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Dialysis Fluid Endotoxin Level and Mortality in Maintenance Hemodialysis: A Nationwide Cohort Study

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Background: The quality of dialysis fluid water might play an important role in hemodialysis patient outcomes. Although targeted endotoxin levels of dialysis fluid vary among countries, evidence of the contribution of these levels to mortality in hemodialysis patients is lacking.

Study Design: Retrospective cohort study using data from the Japan Renal Data Registry, a nationwide annual survey.

Setting & Participants: 130,781 patients receiving thrice-weekly in-center hemodialysis for more than 6 months were enrolled at 2,746 facilities in Japan at the end of 2006. None of the patients changed facility or treatment modality during 2007.

Predictor: Highest endotoxin level in dialysis fluid reported by each facility during 2006. Patients were categorized by facility endotoxin level into the following groups: <0.001, 0.001 to <0.01, 0.01 to <0.05, 0.05 to <0.1, and ≥0.1 EU/mL. Age, sex, dialysis vintage, diabetes mellitus as a primary cause of end-stage renal disease, Kt/V, normalized protein catabolic rate, dialysis session duration, serum albumin, and hemoglobin were measured as potential confounders.

Outcome: All-cause mortality, censored by transplantation; withdrawal from dialysis treatment; or end of follow-up.

Results: Of 130,781 hemodialysis patients, 91.2% had facility endotoxin levels below the limit set for dialysis fluid in Japan (<0.05 EU/mL). During a 1-year follow-up, 8,978 (6.9%) patients died of all causes. The rate of all-cause mortality at 1 year was highest in the ≥0.1-EU/mL category (88.0 deaths/1,000 person-years). Patients in the ≥0.1-EU/mL group exhibited an increased risk of all-cause mortality of 28% (95% CI, 10%-48%) compared to the <0.001-EU/mL group.

Limitations: Endotoxin level in dialysis fluid is reported as categorical data. No information about variation in endotoxin levels in dialysis fluid over time.

Conclusions: Higher facility endotoxin levels in dialysis fluid may be related to increased risk for all-cause mortality among hemodialysis patients. Correcting this modifiable facility water management practice might improve the outcome of hemodialysis patients.

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INDEX WORDS: Hemodialysis (HD); end-stage renal disease (ESRD); mortality; dialysis fluid; ultrapure dialysate; water quality; endotoxin level; bacterial contamination; microbial contamination; dialysis facility; Japan Renal Data Registry (JRDR).

Editorial, p. 817

Because hemodialysis (HD) patients are exposed to a relatively large volume (350-500 L) of dialysis fluid every week, depending on session duration and flow rate, they are particularly vulnerable to contaminants in this fluid. Furthermore, the thin dialysis membrane between blood and dialysis fluid for HD patients might facilitate transfer of toxins directly into the bloodstream, adversely affecting the outcome of HD patients.

The Japanese Society for Dialysis Therapy (JSDT) therefore has advocated strict standards to ensure the cleanliness of dialysis fluid,[1] with most dialysis facilities in Japan achieving high-quality water management practices for dialysis fluid.[2]

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A number of benefits of ultrapure dialysis fluid in HD patients have been reported in case-series studies for surrogate clinical outcomes, such as prevention of carpal tunnel syndrome,[3] improvement in nutritional and inflammatory parameters,[4] increase in erythropoietin responsiveness,[5] and preservation of residual kidney function.[6] However, to our knowledge, few large cohort studies have evaluated the association between water quality and mortality in HD patients.

The relatively low mortality rate of Japanese HD patients compared with those of other countries might be due in part to the high standard of quality control for dialysis fluid. In this study, we investigated the impact of endotoxin levels in dialysis fluid, one of the most important water quality indexes, on the mortality of in-center HD patients using the Japan Renal Data Registry (JRDR) of the JSDT.

METHODS

Data Sources and Participant Selection

In this cohort study, data were obtained from the annual JRDR census, which is conducted nationwide by the JSDT. This census of dialysis patients in Japan is conducted on a voluntary basis at the end of each year. The JRDR contains data including demographics (eg, age, sex, primary cause of end-stage renal disease [ESRD], and dialysis vintage), laboratory examination findings (eg, serum creatinine, serum urea nitrogen, serum albumin, and hemoglobin values), and clinical indexes regarding dialysis treatment (eg, Kt/V, normalized protein catabolic rate [nPCR], and session duration). Detailed information of the JRDR has been described previously.[7] Using a standard analysis file (JRDR09103) obtained with permission from the JSDT (Fig 1), we analyzed 130,781 in-center HD patients who received dialysis fluid with an endotoxin level at baseline, received 3 HD sessions per week, underwent at least 6 months of treatment by the end of 2006, and did not change dialysis facilities or modalities during 2007.[8,9] A nationwide statistical survey of the JRDR for 2007 was conducted for all dialysis facilities in Japan (n = 4,098), with 4,052 (98.9%) facilities responding to the survey.[8]

Definition of Main Exposure: Endotoxin Level in Dialysis Fluid

The main exposure to be tested was facility-reported endotoxin level in dialysis fluid, which is a facility water quality indicator, reported by 2,746 facilities at the end of 2006. The highest endotoxin level in dialysis fluid during a year at each facility was reported by the manager in the annual questionnaire. Facility endotoxin levels were categorized into 5 groups on the JRDR questionnaire: <0.001 (including undetectable level) as reference, 0.001 to <0.01, 0.01 to <0.05, 0.05 to <0.1, and ≥0.1 EU/mL.

Outcome Measures

All-cause mortality during the study period was set as the main outcome measure. Mortality data were collected from the JRDR from December 31, 2006, through December 31, 2007 (follow-up, 1-12 months). Time at risk was from the start date of observation (January 1, 2007) until all-cause death, departure from the observation (as a result of transplantation or withdrawal from dialysis treatment), or end of follow-up (December 31, 2007).

Statistical Analysis

The hazard ratio (HR) of all-cause death during the study period was estimated using Cox proportional hazard regression analysis. The adjusted model was controlled for age, sex, dialysis vintage, diabetes mellitus as a primary cause of ESRD or not, Kt/V, nPCR, session duration, serum albumin level, and hemoglobin level. All models accounted for facility clustering effect. Proportional hazard assumptions of Cox regression analysis were checked graphically and with Schoenfeld residuals. An ordinal variable was created to assess the trend across the 5 different endotoxin levels in dialysis fluid. Multiple imputation using chained equations was used to estimate the models with missing covariates.[10] Complete data set analysis with list-wise deletion also was performed as a sensitivity analysis. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with SAS (version 9.2; SAS Institute Inc) and Stata/SE (version 12.1; StataCorp LP) for Microsoft Windows software.

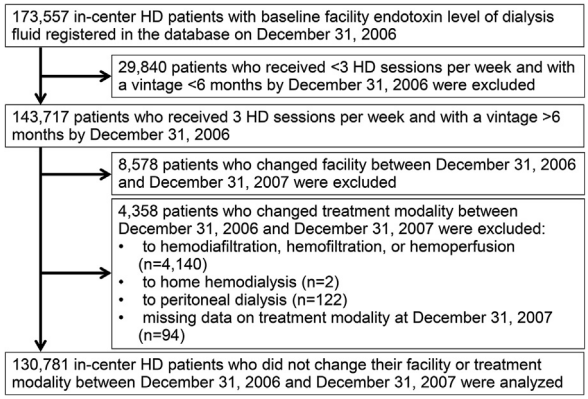

Figure 1. Selection process for analyzed patients using data from the Japan Renal Data Registry. Abbreviation: HD, hemodialysis.

RESULTS

Study Population and Demographic Characteristics of Patients

A total of 130,781 patients receiving in-center HD were analyzed. Figure 1 shows the selection process for these patients using JRDR data from 2006 through 2007. Table 1 lists patient characteristics at baseline overall and stratified by the 5 categories of facility endotoxin level in dialysis fluid.

Of 130,781 HD patients, mean age was 64.8 years, 61.3% were men, mean dialysis vintage was 7.47 years, and diabetes was diagnosed as a primary cause of ESRD in 32.4% (Table 1). Clinical index of dialysis treatment and laboratory findings also are listed in Table 1. Overall mean Kt/V was 1.37, nPCR was 0.90 g/kg/d, dialysis session duration was 234 minutes, serum albumin level was 3.78 g/dL, and hemoglobin level was 10.3 g/dL. Clinical characteristics were similar across endotoxin level groups.

Distribution of Endotoxin Levels in Dialysis Fluid

Figure 2 shows the distribution of facility endotoxin levels in dialysis fluid used to treat in-center HD patients in Japan. The JSDT sets the maximum acceptable level of endotoxins at 0.05 EU/mL in standard dialysis fluid and 0.001 EU/mL in ultrapure dialysis fluid. The proportion of Japanese HD patients

Table 1. Patient Demographic Characteristics by Facility Dialysis Fluid Endotoxin Level

	Overall (N = 130,781)	<0.001 EU/mL (n = 43,436)	0.001-<0.01 EU/mL (n = 50,284)	0.01-<0.05 EU/mL (n = 25,527)	0.05-<0.1 EU/mL (n = 6,392)	≥0.1 EU/mL (n = 5,142)
Age (y)	64.8 ± 12.6	64.7 ± 12.5	64.9 ± 12.6	64.7 ± 12.7	64.8 ± 12.6	65.0 ± 12.5
Male sex	61.3	61.3	60.8	61.7	62.6	60.9
Dialysis vintage (y)	7.47 ± 6.47	7.33 ± 6.39	7.46 ± 6.46	7.65 ± 6.55	7.52 ± 6.40	7.74 ± 6.78
Diabetes as cause of ESRD	32.4	32.9	32.5	31.8	33.0	30.9
Kt/V	1.37 ± 0.28	1.37 ± 0.28	1.39 ± 0.28	1.36 ± 0.29	1.35 ± 0.30	1.35 ± 0.29
nPCR (g/kg/d)	0.90 ± 0.19	0.91 ± 0.19	0.90 ± 0.19	0.91 ± 0.18	0.89 ± 0.18	0.90 ± 0.19
Session duration (min)	234 ± 28.1	234 ± 27.9	235 ± 28.0	234 ± 28.1	232 ± 27.7	231 ± 29.2
Serum albumin (g/dL)	3.78 ± 0.42	3.78 ± 0.42	3.76 ± 0.41	3.79 ± 0.43	3.80 ± 0.40	3.80 ± 0.42
Hemoglobin (g/dL)	10.3 ± 1.27	10.4 ± 1.25	10.3 ± 1.27	10.3 ± 1.29	10.2 ± 1.31	10.2 ± 1.28

Note: Values for categorical variables are given as percentages; values for continuous variables, as mean ± standard deviation.
Abbreviations: ESRD, end-stage renal disease; nPCR, normalized protein catabolic rate.

exposed to the JSDT-set acceptable endotoxin level in standard dialysis fluid (<0.05 EU/mL) was 91.2%. The endotoxin level in dialysis fluid of nearly one-third of Japanese HD patients met the ultrapure target level of the JSDT (<0.001 EU/mL).

Outcome Data

During 125,610.2 person-years of follow-up (n = 130,781 patients), 8,978 (6.9%) deaths were recorded (Table 2). Of 130,781 patients analyzed in total, 142 were censored due to transplantation (n = 116) or withdrawal from dialysis treatment (n = 26) during 1 year. Table 2 lists rates of all-cause mortality for in-center HD patients at 1 year stratified by endotoxin level in dialysis fluid. Overall all-cause mortality rate of patients at 1 year was 71.5 (95% confidence interval [CI], 70.0-73.0) deaths/1,000 person-years. All-cause mortality rate at 1 year was highest for patients in the ≥0.1-EU/mL group (88.0 [95% CI, 80.1-96.7] deaths/1,000 person-years) and lowest in the <0.001-EU/mL (JSDT ultrapure) group (66.6 [95% CI, 64.2-69.1] deaths/1,000 person-years).

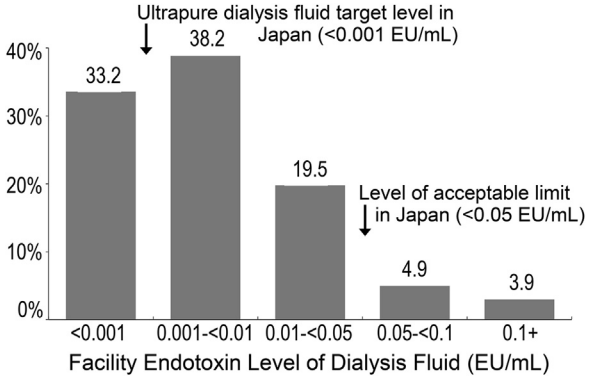

Figure 2. Distribution of facility dialysis fluid endotoxin levels. Data relate to in-center hemodialysis patients in Japan.

Endotoxin Level in Dialysis Fluid and Mortality Risk

HRs of all-cause mortality for in-center HD patients stratified by facility endotoxin level in dialysis fluid are shown in Fig 3. For all-cause mortality in comparison to the reference category (<0.001 EU/mL; JSDT ultrapure), Fig 3A (crude model) illustrates that increased dialysis fluid endotoxin levels had the following HRs for each dialysis fluid endotoxin level category: 1.08 (95% CI, 0.99-1.18) for 0.001 to <0.01 EU/mL, 1.12 (95% CI, 1.01-1.24) for 0.01 to <0.05 EU/mL, 1.14 (95% CI, 0.96-1.35) for 0.05 to <0.1 EU/mL, and 1.32 (95% CI, 1.10-1.59) for ≥0.1 EU/mL (P for trend < 0.001).

A monotonic increase in all-cause mortality risk as facility dialysis fluid endotoxin level increases was found after adjusting for potential confounding factors such as age, sex, dialysis vintage, diabetes mellitus as a primary cause of ESRD, dialysis session duration, Kt/V, nPCR, and serum albumin and hemoglobin levels (Fig 3B). HRs for all-cause mortality were slightly attenuated after multivariable adjustment for each endotoxin level in dialysis fluid category versus that of <0.001 EU/mL, as follows: 1.04 (95% CI, 0.96-1.13) for 0.001 to <0.01 EU/mL, 1.10 (95% CI, 1.00-1.20) for 0.01 to <0.05 EU/mL, 1.14 (95% CI, 0.98-1.32) for 0.05 to <0.1 EU/mL, and 1.28 (95% CI, 1.10-1.48) for ≥0.1 EU/mL (P for trend < 0.001). In other words, in-center HD patients exposed to a dialysis fluid endotoxin level ≥ 0.1 EU/mL had a 28% (95% CI, 10%-48%) increased risk of all-cause mortality compared with those with a dialysis fluid endotoxin level < 0.001 EU/mL (JSDT ultrapure), which was the reference group.

Results were obtained using a multiple imputation approach (n = 130,781) and were near-identical to those based on complete data set analysis using listwise deletion (n = 114,207). The corresponding values in complete data set analysis (n = 114,207)

Table 2. All-Cause Mortality Rate at 1 Year for In-center Hemodialysis Patients by Facility Dialysis Fluid Endotoxin Level

	No. of Deaths	Person-y	Rate/1,000 Person-y (95% CI)
Overall	8,978	125,610.2	71.5 (70.0-73.0)
By endotoxin level			
<0.001 EU/mL	2,786	41,820.9	66.6 (64.2-69.1)
0.001-<0.01 EU/mL	3,469	48,281.3	71.8 (69.5-74.3)
0.01-<0.05 EU/mL	1,827	24,485.8	74.6 (71.3-78.1)
0.05-<0.1 EU/mL	465	6,123.5	75.9 (69.3-83.2)
≥0.1 EU/mL	431	4898.7	88.0 (80.1-96.7)

Abbreviation: CI, confidence interval.

were 1.03 (95% CI, 0.0.94-1.12) for 0.001 to <0.01 EU/mL, 1.11 (95% CI, 1.00-1.23) for 0.01 to <0.05 EU/mL, 1.15 (95% CI, 0.98-1.34) for 0.05 to <0.1 EU/mL, and 1.25 (95% CI, 1.06-1.47) for ≥0.1 EU/mL.

DISCUSSION

To investigate the influence of the facility endotoxin level in dialysis fluid on mortality of in-center HD patients, we analyzed data from a nationwide JRDR annual inventory survey of Japanese dialysis patients conducted by the JSDT. We found that HD patients exposed to higher endotoxin levels in dialysis fluid had an increased risk of all-cause mortality compared with those exposed to lower levels. Specifically, patients exposed to ≥0.1 EU/mL of endotoxin in dialysis fluid had a 28% higher risk of all-cause mortality than those exposed to <0.001 EU/mL (ultrapure endotoxin target level set by the JSDT for dialysis fluid). Our results indicate a high achievement rate for the strict standards of dialysis fluid purity set by the JSDT for Japanese in-center HD patients, with 91.2% of patients exposed to an endotoxin level meeting the acceptable limit for dialysis fluid (<0.05 EU/mL). This strict management of dialysis fluid water quality may be a factor in the increased rate of survival of HD patients in Japan.

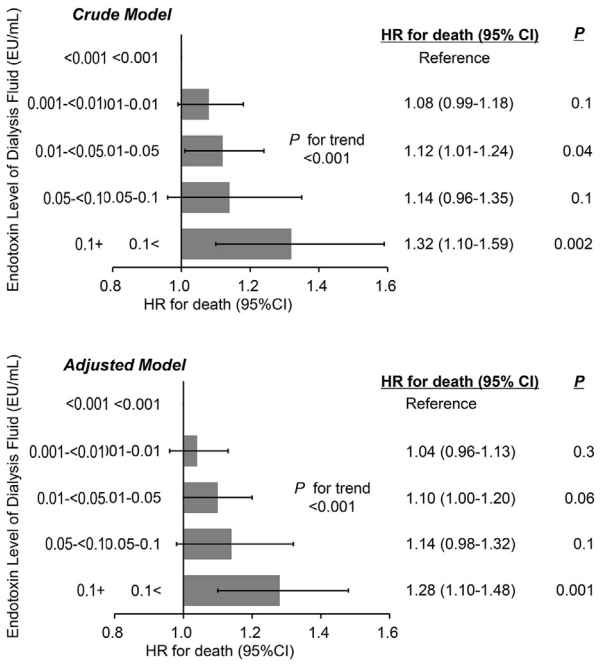

Figure 3. Hazard ratio (HR) of all-cause mortality for in-center hemodialysis patients stratified by facility dialysis fluid endotoxin level, according to crude (upper panel) and adjusted (lower panel) models. Adjusted covariates included age, sex, dialysis vintage, diabetes mellitus as primary cause of end-stage renal disease, Kt/V, normalized protein catabolic rate, dialysis session duration, serum albumin level, and hemoglobin level. Abbreviation: CI, confidence interval.

A variety of high-flux dialysis membranes were applied to HD patients in the 1980s. Since then, there have been warnings about the risk of backfiltration or backdiffusion of bacterial contamination through these high-flux membranes.[11] One hypothesis is that this contamination might play an important role in reducing the biocompatibility of HD therapy and in the release of inflammatory cytokines, which might result in patient outcomes such as hypotension and fever during and after HD sessions.[12] Contaminated dialysis fluid therefore is recognized as one of the most influential factors in poor HD patient outcomes. For example, complications of dialysis-related amyloidosis, such as carpal tunnel syndrome, were reported among the various complications that might be induced by contaminated dialysis fluid, the onset of which was reduced by using ultrapure dialysis fluid.[3] Biological contamination of dialysis fluid might lead to chronic inflammatory responses in HD patients.[13] Chronic inflammation generally is acknowledged as one of the risk factors for mortality in HD patients.[14] Ultrapure dialysis fluid recently was reported to improve the chronic inflammatory status indicated by high-sensitivity C-reactive protein level, and a possible contribution to reduced risk of mortality for HD patients was proposed.[15]

To our knowledge, no reports have been published regarding the association of dialysis fluid endotoxin level with risk of mortality among HD patients or an acceptable level of endotoxins. Our results therefore might provide a possible threshold level for endotoxins in dialysis fluid, which when breached, results in worsened prognosis. Conversely, refining the management of dialysis fluid water might improve the prognosis of HD patients. Management practices for dialysis fluid water can be improved by constructing a high-quality water supply system, but this will require the commitment of skilled clinical engineers.

The data source of the present study is the nationwide JRDR survey of Japanese dialysis patients, which provides an entire picture of HD patients in Japan.

More than 130,000 patients receiving in-center HD were examined. Consistency in results was improved following adjustment for important possible confounders, such as patient demographics, primary cause of ESRD, dialysis prescription and indicators, and laboratory examination data.

The present study contains a number of limitations that warrant attention. First, center effects unrelated to the facility dialysis fluid endotoxin level may have occurred. The key exposure to be tested in this study—the highest dialysis fluid endotoxin level during 2006—was obtained only from the annual questionnaire reported by the manager of each facility. The validity of this measurement therefore might be limited. The annual questionnaire asked for the highest endotoxin level in the facility's dialysis fluid during 2006, capturing peak exposure but not average levels or interfacility variability in endotoxin levels during the study period. Furthermore, other indicators of water quality management for HD patients, such as microbial contamination of dialysis fluid represented by bacterial count, were not considered. However, while the endotoxin level in dialysis fluid can be determined in a few hours, bacterial culture of dialysis fluid requires approximately 1 week of incubation. The dialysis fluid endotoxin level therefore is considered the most reliable, convenient, and representative marker for the evaluation of bacteriological water quality in dialysis units. However, discrepancies may remain between the dialysate endotoxin level and bacterial count in dialysis fluid.[16]

In the present study, only data from the JRDR survey were analyzed. The distribution of facility endotoxin concentrations in dialysis fluid in Japan might differ from that of other countries due to the variation in the limit of the endotoxin level in dialysis fluid. In contrast to the threshold of the dialysis fluid endotoxin level advocated by the JSDT (<0.05 EU/mL), the European Best Practice Guidelines set its maximum limit for the dialysis fluid endotoxin level to be 0.25 EU/mL.[17] In the United States, the Association for the Advancement of Medical Instrumentation advocates its revised standards for water to prepare dialysis fluid. Under this guideline, a 2-grade endotoxin level in dialysis fluid was proposed, with an action level of 1 EU/mL and limit level of 2 EU/mL.[18] Comparison of the standards for dialysis fluid for HD and related therapies between countries is summarized in Table 3. In Japan, the JSDT-defined target level for ultrapure dialysis fluid (<0.001 EU/mL) applies not only in hemodiafiltration but also in standard HD treatment.[19] Our findings suggest that this ultrapure level of endotoxin in dialysis fluid should be aimed for as much as possible.

The survival rate of HD patients in Japan is the highest among developed countries[20] and baseline hazards of mortality markedly differ among these countries. In addition, the central dialysis fluid delivery system (CDDS) is commonly used in Japan, a practice that is distinct from other countries in which personal dialysis machines are mostly used. Thus, results of the present study might be difficult to extrapolate to the different settings in other countries.

Table 3. Comparison of Water Quality for Hemodialysis and Related Therapies

	JSDT (2008)	AAMI (2004)	EBPG (2002)
Endotoxin level (EU/mL)			
Dialysis water	<0.05	<2	<0.25
Standard dialysis fluid	<0.05	<2	<0.25
Ultrapure dialysis fluid	<0.001 (ND)	<0.03 (ND)	<0.03 (ND)
Online-prepared substitution	<0.001 (ND)	<0.001 (ND)	<0.001 (ND)
Bacterial count (CFU/mL)			
Dialysis water	<100	<200	<100
Standard dialysis fluid	<100	<200	<100
Ultrapure dialysis fluid	<0.1	<0.1	<0.1
Online-prepared substitution	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$

Abbreviations: AAMI, Association for the Advancement of Medical Instrumentation; CFU, colony-forming unit; EBPG, European Best Practice Guidelines; JSDT, Japanese Society for Dialysis Therapy; ND, not detectable.

Because this is an observational study, the association found might have unmeasured or unidentified confounding factors. However, patient demographic characteristics were nearly identical across groups regardless of the endotoxin level in dialysis fluid, which might provide a natural quasi-experimental setting. The impact of the residual confounding therefore might be limited. Finally, observational studies can only infer an association between the exposure to be tested and outcomes—not causality. Because randomized controlled trials are the gold standard for determining the efficacy of interventional treatments, a well-designed clinical trial to clarify the impact of dialysis fluid endotoxin level on in-center HD patient outcomes is required. However, at present, our observational study might provide some useful insights.

In conclusion, our results suggest that an increased facility endotoxin level in dialysis fluid, which is a water quality index, is indicative of poor water quality management practices and related to an increased risk of all-cause mortality among in-center Japanese HD patients. Improving facility practice regarding water quality management might improve the outcomes of HD patients.

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Contributions: Study concept and design: TH, SN, IM; data analysis/interpretation: TH, SN, IM; supervision: YW, KI, YT, TA. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. TH, SN, and KI take responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

REFERENCES

1. Kawanishi H, Akiba T, Masakane I, et al. Standard on microbiological management of fluids for hemodialysis and related therapies by the Japanese Society for Dialysis Therapy 2008. *Ther Apher Dial*. 2009;13(2):161-166.

2. Masakane I, Tsubakihara Y, Akiba T, Watanabe Y, Iseki K. Bacteriological qualities of dialysis fluid in Japan as of 31 December 2006. *Ther Apher Dial*. 2008;12(6):457-463.

3. Baz M, Durand C, Ragon A, et al. Using ultrapure water in hemodialysis delays carpal tunnel syndrome. *Int J Artif Organs*. 1991;14(11):681-685.

4. Schiffl H, Lang SM, Stratakis D, Fischer R. Effects of ultrapure dialysis fluid on nutritional status and inflammatory parameters. *Nephrol Dial Transplant*. 2001;16(9):1863-1869.

5. Matsuhashi N, Yoshioka T. Endotoxin-free dialysate improves response to erythropoietin in hemodialysis patients. *Nephron*. 2002;92(3):601-604.

6. Schiffl H, Lang SM, Fischer R. Ultrapure dialysis fluid slows loss of residual renal function in new dialysis patients. *Nephrol Dial Transplant*. 2002;17(10):1814-1818.

7. Iseki K, Nakai S, Yamagata K, Tsubakihara Y. Tachycardia as a predictor of poor survival in chronic haemodialysis patients. *Nephrol Dial Transplant*. 2011;26(3):963-969.

8. Nakai S, Masakane I, Shigematsu T, et al. An overview of regular dialysis treatment in Japan (as of 31 December 2007). *Ther Apher Dial*. 2009;13(6):457-504.

9. Nakai S, Masakane I, Akiba T, et al. Overview of regular dialysis treatment in Japan as of 31 December 2006. *Therc Apher Dial*. 2008;12(6):428-456.

10. Royston P, White I. Multiple imputation by chained equations (MICE): implementation in Stata. *J Stat Software*. 2011;45(4):1-20.

11. Baurmeister U, Travers M, Vienken J, et al. Dialysate contamination and back filtration may limit the use of high-flux dialysis membranes. *ASAIO Trans*. 1989;35(3):519-522.

12. Henderson LW, Koch KM, Dinarello CA, Shaldon S. Hemodialysis hypotension: the interleukin hypothesis. *Blood Purif*. 1983;1(1):3-8.

13. Lonnemann G. Chronic inflammation in hemodialysis: the role of contaminated dialysate. *Blood Purif*. 2000;18(3):214-223.

14. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int*. 1999;55(2):648-658.

15. Honda H, Suzuki H, Hosaka N, et al. Ultrapure dialysate influences serum myeloperoxidase levels and lipid metabolism. *Blood Purif*. 2009;28(1):29-39.

16. Ledebo I, Nystrand R. Defining the microbiological quality of dialysis fluid. *Artif Organs*. 1999;23(1):37-43.

17. European Best Practice Guidelines. Section IV. Dialysis fluid purity. *Nephrol Dial Transplant*. 2002;17(suppl 7):45-62.

18. AAMI Renal Disease and Detoxification Committee. Dialysate for hemodialysis. Arlington, VA: AAMI; 2004:24-27.

19. Masakane I. Review: clinical usefulness of ultrapure dialysate—recent evidence and perspectives. *Ther Apher Dial*. 2006;10(4):348-354.

20. Goodkin DA, Bragg-Gresham JL, Koenig KG, et al. Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *J Am Soc Nephrol*. 2003;14(12):3270-3277.