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Dialyzer membrane type and reuse practice influence polymorphonuclear leukocyte function in hemodialysis patients

MADHUMATHI RAO, DAQING GUO, BERTRAND L. JABER, SUMUK SUNDARAM, MIGUEL CENDOROGLO, ANDREW J. KING, BRIAN J.G. PEREIRA, VAIDYANATHAPURAM S. BALAKRISHNAN, and THE HEMO STUDY GROUP

Division of Nephrology, Tufts-New England Medical Center, Boston, Massachusetts

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Background. Polymorphonuclear leukocyte (PMNL) production of reactive oxygen species (ROS) has been linked to hemodialysis (HD) associated morbidity. The effect of dialyzer membrane type and reuse on PMNL function has not been clearly defined.

Methods. The present report is a cross-sectional study undertaken in a cohort of patients undergoing regular HD, at enrollment into the Hemodialysis (HEMO) Study, to study the association between patient and dialysis-related factors and PMNL function. PMNL function was assessed by measuring PMA- and N-formyl methionyl-leucyl-phenylalanine (fMLP) -induced respiratory burst, and phagocytic activity toward *Staphylococcus aureus*.

Results. PMNL from patients dialyzed with polysulphone (PS) or cuprophane (CU) membranes showed higher PMA-induced respiratory burst activity compared with those exposed to substituted cellulose (cellulose acetate, cellulose triacetate, CA/CT) membranes, regardless of dialyzer reuse. The use of bleach as a cleansing agent during reuse was associated with higher PMA-induced PMNL superoxide production, as was the use of renalin when compared to aldehydes. In a subgroup of patients using PS dialyzers, reuse itself was associated with higher fMLP-induced superoxide production. The type of bleach-germicide combination during reuse showed that use of renalin as a germicide was also associated with higher PMNL phagocytosis index. The number of years on HD correlated inversely with PMA-induced PMNL superoxide response. Weaker PMNL response to fMLP was associated with greater comorbidity and poor functional status as quantified by Index of Coexisting Diseases (ICED) and Karnofsky scores, respectively.

Conclusion. Our results indicate that dialyzer membrane type and the reuse process influence the oxidative response of PMNL among HD patients. The implications of these observations on clinical morbidity need to be further evaluated in prospective studies.

Key words: PMNL respiratory burst, biocompatibility, dialyzer reuse, dialyzer membrane, ICED score, Karnofsky index.

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Polymorphonuclear leukocyte (PMNL) function among patients on long-term hemodialysis (HD) has been an important focus of study over the past two decades. Clinical correlates of depressed PMNL function have been associated with increased risk of infections and infectious mortality in HD patients [1, 2]. However, heightened phagocyte activation, generation of reactive oxygen species (ROS), and consequent oxidative stress have more recently been linked to complications such as accelerated atherosclerosis [3], malnutrition and inflammation [4, 5], and the deleterious effects of homocysteine on vascular endothelium [6].

Previous work in this area has addressed the effect of uremia [7, 8] and acute intradialytic blood-membrane interactions [8, 9] on PMNL function. Phagocytic cell function has also been used as an index of biocompatibility [10]. Indeed, the metabolic response to phagocytosis was demonstrated to be significantly lower in uremic patients compared to controls, and declined further on initiation of dialysis with complement-activating membranes [11]. Thus, the relationship between PMNL function, clinical, and dialysis-related variables is a dynamic one that merits further exploration. There has been no systematic study on a larger scale to identify the determinants of, and clinical significance of, PMNL function parameters in patients on long-term maintenance HD. In particular, the relationship of PMNL function to dialyzer membrane type, reuse-related variables, demographic characteristics, or indices of comorbidity has not been clearly defined. In the present study, PMNL respiratory burst and phagocytosis were measured in a cohort of patients undergoing maintenance HD, at enrollment into the Hemodialysis (HEMO) Study, before randomization into study limbs took place. Patient- and HD-related factors associated with PMNL function were identified.

METHODS

Patient enrollment and characteristics

The study population included adult patients (18–80 years old) who had completed a minimum of 3 months on

maintenance HD three times a week, who were enrolled into the baseline phase of the HEMO Study from the dialysis units affiliated with the two participating Boston centers. This ancillary study was approved by the Human Investigation Review Committee (HIRC), and all participants provided written, informed consent. The HEMO Study was sponsored by the United States National Institute of Diabetes, Digestive, and Kidney Diseases, and the full-scale trial began in 1995. Details regarding the design of the HEMO Study have been published elsewhere [12]. In brief, this was a multicenter, prospective, randomized clinical trial designed to evaluate the effect of dialyzer urea and β2-microglobulin clearances on the morbidity and mortality of patients. The following were exclusion criteria: patients in acute care or chronic care hospitals; interdialytic urea clearance >1.5 mL/min; pregnancy; scheduled living related renal transplant; <6 months after failed renal transplant; active malignancy; severe cardiac, pulmonary, or liver disease; serum albumin <3.0 g/dL; participation in ongoing investigational drug/device studies; or inability to participate due to incompetence, refusal, or likelihood of geographic move.

Heparinized blood samples were collected from these patients upon enrollment in the HEMO Study. Demographic, clinical, laboratory, and hemodialysis-related data were provided by the HEMO Study Data Coordinating Center at Cleveland, Ohio, and consisted of baseline information collected at enrollment. Clinical data included comorbidity parameters [derived index of coexistent disease (ICED) scores] [13, 14], nutritional indices [body mass index (BMI), serum albumin] [15], and functional status (Karnofsky index) [16]. Hemodialysis-related variables included dialysis adequacy data, type of dialyser used, presence and type of reuse procedure, and number of years on dialysis, and again referred to the dialysis parameters identified at enrollment and before randomization.

PMNL function parameters

Baseline PMNL function was assessed upon enrollment in the HEMO Study. The production of ROS by PMNL after stimulation by phorbol myristate acetate (PMA) or N-formyl methionyl–leucyl–phenylalanine (fMLP) was assessed. In addition, the ability of PMNL to phagocytose *Staphylococcus aureus* was measured. Measurements of ROS production after stimulation with PMA and fMLP were also carried out in PMNLs harvested from 10 healthy volunteers. PMA is a pharmacologic activator of protein kinase C that leads to the phosphorylation of several substrates including p47phox, which are critical in NADPH-oxidase activation and generation of the neutrophil respiratory burst. FMLP, on the other hand, binds to a G-protein coupled receptor, eventually triggering NADPH-oxidase activation [17].

PMNL isolation

Heparinized (10 IU/mL) whole blood was drawn from the fistula needle before the start of dialysis for harvesting PMNL and transported on ice within an hour of collection to the laboratory for processing. Water and other solutions used in the study were subjected to ultrafiltration using a polyamide hollow-fiber ultra-filter (U2000; Gambro AB, Hechingen, Germany) to remove cytokine-inducing agents. The buffy coat was separated using the Ficoll-Hypaque density gradient separation technique [18]. Ten mL of heparinized blood was diluted in 20 mL of normal saline (Abbott Laboratories, Rockford, IL, USA), underlayered with 10 mL of Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO, USA; Nycomed Inc., New York, NY, USA), and centrifuged at 1200 rpm for 45 minutes at room temperature. The buffy coat was harvested and resuspended in 25 mL of Dulbecco's phosphate-buffered saline (PBS) (Gibco BRL-Life Technologies, Grand Island, NY, USA), and 3% dextran (Sigma Chemical Co.) was added to a volume of 50 mL. After 15 minutes, the leukocyte-rich supernatant was harvested and centrifuged at 400*g* for 5 minutes at 4°C. Residual erythrocytes were subjected to hypotonic lysis. This procedure was repeated once or twice until the cell pellet was free of erythrocytes. Cells were suspended in ice-cold PBS and PMNL were counted using a standard hemocytometer. The purity of the PMNL preparation was >95% and viability >99% as judged by the trypan blue exclusion method. PMNL were resuspended at 10×10^6 cells/mL in Hank's balanced salt solution (HBSS).

Measurement of superoxide production

Superoxide production by PMNL was determined by measuring the capacity of cells to reduce ferricytochrome to ferrocytochrome C [18]. PMNL aliquots of 125 μL (1.25×10^6 cells) were incubated in Eppendorf™ tubes (USA/Scientific Plastic, Ocala, FL, USA) in the presence of 25 μL of ferricytochrome C (12.3 mg/mL; Sigma Chemical Co.), 25 μL of stimuli [10 nmol/L solution of 4-β phorbol 12-β myristate 13-α-acetate (PMA) (Sigma Chemical Co.)], or 10 μmol/L solution of N-formyl methionyl-leucyl-phenylalanine (fMLP) (Sigma Chemical Co.) with and without 25 μL of superoxide dismutase (SOD; 1 mg/mL; Sigma Chemical Co.). HBSS was added to finalize the volume at 250 μL. After a 10-minute incubation at 37°C with rotational agitation, the tubes were placed on ice and centrifuged at 14,000 rpm for 1 minute at 4°C. Control experiments were run in parallel using 25 μL of HBSS instead of stimuli. All experiments were performed in duplicate. The amount of superoxide produced was quantitated by measuring the change in spectrophotometric absorbance of the cell-free supernatant at dual wavelength (550 nm test filter with a reference wavelength of 570 nm), using a microplate reader (MRX-500;

Dynatech Laboratories, Inc., Chantilly, VA, USA). The intra-assay coefficient of variation in our laboratory, for measurement of stimulated superoxide production, was <5% in 80% of samples and <10% in 100% of samples. Results are expressed as nanomoles of superoxide produced/1.25 $\times$ 10^6 cells/10 minutes, based on an extinction coefficient for a 1 cm light path (ferro-ferri) of 21.1.

Measurement of phagocytosis

A quantitative assay was used to determine the uptake of opsonized ^{14}C-labeled heat-killed *S. aureus* by phagocytes [18]. In brief, PMNL (500 μL = 5 $\times$ 10^6 cells) were washed in PBS and resuspended in 800 μL of HBSS in a 12 $\times$ 75-mm polypropylene tube (Becton Dickinson Labware, Franklin Lakes, NJ, USA), in the presence of 100 μL each of ^{14}C-labeled S. *aureus* (5 $\times$ 10^7), and 10% autologous serum to make up a final volume of 1 mL. The mixture was incubated at 37°C for 30 minutes using rotational agitation. The final bacteria-to-phagocyte ratio was 10:1. Phagocytosis was stopped by adding 2 mL of ice-cold PBS to the mixture and the mixture was placed on ice for 2 minutes. Phagocyte-associated bacteria were separated by centrifugation at 1200 rpm for 5 minutes. The supernatant was aspirated to remove non-cell–associated bacteria. The pellet was resuspended in 2 mL of PBS. The wash procedure was repeated twice and the cell pellet finally suspended in 500 μL of 0.2N sodium hydroxide solution and incubated overnight at 37°C. To this was added 200 μL of 3% acetic acid and 300 μL of HBSS. The whole amount (1 mL) was aliquotted into a scintillation tube, and 9 mL of scintillation liquid (Ecoscint™ H; National Diagnostics, Atlanta, GA, USA) was added. Phagocyte-associated radioactivity was determined by a 1219 Rackbeta liquid scintillation counter (LK 66 Wallac; Turku, Finland). Phagocytosis was expressed as an index: ratio of cell associated counts/total radioactivity added (estimated from the count on a vial containing 50 μL radiolabeled bacteria and 10 mL of scintillation liquid).

Statistical analysis

Data definitions. Continuous clinical variables such as serum albumin, age, and Kt/V, were divided into quartiles and dichotomized around the 25th percentile. Karnofsky score was also dichotomized around the 25th percentile, the lowest quartile consisting of scores under 80. Patients with Index of Coexisting Diseases (ICED) scores of 3 were categorized as having severe comorbidity as opposed to those with scores of 1 or 2 (mild to moderate). A high-flux dialyzer was defined according to the protocol of the HEMO study as one having minimum values for ultrafiltration coefficient and first-use β2-microglobulin clearance of 14 mL/hr/mm Hg and 20 mL/min, respectively; a low-flux dialyzer was defined as one having ultrafiltration coefficient and first-use β2-microglobulin clearance values lower than these [12]. Membrane type was classified as polysulphone (PS), cuprophane (CU), or substituted cellulose (cellulose acetate, cellulose triacetate, CA/CT). Germicidal agents in reuse were categorized as aldehydes (formaldehyde, glutaraldehyde) or renalin. Bleach-germicide combinations formed three categories: bleach-aldehyde, bleach-renalin, and renalin alone. HD adequacy was measured by the equilibrated Kt/V (eKt/V) using the rate equation of Daugirdas and Schneditz [19]. Vascular accesses were categorized as native arteriovenous fistulae (AVF), grafts (AVG), or venous catheters.

Analysis

Statistical analysis was carried out using a statistical software program (SPSS for Windows, Release 10.1.0, Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation and categorical data as proportions; adjusted means were expressed as mean ± standard error of the mean (SEM).

Measures of PMNL function were used as outcome variables (superoxide response to PMA and fMLP, and % phagocytosis index). Because patient information and PMNL function parameters were collated over a period of 5.5 years (April 1995 to December 2000), all analyses were adjusted for the year of enrollment to account for evolution in practice patterns, standard of delivery of dialysis care over this period of time, and any changes in laboratory personnel and facilities. The association between measures of PMNL function and the following independent variables were studied: dialyzer flux (low- vs. high-flux), reuse-related variables such as the cleansing agent (sodium hypochlorite or bleach) and germicide, and dialysis adequacy (measured by eKt/V), using a general linear model (GLM) adjusted for appropriate factors and covariates. Measures of PMNL function included superoxide response to PMA and fMLP, and the % phagocytosis index. A P value of < 0.05 was considered statistically significant. The Bonferroni adjustment was used for all multiple comparisons.

Hemodialysis practice patterns showed a change during the 5-year period over which enrollment into the HEMO study occurred, with a move to the almost exclusive use of non-complement activating synthetic membranes (PS) in the latter three years of the study. fMLP-induced ROS production was studied only during the latter three-year period in those patients exposed to PS membranes. As membrane type and certain aspects of reuse practice were colinear, analyses using appropriate subsets were carried out.

The relationship between PMNL function and clinical variables (ICED score and Karnofsky index) was also explored. The GLM was used to study PMNL responses in patients at different levels of ICED or Karnofsky scoring. Multivariate analysis using logistic regression and ICED

Table 1. Baseline characteristics of the study population ($N = 206$)

Age *years*	61.9 ± 12.5
Male gender	45%
Race	
Caucasian	55%
African American	45%
Number of years on dialysis	3.7 ± 4.3
<1 year	27%
1–3 years	36%
>3 years	37%
Cause of kidney failure	
Diabetic nephropathy	34%
Hypertensive nephrosclerosis	32%
Glomerular disease	14%
Miscellaneous	20%
Percentage diabetics[a]	42%
Body mass index kg/m^2	24.7 ± 5.1
Serum albumin *g/dL*	3.6 ± 0.4
Hematocrit %	33.2 ± 5.0
Index of Coexistent Disease Scores (ICED)	
1	34%
2	32%
3	34%
Karnofsky score	79.6 ± 17.1
Vascular access	
AV fistula	78%
AV graft	14%
Venous catheter	8%
Equilibrated Kt/V	1.4 ± 0.2
nPCR, g/kg/day	1.0 ± 0.2

Data are expressed as mean ± SD or percentages.
[a] Includes patients that had a diagnosis of diabetes even if diabetic nephropathy was not the cause of ESRD.

score or Karnofsky index as response variables was performed to study the effect of PMA- or fMLP-induced superoxide responses, or% phagocytosis index. A model was created with stepwise selection of the following covariates: year of enrollment, age, gender, race, and number of years spent on HD.

RESULTS

Baseline characteristics

A total of 210 patients were enrolled during the 5.5-year period (April 1995 to December 2000) from the two Boston centers participating in the HEMO Study. Table 1 shows baseline demographic, clinical, and selected laboratory indices, as well as dialysis-related variables of 206 patients. There were 4 patients who were non-Caucasian and non-African American and who were not considered for subsequent analysis. Sixty-nine percent of patients dialyzed with high-flux PS membranes, 10% of the cohort, did not reuse dialyzers, chiefly where the patient did not give consent for reuse. The distribution of membrane type and flux in the present study reflected the status at baseline before randomization to the high- or low-flux limbs of the HEMO Study. Since much of this data was obtained before the advent of automated reprocessing with renalin, the concomitant use of bleach as a cleansing agent with renalin was still prevalent. Indeed, bleach was the cleansing agent in 95.5% of dialyzers, with either formaldehyde or glutaraldehyde as the germicidal agent in 37%, and renalin in 58%. Tables 2 and 3 show the distribution of reuse and related parameters by membrane type and flux property. The effect of flux on PMNL function was assessed only in PS and CA/CT, as all cuprophane (CU) dialyzers were low-flux membranes. The effect of germicidal agent (aldehyde vs. renalin) was assessed only in reused CU and PS dialyzers, as nine of 10 reused CA/CT dialyzers were sterilized with glutaraldehyde.

Table 2. Distribution of reuse by membrane type and flux property

Membrane	N%	Flux	Reuse ($N = 177$)	No reuse ($N = 20$)
CA/CT	12 (6%)	High[a]	8	0
		Low[a]	2	2
CU	20 (10%)	High	0	0
		Low	19	1
PS	165 (84%)	High	132	14
		Low	16	3

Data on reuse is missing in 9 patients. Abbreviations are: CA, cellulose acetate; CT, cellulose triacetate; CU, cuprophane dialyzers; PS, polysulfone dialyzers.
[a] CA, cellulose acetate, classified as low flux; CT, cellulose triacetate, classified as high flux.

Superoxide production in study and control population

Superoxide production (mean ± SEM) by PMNLs harvested from 10 healthy individuals was 52.0 ± 4.3 nmol $O_2^-/1.25 \times 10^6$ cells/10 minutes after stimulation with PMA and 19.0 ± 4.0 nmol $O_2^-/1.25 \times 10^6$ cells/10 minutes after stimulation with fMLP. The corresponding unadjusted values for HD patients in this cohort were 32.4 ± 0.9 nmol $O_2^-/1.25 \times 10^6$ cells/10 minutes and 22.0 ± 1.5 nmol $O_2^-/1.25 \times 10^6$ cells/10 minutes, respectively. PMA-induced superoxide production was significantly lower in HD patients ($P < 0.001$); the difference for fMLP-induced superoxide production between HD and healthy controls did not reach significance.

Dialysis- and reuse-related factors associated with PMNL function

Tables 4 and 5 show the relationship between dialysis- and reuse-associated variables and measures of PMNL function adjusted for year of enrollment.

PMA-induced superoxide production by PMNL

Effect of membrane. PMNL from patients dialyzed with either PS or CU membranes demonstrated higher respiratory burst activity in response to PMA in comparison to those exposed to CA/CT membranes (33.3 ± 0.7, 31.1 ± 2.0 vs. 21.5 ± 2.6; $P < 0.001$ for PS vs. CA/CT, $P = 0.01$ for Cu vs. CA/CT, $P =$ NS for PS vs.Cu) (Table 4). These differences in PMA-induced superoxide

Table 3. Distribution of type of cleansing agent and germicide utilized for reprocessing dialyzers by membrane type and flux property ($N = 177$)

Membrane	N%	Flux	Cleansing agent and germicide			
			Bleach + aldehyde[b]	Bleach + glutaraldehyde[b]	Bleach + renalin	Renalin only
CA/CT	10 (6%)	High[a]	0	7	0	0
		Low[a]	0	2	0	1
CU	19 (11%)	High	0	0	0	0
		Low	1	15	2	1
PS	148 (83%)	High	12	26	88	6
		Low	1	2	13	0

Abbreviations are: CA, cellulose acetate; CT, cellulose triacetate; CU, cuprophane dialyzers; PS, polysulfone dialyzers.
[a]CA, cellulose acetate, classified as low flux; CT, cellulose triacetate, classified as high flux.
[b]Both formaldehyde and glutaraldehyde treatment considered for analysis as a single category.

Table 4. Association between dialysis- and reuse-associated variables and PMNL superoxide production and % phagocytosis index

	PMNL superoxide production (nmol O^{2-}/1.25 × 10^6 cells/10 min)								
	PMA			FMLP					
	N	response[a]	*P*	N	response[a]	*P* value	N	Phagocytosis index (%)[a]	*P* value
Healthy control	10	52.0 ± 4.3		10	19.0 ± 1.6				
All patients	205	32.3 ± 0.7		65	22.3 ± 1.6		203	44.2 ± 1.1	
PMNL function by membrane[b]									
CA/CT	12	21.5 ± 2.6					12	46.0 ± 3.4	0.3
CU	21	31.1 ± 2.0	<0.001				21	37.8 ± 4.4	
PS	172	33.3 ± 0.7		65	22.3 ± 1.6		170	44.5 ± 1.2	
PMNL function by reuse									
No	20	29.4 ± 2.1	0.3	13	15.8 ± 3.5	0.04	19	40.7 ± 3.6	0.3
Yes	176	31.9 ± 0.7		46	24.4 ± 1.9		175	44.4 ± 1.1	
PMNL function by dialysis adequacy									
Kt/V Q-1 (<1.28; range 0.95–1.27)	51	33.8 ± 1.3	0.2	16	22.9 ± 3.3	0.8	56	44.8 ± 2.2	0.9
Kt/V Q2-4 (≥1.28; range 1.28–1.97)	154	31.9 ± 0.8		49	22.1 ± 1.8		147	44.1 ± 1.2	
PMNL function by number of years on dialysis									
≤1 year	56	34.4 ± 1.2	0.05	19	19.9 ± 2.9	0.3	49	44.5 ± 2.0	0.8
>1 year	149	31.6 ± 0.8		46	23.3 ± 1.9		154	44.1 ± 1.3	
PMNL function by flux property									
Patients using CA/CT or PS dialyzers	184	33.0 ± 0.7		182	33.0 ± 0.7		182	33.0 ± 0.7	
Low-flux	35	33.4 ± 1.7	0.8	25	19.3 ± 2.5	0.1	35	46.3 ± 2.8	0.4
High-flux	149	33.0 ± 0.8		40	24.2 ± 2.0		147	43.5 ± 1.5	
PMNL function by cleansing agent-germicide categories									
Patients reusing CU or PS dialyzers	166	31.5 ± 0.7		46	24.5 ± 2.0		165	44.7 ± 1.2	
Bleach + aldehyde[c]	57	29.9 ± 1.1	0.003[d]	6	25.0 ± 6.5	0.9	57	41.5 ± 2.0	
Bleach +renalin	102	33.1 ± 0.8		34	24.5 ± 2.5		101	45.7 ± 1.5	0.05[e]
Renalin alone	7	23.0 ± 3.5		6	21.9 ± 5.7		7	56.7 ± 6.3	

Abbreviations are: CA, cellulose acetate; CT, cellulose triacetate; CU, cuprophane dialyzers; PS, polysulfone dialyzers. fMLP-induced responses were only studied in 65 patients using PS dialyzers. PMA-induced responses were available in 205 patients; % phagocytosis index was available in 203 patients; reuse data was not available in 9 patients.
[a]Adjusted for year of enrollment; $P < 0.001$ in models for PMA-induced superoxide response as outcome.
[b]PS vs. CA/CT: $P < 0.001$; CU vs. CA/CT: $P = 0.01$; PS vs. CU: P = NS.
[c]Aldehyde includes formaldehyde or glutaraldehyde.
[d]Renalin alone vs. bleach + renalin: $P = 0.006$; bleach + renalin vs. bleach + aldehyde: $P = 0.03$.
[e]Renalin alone vs. bleach + renalin: $P = 0.09$; bleach + renalin vs. bleach + aldehyde: $P = 0.09$; renalin alone vs. bleach + aldehyde: $P = 0.03$.

production between CA/CT and either CU or PS membranes remained significant ($P < 0.001$), after further adjustment for Kt/V, number of years spent on HD, and reuse. Of these covariates, number of years spent on HD showed a significant inverse relationship with PMA-induced superoxide production.

Effect of flux. The effect of flux on PMA-induced superoxide production was studied in CA/CT and PS membranes. On adjustment for year of enrollment alone (Table 4), as well as further adjustment for membrane type, reuse, Kt/V, and number of years spent on HD, there was no significant difference in PMA-induced superoxide production between high- and low-flux dialyzers. However, PMA-induced superoxide production remained significantly higher in PMNL from patients using PS dialyzers compared to CA/CT dialyzers ($P < 0.001$), as demonstrated in the preceding section. Other variables—number of years spent on HD, dialyzer reuse, and eKt/V—showed no significant association with PMA-induced respiratory burst.

Effect of membrane and flux. High- and low-flux membranes were analyzed as separate strata. Fifty-six

Table 5. Multivariate analysis (multiple linear regression) to show the effect of dialysis- and reuse-associated variables on fMLP-stimulated superoxide production by PMNL among patients using PS dialyzers ($N = 65$)

Variable	Parameter estimate (PE)	95% CI for PE	P value
Dialzyer reuse (vs. single use)	9.1	0.8–17.4	0.03
No. of years on dialysis (per year)	0.1	−0.6–0.9	0.7
Kt/V (per 1.0 increase)	8.9	−11.4–29.3	0.4

Adjusted for year of enrollment (P = NS).

patients used low-flux membranes (CA = 4, CU = 21, and PS = 31), and 150 patients used high-flux membranes (8 CT and 142 PS). In the high-flux category, PMA-induced superoxide production was significantly lower in CT membranes compared with PS on adjustment for Kt/V, number of years spent on HD, and reuse (19.2 ± 3.0 vs. 31.0 ± 0.7; $P < 0.001$). In the low-flux category, the difference in PMA-induced superoxide production remained significant across membrane type (CA, CU, PS: 23.6 ± 5.2, 30.8 ± 2.9, 39.5 ± 2.3; $P = 0.03$). The comparison between CA and CU was significant ($P = 0.01$), although that between PS and CU was not ($P = 0.06$).

Stratification by membrane type showed no difference in PMA-induced superoxide production between high- and low-flux substituted cellulose membranes (CA vs. CT), or between high- and low-flux PS membranes (data not shown).

Effect of the three cleansing agent-germicide categories (bleach + aldehyde, bleach + renalin, renalin alone). This was studied in either CU or PS reused dialyzers. There were only 7 dialyzers reprocessed with renalin alone, without the concomitant use of bleach as a cleansing agent. However, the use of renalin alone was associated with significantly lower PMA-induced superoxide production compared with dialyzers in which both renalin and bleach were used (23.0 ± 3.5 vs. 33.1 ± 0.8; $P = 0.006$), or compared with dialyzers reprocessed with aldehydes and bleach (23.0 ± 3.5 vs. 29.9 ± 1.1; $P = 0.03$) (Table 4). These differences persisted when the effect of the bleach-germicide categories was further adjusted for Kt/V, number of years spent on HD, and membrane type (renalin alone vs. bleach + renalin: 24.5 ± 3.6 vs. 32.9 ± 0.9; $P = 0.02$; bleach + aldehyde vs. bleach + renalin: 29.9 ± 1.2 vs. 32.9 ± 0.9; $P = 0.05$). Of the above covariates, number of years spent on HD showed an inverse relationship with PMA-induced superoxide production ($P = 0.02$).

Effect of number of years spent on HD. Patients who had been on HD <1 year had higher respiratory burst activity in response to PMA in comparison to those on HD >1 year (34.4 ± 1.2 vs. 31.6 ± 0.8; $P = 0.05$). As demonstrated in the earlier adjusted analyses for effects of membrane, flux and bleach-sterilant combinations, respectively, number of years spent on HD used as a covariate, showed an inverse relationship with PMA-induced superoxide production. The relationship was significant when all patients were studied (parameter estimate, PE = −0.3, $P = 0.03$), the model being adjusted for membrane type, Kt/V, and reuse. Similar analysis of the subset of patients reusing CU or PS dialyzers, adjusted for membrane type, bleach-germicide combination, and Kt/V were studied, showed an inverse relationship between number of years spent on HD and PMA-induced superoxide production (PE = −0.3, $P = 0.02$). Thus, each additional year on dialysis was associated with a decline in PMA-induced superoxide production roughly the magnitude suggested by the respective coefficients. Indeed, when patients were categorized by duration on HD of <1 year, 1 to 3 years, and >3 years, a graded decrease in the level of PMA-induced superoxide production was apparent (34.4 ± 1.2, 33.0 ± 1.1, 30.2 ± 1.1; $P = 0.03$). When only the subset of patients using CA/CT or PS dialyzers was analyzed, after adjustment for membrane flux there was a trend toward an inverse relationship between PMA-induced superoxide production and number of years spent on HD (PE = −0.3; $P = 0.07$).

Stratification for flux property showed that the inverse relationship between PMA-induced superoxide production and number of years spent on HD remained significant in high-flux dialyzers (PE = −0.4; $P = 0.03$), but not when only low-flux dialyzers were considered.

fMLP-induced superoxide production by PMNL among patients using PS dialyzers

Reuse was associated with significantly higher fMLP-stimulated superoxide production (24.4 ± 1.9 vs. 15.8 ± 3.5 for patients not reusing, $P = 0.04$) in 65 patients using PS dialyzers (Table 4). This difference remained significant ($P = 0.03$) after further adjustment for Kt/V and number of years spent on HD (Table 5). The model was not adjusted for flux, because all high-flux PS dialyzers and about half the low-flux PS dialyzers were reused. When the effect of reuse was assessed in only the subset of patients using low-flux dialysers, the association with higher fMLP-stimulated superoxide production did not reach statistical significance (23.2 ± 3.1 vs. 15.5 ± 3.0 for patients not reusing, $P = 0.09$). Further, the model also showed that the flux property of the dialyzer and bleach-germicide combination did not influence fMLP-stimulated superoxide production. This was also true when the subset of reused PS dialyzers were considered.

% Phagocytosis index

No association was demonstrable between membrane type, flux, or reuse, and % phagocytosis index. However, in those patients reusing (i.e., either CU or PS

membranes), the % phagocytosis index was significantly different in the three bleach-germicide categories, being highest with the use of renalin alone, followed by bleach + renalin and then bleach + aldehyde ($P = 0.05$). The difference between renalin alone versus bleach + renalin, and bleach + renalin versus bleach + aldehyde did not reach statistical significance ($P = 0.09$), whereas use of renalin alone versus bleach + aldehyde was significantly different ($P = 0.03$) (Table 4). On further adjustment for Kt/V, number of years spent on HD, and membrane type as covariates, the differences among the three bleach-germicide categories remained significant ($P = 0.05$). The use of renalin alone was associated with a higher % phagocytosis index than bleach + aldehyde (55.8 ± 6.5 vs. 41.2 ± 2.1; $P = 0.04$). While the use of bleach + renalin showed higher % phagocytosis index than bleach + aldehyde, this did not reach statistical significance (46.0 ± 1.6 vs. 41.2 ± 2.1; $P = 0.08$). The difference between use of renalin alone versus bleach + renalin did not reach statistical significance.

"Center effects"

Patients enrolled from a total of 10 dialysis units. Univariate analysis showed that significant differences in PMA-induced superoxide production ($P < 0.001$), and phagocytosis index ($P = 0.05$) existed among patients drawn from different units, although fMLP-induced superoxide production did not show a difference. This was attributable to the significant differences in dialysis- and reuse-related factors among these units. The type of membrane used differed: six units used exclusively PS membranes; at least two units did not practice reuse; and in those that did, at least three units used only a renalin-bleach combination, and two an aldehyde-bleach combination. As the dialysis unit appeared to cosegregate almost completely with one or more dialysis- or reuse-related factor, is was not included in multivariate adjustments.

Patient-related factors and clinical comorbidity associated with PMNL function

PMA-induced superoxide production by PMNL and patient related factors. PMA-induced superoxide production did not differ by age, gender, race, presence of diabetes, or type of vascular access. All analyses were adjusted for the year of enrollment into the study.

fMLP-induced superoxide production by PMNL and clinical comorbidity. fMLP-induced superoxide production was found to be significantly higher in African American than Caucasian subjects (27.9 ± 2.1 vs. 17.2 ± 2.0; $P < 0.001$), and marginally higher in female subjects (24.0 ± 1.9 vs. 18.2 ± 2.9 in males; $P = 0.09$). There was no relationship to age or number of years spent on HD. Patients dialyzing through AVG had the highest levels of fMLP-induced superoxide production, followed by patients dialyzing through AVF, with the lowest levels seen in patients with venous catheters (34.8 ± 4.1, 21.0 ± 1.7, 15.6 ± 4.8; $P = 0.005$). Patients with the highest ICED score showed significantly lower levels of fMLP-induced superoxide production by PMNL (17.2 ± 2.8 in patients with ICED score of 3 vs. 24.6 ± 1.8 in patients with ICED score of 1 or 2; $P = 0.03$). Similarly, fMLP-induced superoxide production by PMNL was lower in patients in the lowest quartile of Karnofsky scores, consisting of patients with scores of 40–70 (Q 1), versus those with scores of ≥80 (Q 2–4) (16.3 ± 2.9 in Q 1 vs. 24.6 ± 1.8 in Q 2–4; $P = 0.02$).

Multivariate analysis using logistic regression identified higher fMLP-induced superoxide production as the only significant factor associated with lower odds of the outcome of highest ICED score of 3 (OR = 0.94, 95% CI = 0.89–0.99; $P = 0.03$). Other independent variables that were not significant included year of enrollment, age, gender, race, and number of years spent on HD. When being in the lowest quartile of Karnofsky scoring was considered as the outcome variable, the significant variables in the equation were fMLP-induced superoxide production (OR = 0.91, 95% CI = 0.85–0.98; $P = 0.007$), age (OR = 1.07, 95% CI = 1.01–1.15; $P = 0.04$), and race (Caucasian vs. African American: OR = 0.24, 95% CI = 0.06–0.95; $P = 0.04$). The year of enrollment, gender, and number of years spent on HD were not significantly associated with the outcome.

% Phagocytosis index did not show any relationship to clinical or demographic parameters.

DISCUSSION

Bacterial infections are a frequent cause of morbidity and mortality in patients with end-stage renal disease (ESRD) on maintenance dialysis, and most studies indicate they are the second leading cause of death in this patient population [20]. PMNL are an integral component of host defense against bacterial infections, and there is substantial clinical evidence that indicates profound disturbances in PMNL function in patients on chronic hemodialysis. Impaired chemotaxis, phagocytic capacity, bactericidal activity, and metabolic dysfunction of these cells in HD patients have been reported [21]. Mechanisms ascribed to altered PMNL function in uremia include malnutrition, iron overload, and increased intracellular calcium [22]. In the present study, we examined the relationship of dialysis-related, as well as other clinical variables, to PMNL function in a cohort of patients with ESRD on long-term maintenance hemodialysis enrolled in the National Institutes of Health-sponsored HEMO Study.

Our results showed that use of synthetic dialyzer membranes (PS) and CU membranes were associated with

higher PMA-stimulated respiratory burst than CA/CT membranes. The flux property of the membrane did not appear to be important in influencing the PMNL functional parameters measured. While a difference between fMLP-induced superoxide production was demonstrable between reused and single-use PS dialyzers, we were unable to show a similar difference in PMA-induced superoxide production or the % phagocytosis index. However, when only reused dialyzers were analyzed, the use of bleach and renalin combination appeared to be associated with higher PMA-induced superoxide production, and the use of renalin alone with higher % phagocytosis index. In addition, there was also a significant inverse relationship between the number of years spent on HD and PMA-induced superoxide production, especially in high-flux dialyzers. Therefore, dialyzer membrane type (CA/CT vs. CU or vs. PS membranes), dialyzer reprocessing, and the number of years on HD influence PMNL function in patients on maintenance HD.

There is only limited information on the long-term impact of dialyzer membrane biocompatibility on PMNL function. Previous studies indicate that PMNL exposed to complement-activating dialyzer membranes generate ROS during dialysis [23]. Studies utilizing ex vivo models of hemodialysis have demonstrated significant priming of PMNL respiratory burst by CU, but not PS dialyzer membranes [24]. In the present study, patients dialyzing with CU membranes showed higher PMA responses compared to those dialyzing with substituted CA/CT membranes. Intradialytic generation of ROS has been observed 15 minutes after initiation of hemodialysis with CU membranes. These cells, however, become refractory to subsequent stimulation by complement products and fMLP [23]. Vanholder et al [11] studied the metabolic response of PMNL (glucose-1-C14 utilization in response to latex and zymosan) sequentially in a small cohort of patients with ESRD initiated on maintenance HD. In contrast to synthetic membranes (PS and polymethylmethacrylate), initiation of dialysis with an unsubstituted cellulose membrane (CU) led to a sharp decline in the metabolic response of these cells to phagocytic challenge after four weeks of hemodialysis. Further, this attenuated metabolic response was reversed after two weeks of hemodialysis with a synthetic membrane [11]. The abnormal response of PMNL exposed to CU membranes was believed to be related to a state of "deactivation" arising from recurrent complement activation and subsequent intradialytic PMNL stimulation. Over time, these cells could become relatively refractory to further challenge, particularly during the interdialytic period [25]. These latter observations are at variance with our data, as higher ROS production was still demonstrable with chronic use of CU membranes compared to CA/CT membranes. Our observations were based on the small proportion of patients who dialyzed with CU (20/206), were not sequential, and were drawn on patients who had been on long-term maintenance dialysis. However, a stratified analysis did suggest lower PMNL oxidative response to PMA with CU membranes compared to low-flux PS membranes.

Among the many partly interdependent factors contributing to PMNL dysfunction in advanced uremia, certain middle molecular weight uremic toxins appear to play an important role [22]. Plasma levels of many of these peptides, such as granulocyte inhibitory protein I and II, degranulation inhibiting protein I (identical to angiogenin), and degranulation inhibiting protein II (complement factor D), are markedly elevated in ESRD patients and interfere with several facets of PMNL function, including chemotaxis, oxidative response to PMA, and fMLP and bactericidal activity [21, 22]. High-flux synthetic dialyzers (PS and AN69) have been shown to produce significant reduction in plasma levels of these middle molecular weight proteins [21]. While bioincompatibility could explain the higher oxidative response seen with CU membranes, more effective clearance of middle molecular weight proteins may explain that observed with PS membranes in this study.

The influence of dialyzer reprocessing on PMNL function has not been previously reported. We observed a relationship between reuse and associated variables and enhanced PMNL response to PMA and fMLP, as well as phagocytosis. Recent data from the Hemodialysis Study Group showed increased middle molecule clearance of synthetic membranes after reuse with bleach, particularly for high-flux PS membranes [26]. Other studies have indicated that high-flux dialyzer membranes reprocessed with renalin show a reduction in clearance of β2-microglobulin [27]. However, in the present study, for the majority of patients using dialyzers reprocessed with renalin, bleach was utilized as a cleansing agent (Table 3). It is possible that a reuse procedure that improves dialyzer flux property may result in improved clearance of these PMNL inhibitory proteins and consequently lead to improved PMNL functional response. On the other hand, there have been a few large representative cohort studies linking reuse with infection risk, and enhanced PMNL functional response with reuse may reflect PMNL priming by bacterial products. In a longitudinal cohort study of incident ESRD patients in the case-mix study of the USRDS, Powe et al [28] observed that patients who reused had a 28% higher risk of septicemia than patients who did not reuse dialyzers over the entire follow-up period of seven years. However, the relative effects of specific sterilants or bleach were not evaluated in this study. Indeed, in the USRDS Dialysis Morbidity and Mortality Study, Port et al [29] observed substantially lower mortality for synthetic dialyzers treated with bleach compared with no bleach during the reuse procedure. To date, few clinical studies have investigated the influence of dialyzer biocompatibility on susceptibility to bacterial infections.

Two studies have shown lower infective morbidity with PS dialyzers compared to CU dialyzers [11]. The clinical impact of dialyzer membrane type and reuse associated effects on PMNL function requires further elucidation.

It is therefore apparent that several mechanisms, including biocompatibility, middle molecule clearance, neutrophil priming by bacterial products, and development of a relative refractoriness to oxidant stress over time may all impact on PMNL oxidative response. The association between decrease in PMA-induced superoxide production, especially in high-flux dialyzers, and number of years spent on HD may therefore reflect interplay between any of the above factors and will be difficult to resolve in a cross-sectional study. Previous work from this center demonstrated an inverse correlation between cytokine (interleukin-1 receptor antagonist) synthesis by peripheral blood mononuclear cells (PBMC) and number of years spent on HD [30]. These observations may be an indication of impaired host defense mechanisms in patients on long-term HD, and prospective outcome-related evaluation is required to study this further.

We also observed a strong relationship between lower response of PMNLs to fMLP, and worse comorbidity in terms of higher ICED scores and lower Karnofsky indices. These associations remained significant even after adjustment for age, gender, race, and duration spent on HD, indicating a link between diminished PMNL respiratory burst activity and chronic comorbidity in long-term HD patients. However, due to the cross-sectional nature of this study, a causal relationship cannot be inferred. Further, our observation that African American subjects showed an enhanced response of PMNL to fMLP is consistent with previous observations that African American patients on HD have lower infection-related mortality compared to Caucasians [20]. It is also significant that patients dialyzing off venous catheters had lower levels of fMLP-induced superoxide production, considering the higher risks of infection with this access type.

There are several limitations in this study that require mention. First, it is cross-sectional and observational; hence, inferences can only be drawn regarding associations between PMNL function and explanatory variables addressed in the study. Second, being ancillary to the HEMO Study, patient selection and design could not be controlled to answer specific questions related to PMNL function. Specifically, information regarding characteristics of reuse practice, differences between facilities, and duration of exposure to a particular dialyzer cleansing or germicidal agent may have had an impact on the relationship between reuse and PMNL function, and could not be accounted for in the analysis. Although there were significant associations between the individual dialysis unit and PMNL function, the "Center effect" could not be dissociated from other dialysis- and reuse-related factors because of practice differences. Further, HEMO Study participants represented a very select population with predominantly prevalent patients (average duration on HD >3years), and the results may not be easily generalizable. Finally, while we were able to demonstrate relationships between diminished PMNL ROS production and more severe comorbidity, the ability to interpret the clinical significance of changes in PMNL ROS production is limited.

CONCLUSION

Clearly, our observations on the association of membrane biocompatibility and reuse characteristics with PMNL function, as well as the association with comorbidity indices, require confirmation in a prospective study. This is currently in progress with our cohort of patients enrolled in the Hemodialysis Study.

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Reprint requests to Vaidyanathapuram S. Balakrishnan, M.D., Assistant Professor of Medicine, Division of Nephrology, Tufts-New England Medical Center, 750 Washington Street, Box 391 Boston, MA 02111.
E-mail: vbalakrishnan@tufts-nemc.org

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