 (0.2%).

Microbial and Endotoxin Surveillance of Dialysis Fluids

Bacterial and endotoxin concentrations in dialysis fluids collected weekly throughout the study did not differ significantly between centers, and therefore, the results of testing were combined. The mean bacterial concentration in processed water throughout the study period was 79 CFU/mL (range, 5 to 1,060 CFU/mL [Figure 2]) and was usually below the Association for the Advancement of Medical Instrumentation (AAMI) recommended level of ≤200 CFU/mL (11). The mean endotoxin concentration in processed water was 45 pg/mL (Figure 3).

The mean bacterial and endotoxin concentrations in the bicarbonate concentrate, sampled on the fourth day of storage, were substantially higher than those in the processed water (132,000 CFU/mL and 6,300 pg/mL, respectively) (Figures 2 and 3). The mean bacterial and endotoxin concentrations in the bicarbonate dialysate were 19,000 CFU/mL and 380 pg/mL, respectively, and were usually above the AAMI recommendations of ≤2,000 CFU/mL for bacteria levels in dialysate (Figures 2 and 3). The most prevalent bacteria isolated from dialysis fluids were *Pseudomonas* species (*P. paucimobitus*, *P. cepacia*, *P. stutzeri*, *P. fluorescence*, *P. pickettii*, and *P. putida*), *Xanthomonas maltophilia*, and *Alcaligenes denitrificans*.

The concentration of Renalin® used during a reprocessing cycle was determined for each of the automated reprocessing machines at the three centers on two different occasions and was found to average 4.1% (range, 2.6 to 5.1%).

The Positive Predictive Value of the Plasma LAL-Endotoxin Test for Detecting PR

A total of 12 of the 18 patients experiencing PR and 12 control patients participated in the case-control study. There were no significant differences between case and control patients for age, race, sex, or number of prior reuses of the dialyzer. Bacteria were isolated from the blood of one study patient during a PR; *P. paucimobilis* was isolated from two of two blood cultures in this patient. There were no significant differences between case and control patients for bacterial or endotoxin concentrations of dialysate collected at point-of-use at the time of the PR (log 4.1 versus log 3.6 CFU/mL; 1.4 versus 0.9 ng/mL; *P* = 0.5) (Figure 4).

The sensitivity and specificity of the plasma LAL test for endotoxin to detect PR for the 12 true PR and 12 true non-PR patients at a level of 10 pg/mL were 37 and 82%, respectively. The positive predictive value was 67%, with a negative predictive value of 56%.

DISCUSSION

Despite high levels of bacteria and endotoxin in bicarbonate dialysate at point-of-use, the incidence of PR among patients receiving chronic hemodialysis by using the three different modalities was low (0.7

Figure 2. Mean microbial concentrations in dialysis fluids by week at three hemodialysis centers from September 25, 1988, to August 1, 1989.
PR/1,000 treatments). We found no association between the type of hemodialysis treatment or the number of prior reuses of a hemodialyzer and the risk of PR during treatment. The bicarbonate concentrate used to prepare the dialysate at the centers was the principal source of bacterial and endotoxin contamination of the dialysate (mean levels of 19,000 CFU/mL and 380 pg/mL, respectively). The processed water used to prepare the dialysate and to reprocess dialyzers was prepared in a similar manner at all three centers and usually contained levels of bacteria <200 CFU/mL.

The low incidence of PR and bacteremia among patients receiving hemodialysis at these centers sug-
suggests that intact membranes of various types of hollow-fiber dialyzers used during HF, HE, or conventional hemodialysis are impermeable to bacteria and endotoxin in dialysate. The data also suggest that the polysulfone membranes of properly reprocessed hollow-fiber hemodialyzers used in HF dialysis treatments are as effective a barrier to bacteria and endotoxin as are the hollow-fiber dialyzers that are constructed from cellulose-derived or cellulose membranes and that are used in conventional and HE dialysis. Our findings support recent reports that also could not detect the passage of endotoxin in dialysate across intact dialyzer membranes during conventional dialysis or across intact polysulfone membranes during HF hemodialysis (12-15).

Numerous studies have demonstrated that endotoxin induces the production of interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) in macrophages and monocytes and that these cytokines are probably responsible for the clinical signs and symptoms associated with PR. IL-1 may also be induced during hemodialysis by complement-activating dialyzer membranes (16,17). The low positive predictive value of the plasma LAL-endotoxin assay we observed in patients experiencing PR suggests that detectable levels of endotoxin were not the cause of PR but does not rule out the induction of cytokines as a consequence of interactions between endotoxin and peripheral blood mononuclear cells across the dialyzer membrane. The delay observed in patients with PR between the initiation of hemodialysis and the onset of clinical signs and symptoms of PR is consistent with observations of peak TNF-α and fever 90 min after experimental endotoxin fever in human volunteers (18-20).

Our study was not designed to identify the cell mediators involved in the pathogenesis of PR but to determine the incidence rates of PR for three types of hemodialysis treatments with bicarbonate dialysate with high levels of endotoxin and bacteria. The low rate of PR for each method of hemodialysis suggests that dialysate contaminated with high levels of endotoxin and bacteria does not commonly result in febrile reactions among patients receiving dialysis treatments from dialyzers with intact membranes.

Because PR is a gross clinical measure of the magnitude of host response, a low incidence of PR does not exclude interactions at the dialysis membrane resulting in "subclinical" elevations of TNF-α and IL-1, which may have deleterious biologic effects in patients subjected to chronic hemodialysis. Cytokine activity in vivo is probably dependent upon a variety of factors (20) including the duration and amount of exposure to endotoxin or the triggering of cytokine release by peripheral blood mononuclear cells; the duration and elevation of plasma cytokines (most notably TNF-α), the binding sites in target tissues to cytokines; and the effect of other synergistic or inhibitory mediators (e.g., cyclooxygenase inhibitors).

An important limitation of our study is the lack of a "standard definition" of a PR. We chose a sensitive definition, based upon clinical signs and symptoms, so that it could be used in any dialysis center. The high rate of patient treatment records having three recorded temperatures and the incidence rates of signs and symptoms recorded during hemodialysis, which were not specifically related to PR (e.g., hypotension, cramping, headache), indicate that the surveillance system for PR was both active and sensitive. The addition of a temperature recording at 1 h after the onset of treatment increased the sensitivity for detecting PR by 50%.

Another limitation of the study was the representativeness of sampling of bicarbonate concentrate, which usually occurred on the final day of use (day 4) and, therefore, during the highest levels of bacterial growth and endotoxin production. Previous studies of sterile liquid bicarbonate concentrate formulated at these same dialysis centers demonstrated mean bacterial levels of 10⁹ CFU/mL after 24 or 48 h of storage (3). In addition, the mean bacterial count in dialysate that was obtained during dialysis in the case-control study, and not taken on the fourth day of storage, was 10⁵ CFU/mL. Therefore, we believe that the elevated levels of bacteria and endotoxin were representative of dialysate used for treatments of all patients in the study.

Because only one type of hollow-fiber dialyzer (polysulfone membrane) was used for HF hemodialysis treatments, our findings may not be able to be generalized to other types of dialyzers. In addition, because HF treatments accounted for only 10% of the study treatments, it is possible that a longer period of observation may suggest a greater difference in adverse events between conventional and HF treatments. We are continuing active surveillance for PR at all three centers.

During reprocessing of a hollow-fiber dialyzer, bacterial or endotoxin contamination of the dialyzer interior can occur when the dialyzer is rinsed with water containing endotoxin or bacteria or when the germicide used for disinfection is diluted with water containing endotoxin or bacteria. If the blood compartment of the dialyzer is contaminated, PR may result during its subsequent use (21). Throughout the study period, the processed water at all three centers consistently met AAMI bacterial and endotoxin standards for the reuse of hemodialyzers (22). In addition, the reuse procedure included a membrane integrity check of all dialyzers with an automated reprocessing machine. This prevented the inadvertent use of damaged or "leaky" dialyzer membranes, which could contribute to PR and bactereimias in the presence of a high dialysate bacterial load.
AAMI guidelines for monitoring bacterial concentrations in dialysate are based in part upon investigations of PR in the early 1970s, which suggested that levels of bacteria >2,000 CFU/mL in the dialysate were associated with an increased risk of PR among patients receiving hemodialysis in which parallel plate dialyzers were used (6). However, we did not find a high incidence of PR among patients receiving conventional, HE, on HF dialysis despite significant bicarbonate dialysate bacterial contamination. Our results suggest that if processed water meets AAMI standards and if dialyzers are carefully and adequately reprocessed, then the use of bicarbonate dialysate containing high bacterial and/or endotoxin levels may not increase the risk of PR in hemodialysis patients receiving conventional, HE, or HF hemodialysis.

It is important to note that a recent CDC survey of hemodialysis centers in the United States reported an association between PR in patients and the reuse of hemodialyzers or the use of HF hemodialyzers (8). In contrast to the current study, that survey was retrospective, relying on passive surveillance of adverse outcomes during dialysis and self-reporting by personnel at each center, without a stated case definition of PR. The survey did not examine rates of PR, only whether or not a center had reported any PR. Therefore, the larger dialysis centers (>40 patients), which used HF dialyzers and practiced reuse of hemodialyzers more commonly than did the smaller centers, also had a greater chance of having patients experience PR because they had more patients. In addition, the surveillance and reporting of adverse events during hemodialysis may have been better in larger centers. We believe that the overall quality of the dialysis procedures, including dialyzer reprocessing, at individual centers may be more important in preventing PR during hemodialysis than is any single factor, such as the bacteriologic quality of the dialysate fluids.

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"Uroscopy was the art (if we may call it such) of making a diagnosis and prescribing therapy solely on the evidences afforded by looking at the urine, for which purpose the fluid was studied carefully in a specially shaped flask and by an almost ritualistic procedure . . . . . . . . The uroscopists were largely ignorant of both anatomy and therapeutics, and their activity ultimately expanded into that form of divination known as uromancy; yet it was a day when, apart from taking the patient's pulse and listening to his history, there was little that the physician could do in the way of therapy, and the uroscopist, or water caster, water juggler, water judge, or as he was later called, the piss-prophet with his elaborate gown and headdress, mysterious urine glass and solemn mien, must have had a strong appeal for the sick and in many instances probably imparted to them some measure of psychosomatic benefit."