Tanemoto et al. Renal Replacement Therapy (2018) 4:42
https://doi.org/10.1186/s41100-018-0188-1

Renal Replacement Therapy

RESEARCH Open Access

Comparison of intradialytic plasma volume change between online hemodiafiltration and standard hemodialysis

Masayuki Tanemoto[1,2*], Yu Ishimoto[2], Yukio Kosako[2] and Yukio Okazaki[2]

Abstract

Background: Intradialytic plasma volume change (IPVC) owing to ultrafiltration is a dominant cause of intradialytic hypotension (IDH). Lower occurrence of IDH in hemodiafiltration (HDF) than in standard hemodialysis (std-HD) indicates that HDF has preventive effect on IPVC. This study examined whether online HDF (ol-HDF) prevented IPVC compared with std-HD.

Methods: In 115 std-HD, 59 push-pull ol-HDF (pp-ol-HDF), 39 pre-dilution ol-HDF (pre-ol-HDF), and 33 post-dilution ol-HDF (post-ol-HDF) sessions, we calculated IPVC using intradialytic change of hemoglobin ($IPVC_{Hb}$) and also using the change of hematocrit ($IPVC_{Ht}$), and compared them in association with intradialytic body weight loss (IBWL) between the dialysis modes.

Results: Both $IPVC_{Hb}$ and $IPVC_{Ht}$ were not different between the modes; in std-HD, pp-ol-HDF, pre-ol-HDF, and post-ol-HDF, $IPVC_{Hb}$ were 0.10 ± 0.06, 0.11 ± 0.07, 0.10 ± 0.05, and 0.10 ± 0.06, respectively, and $IPVC_{Ht}$ were 0.10 ± 0.07, 0.11 ± 0.07, 0.10 ± 0.06, and 0.10 ± 0.06, respectively. IPVCs divided by relative IBWL to DW (IBWL/DW) were also not different between the modes; in std-HD, pp-ol-HDF, pre-ol-HDF, and post-ol-HDF, $IPVC_{Hb}$/IBWL/DW were 1.9 ± 2.0, 1.7 ± 4.6, 2.1 ± 0.9, and 2.1 ± 1.8, respectively, and $IPVC_{Ht}$/IBWL/DW were 1.9 ± 2.2, 1.9 ± 3.7, 2.1 ± 1.1, and 2.1 ± 2.0, respectively.

Conclusion: Irrespective of the modes, ol-HDF has the same level of IPVC as std-HD.

Keywords: Hemodiafiltration, Hemodialysis, Hypotension, Plasma volume, Ultrafiltration

Background

Hemodialysis is a renal replacement therapy for end-stage renal disease [1]. As an outpatient-based treatment, maintenance hemodialysis is performed intermittently (2–3 sessions per week) and for short duration (3–5 h) per session. The intermittent treatment inevitably leads to fluid accumulation between sessions, which needs to be removed by ultrafiltration during each session [2]. The removal induces intradialytic plasma volume change (IPVC), which is a dominant cause of intradialytic hypotension (IDH); the reduction in systolic blood pressure was related to the reduction in IPVC, and an individual threshold of IPVC was noted for IDH [3–5].

Hemodiafiltration (HDF) is a mode of hemodialysis performed by a combination of diffusive transport and convective transport where appropriate fluid balance is maintained by infusing substitution fluid [6]. Lower occurrence of IDH in HDF than in standard hemodialysis (std-HD), where only diffusive transport is performed, indicates that convective transport can provide preventive effect on IPVC [7–9]. Several different modes of HDF are available depending on the site of substitution fluid infusion; the fluid is infused upstream, downstream, and through the membrane of the dialyzer in pre-dilution mode (pre-HDF), post-dilution mode (post-HDF), and push-pull mode (pp-HDF), respectively [6]. Preparation of infusion fluid from non-pyrogenic dialysate further enabled an 'online' option for HDF (ol-HDF) [10]. The preventive effect of HDF on IPVC would differ depending on the modes. In the present report, we

* Correspondence: mtanemoto-tky@umin.ac.jp
[1]Division of Nephrology, Department of Internal Medicine, 418-1 Kamihayami, Kuki, Saitama 346-8530, Japan
[2]Dialysis Unit, Shin-Kuki General Hospital, 418-1 Kamihayami, Kuki, Saitama 346-8530, Japan

compared IPVC between std-HD and three different modes of online HDF.

Methods

Sessions and dialysis conditions

This study was a retrospective analysis of dialysis sessions at a single dialysis center. In addition to std-HD, three different modes of online HDF (pp-ol-HDF, pre-ol-HDF, post-ol-HDF) had been performed. Sessions after long dialysis interval in adult (> 18 years old) patients under maintenance dialysis (≥ 3.5 h/session, 2–3 sessions/week, > 6 months), where complete blood cell count tests were performed for blood samples at the start and end of sessions, were included. Sessions with blood transfusion or fluid infusion during dialysis treatment were excluded. The hospital ethics committee approved the study.

Dialysis sessions were performed using bicarbonate dialysate (Na^+ 140, K^+ 2.0, Ca^{2+} 1.5, Mg^{2+} 0.5, Cl^- 111, HCO_3^- 35, and glucose 8.3 mmol/L). The dialysate temperature and flow rate were set at 35–36 °C and 500 mL/min, respectively. Blood flow rates were individually prescribed to achieve a dialyzer clearance of urea to volume of distribution area (Kt/V) of > 1.2 [11], and ranged from 180 to 280 mL/min. For HDF, one of the following online modes, which are regularly performed in Japan using dialysate as substitution fluid, was selected; pre-ol-HDF with a constant replacement rate of 10 L/h, post-ol-HDF with a constant replacement rate of 2.5 L/h, and pp-ol-HDF with intermittent replacement of 0.2 L every 30 min [12].

Data collection and definitions

Pre-dialysis and post-dialysis blood samplings were performed just after the venipuncture at the start of the sessions and just before the wash-back at the end of the sessions, respectively. To avoid the influence of postural and food-intake effects on hemodynamics, the patients were placed in a supine position for > 20 min before the pre-dialysis sampling, kept lying on beds, and not allowed to eat or drink until the end-of-session [13, 14]. Pre-dialysis and post-dialysis body weight (BW) was measured before the start of the sessions and after the end of the sessions, respectively. Intradialytic BW loss (IBWL) was calculated as "IBWL = pre-dialysis BW − post-dialysis BW," and relative IBWL to DW was calculated as "IBWL/DW." IPVC was calculated by using the intradialytic change of hemoglobin concentration (Hb) as "$IPVC_{Hb}$ = 1 − (pre-dialysis Hb)/(post-dialysis Hb)" and by using the change of hematocrit (Ht) as "$IPVC_{Ht}$ = 1 − (pre-dialysis Ht)/(post-dialysis Ht)." To indicate IPVCs relative to IBWL, IPVCs divided by IBWL ($IPVC_{Hb}$/IBWL and $IPVC_{Ht}$/IBWL) were used.

Dry weight (DW) was adjusted for each patient to maintain cardio thoracic ratio < 52% and no pulmonary congestion on chest radiography. For the participants with valvular heart disease, vena cava diameter normalized for body surface area < 11.5 mm/m^2 and its respiratory fluctuation under the adjusted DW were confirmed on echocardiography. Relative post-dialysis BW to DW (post-dialysis BW/DW) was used as a measure of the fluid status. The plasma level of atrial natriuretic peptide (ANP) was measured at the end of dialysis sessions where the post-dialysis BW was <DW + 0.3 kg. Measurements of ANP with cardiac arrhythmia were excluded from the analysis.

Statistical analysis

Continuous variables were reported as the mean ± standard deviation. Comparisons were done by the one-way analysis of variance and then the Tukey's post hoc tests for patient characteristics and by a mixed-effects model analysis for intradialytic measures. Discrete variables were reported as counts, and comparisons were done by the Pearson's chi-square test. A correlation between parameters was analyzed by using the Pearson's correlation test, and R of the prediction model for linear regression was obtained. All statistical analyses were performed using the SPSS software package (Dr. SPSS II, SPSS Inc., Chicago, IL, USA). A probability value of $P < 0.001$ was considered statistically significant.

Results

At most, 5 sessions per patient from 75 patients were included in the study. Dialysis mode was changed in 31 patients, and 115 std-HD, 59 pp-ol-HDF, 39 pre-ol-HDF, and 33 post-ol-HDF sessions (a total of 246 sessions) were included in the analyses. Table 1 shows the summary of patient characteristics. None of the characteristics examined (age, gender proportion, dialysis lengths, diabetes mellitus prevalence, and the proportion of anti-hypertensives treatment) were significantly different between the modes.

Laboratory measures of pre-dialysis and post-dialysis blood samples and indicators of intradialytic body fluid status were compared between the modes (Table 2). None of the measures and the indicators examined were significantly different between the modes. While the levels of pre-dialysis Hb and Ht were generally lower in std-HD than in ol-HDFs, the levels of post-dialysis Hb and Ht were also generally lower in std-HD than in ol-HDFs, and the levels of $IPVC_{Hb}$ and $IPVC_{Ht}$ were not significantly different between the modes. The levels of post-dialysis ANP compared in 151 out of the 246 sessions were also not significantly different between the modes.

Table 1 Patient characteristics

Characteristic	std-HD (n = 55)	ol-HDF			P
		pp (n = 23)	pre (n = 24)	post (n = 27)	
Age (years)	67 ± 10	71 ± 11	65 ± 10	66 ± 10	0.25
Sex (% female)	18	26	17	15	0.76
Dialysis (months)	63 ± 67	52 ± 58	71 ± 71	71 ± 70	0.72
Diabetes mellitus (%)	53	39	46	44	0.71
Antihypertensives (%)					
RASI	36	22	33	30	0.64
CCB	33	35	29	30	0.97
β-blockers	15	4	17	15	0.58
Diuretics	38	35	38	41	0.98

Data are presented as either mean ± SD or % in each group
Abbreviations: CCB calcium channel blockers, *ol-HDF* online hemodiafiltration, *std-HD* standard hemodialysis, *post* post-dilution mode, *pp* push-pull mode, *pre* pre-dilution mode, *RASI* renin-angiotensin-system inhibitors

Table 2 Comparison between dialysis modes

Measure	std-HD (n = 115)	ol-HDF			P
		pp (n = 59)	pre (n = 39)	post (n = 33)	
Pre-dialysis					
Hb (g/dL)	10.5 ± 1.2	10.8 ± 0.9	10.7 ± 1.1	10.8 ± 1.2	0.19
Ht (%)	32.0 ± 3.7	33.0 ± 2.8	32.6 ± 3.3	33.2 ± 3.6	0.28
Alb (g/dL)	3.4 ± 0.3	3.4 ± 0.3	3.5 ± 0.3	3.5 ± 0.3	0.04
Na (mmol/L)	139 ± 3	139 ± 2	140 ± 3	140 ± 3	0.43
Post-dialysis					
Hb (g/dL)	11.7 ± 1.5	12.2 ± 1.2	11.9 ± 1.3	12.1 ± 1.5	0.14
Ht (%)	35.9 ± 4.7	37.3 ± 3.7	36.4 ± 3.9	37.1 ± 4.6	0.22
Alb (g/dL)	3.8 ± 0.4	3.9 ± 0.6	3.9 ± 0.3	3.9 ± 0.4	0.25
BW/DW	1.005 ± 0.020	1.007 ± 0.011	1.003 ± 0.008	1.002 ± 0.018	0.76
ANP (pg/mL)	66.0 ± 65.4 (n = 61)	69.3 ± 41.8 (n = 35)	63.8 ± 53.7 (n = 29)	90.2 ± 78.0 (n = 26)	0.41
DW (kg)	54.4 ± 10.3	51.5 ± 7.0	56.2 ± 9.9	56.6 ± 11.0	0.15
IBWL (kg)	2.5 ± 0.7	2.4 ± 0.9	2.6 ± 0.8	3.0 ± 0.8	0.19
IBWL/DW	0.048 ± 0.013	0.046 ± 0.016	0.047 ± 0.014	0.049 ± 0.013	0.16
UFR (L/h/kg)	0.013 ± 0.004	0.013 ± 0.004	0.013 ± 0.004	0.013 ± 0.003	0.16
IPVC					
Hb	0.10 ± 0.06	0.11 ± 0.07	0.10 ± 0.05	0.10 ± 0.06	0.73
Ht	0.10 ± 0.07	0.11 ± 0.07	0.10 ± 0.06	0.10 ± 0.06	0.48
IPVC/IBWL/DW					
Hb	1.9 ± 2.0	1.7 ± 4.6	2.1 ± 0.9	2.1 ± 1.8	0.95
Ht	1.9 ± 2.2	1.9 ± 3.7	2.1 ± 1.1	2.1 ± 2.0	0.99

Data are presented as mean ± standard deviation
Abbreviations: Alb serum albumin concentration, *ANP* plasma atrial natriuretic peptide concentration, *BW* body weight, *DW* dry weight, *Hb* hemoglobin, *Ht* hematocrit, *IBWL* intradialytic BW loss, *IPVC* intradialytic plasma volume change, *Na* serum sodium concentration, *ol-HDF* online hemodiafiltration, *post* post-dilution mode, *pp* push-pull mode, *pre* pre-dilution mode, *std-HD* standard hemodialysis, *UFR* ultrafiltration rate

Irrespective of the dialysis modes, $IPVC_{Hb}$ and $IPVC_{Ht}$ correlated well to each other (Fig. 1, $R = 0.97$, $P < 0.001$) and similarly correlated to IBWL/DW (Fig. 2). There were positive correlations between IBWL/DW and $IPVC_{Hb}$ (left panel, $R = 0.51$, $P < 0.001$) and between IBWL/DW and $IPVC_{Ht}$ (right panel, $R = 0.50$, $P < 0.001$). The slopes of the least squares line were 2.3 for both $IPVC_{Hb}$/IBWL/DW and $IPVC_{Ht}$/IBWL/DW, and the levels of IPVCs and IPVCs/IBWL/DW were similar between the modes (Table 2).

Discussion

The present findings revealed that ol-HDF in either push-pull, pre-dilution, or post-dilution mode did not prevent IPVC compared with std-HD; IPVC and IPVC/UFR in ol-HDFs were not different from those in std-HD.

In the present study, both $IPVC_{Hb}$ and $IPVC_{Ht}$ had positive correlations to IBWL/DW. The findings support the notion that intradialytic fluid removal by ultrafiltration induces IPVC and causes IDH [2–4]. However, there was wide variation in IPVCs, which indicated that factors other than ultrafiltration influenced IPVC [5, 14–16]. Vascular refilling by interstitial fluid, which occurs during dialysis sessions, would be a dominant factor among them, and its rate would differ owing to individual difference. Thus, patients' individual difference in the vascular refilling rate could have caused the variation in IPVCs.

Relative volume excess to DW could also facilitate the vascular refilling [3, 14, 15], and inappropriate DW might influence IPVC. However, inappropriate DW would not have influenced the present findings significantly, because not only the levels of post-dialysis BW/DW but also those of ANP, which reflect volume status, were not different between the modes. Furthermore, independency of the vascular refilling from the volume status relative to DW was reported previously [17].

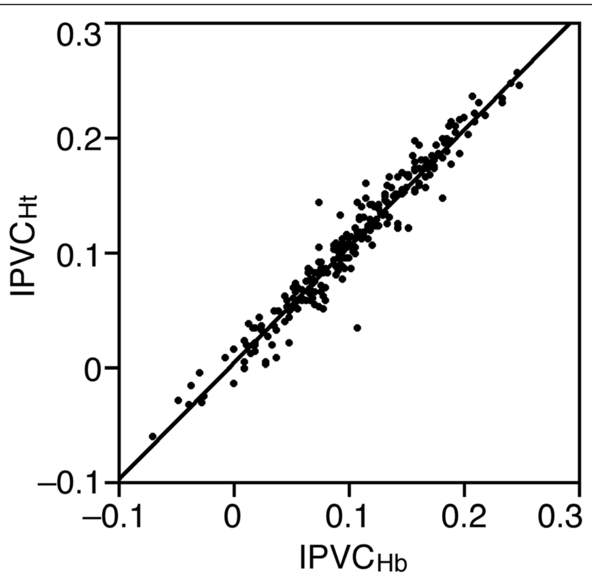

Fig. 1 Scatter plot comparing $IPVC_{Hb}$ with $IPVC_{Ht}$. There was a positive correlation between $IPVC_{Hb}$ and $IPVC_{Ht}$ with a linear approximation of $y = 1.02\,x–0.0003$ ($R = 0.97$, $P < 0.001$). $IPVC_{Hb}$ intradialytic plasma volume change based on hemoglobin, $IPVC_{Ht}$ intradialytic plasma volume change based on hematocrit

Reduction of core-temperature by not-pre-warmed substitution fluid, i.e., thermal effect, was proposed to be a dominant factor preventing IDH in HDF [18, 19]. The present findings might support the proposal of thermal effect. Online HDFs, where pre-warmed dialysate was used as substitution fluid and thermal effect would not have occurred, had no preventive effect on IPVC compared with std-HD. However, factors other than thermal effect could contribute the IDH prevention by ol-HDF. Online HDF may increase the vascular tone or facilitate the vascular refilling between dialysis sessions, because it was reported to have increased pre-dialysis blood pressure compared with std-HDF [8].

Intradialytic loss of albumin can decrease the vascular refilling, which increases IPVC. Thus, the possible loss of albumin, which is larger in ol-HDF than in std-HD, will overestimate IPVC in ol-HDF than in std-HD [20]. The present findings that post-dialysis Alb levels were similar while pre-dialysis Alb levels were generally lower in std-HD than in ol-HDFs would indicate difference in intradialytic albumin loss between std-HD and ol-HDFs. Therefore, the present findings of same IPVCs levels between the modes might have indicated smaller IPVC in ol-HDFs than in std-HD. However, intradialytic albumin loss would not have influenced IPVC, because recent report showed that inward oncotic force had little effect on the vascular refilling [15].

Inducing intracellular-to-extracellular water movement, an increase in Na can facilitate the vascular refilling [21]. During dialysis session, Na is reaching the sodium concentration of dialysate. Thus, differences in pre-dialysis Na could have influenced IPVC. However, in the present study, the levels of pre-dialysis Na were not different between the modes. Additionally, recent data suggest that inward osmotic force has little effect on the vascular refilling [15]. Therefore, intradialytic Na change would not have influenced IPVCs significantly in the present study.

The convection volume, which indicates the effectiveness of convective transport in HDF, can influence IPVC [5, 22]. However, the convection volume would not have influenced the present findings significantly; in spite of differences in the convection volume, the levels of IPVCs were not different between the modes of HDF. Furthermore, in spite of inferiority in convective transport, std-HD had same levels of IPVCs as those of HDFs.

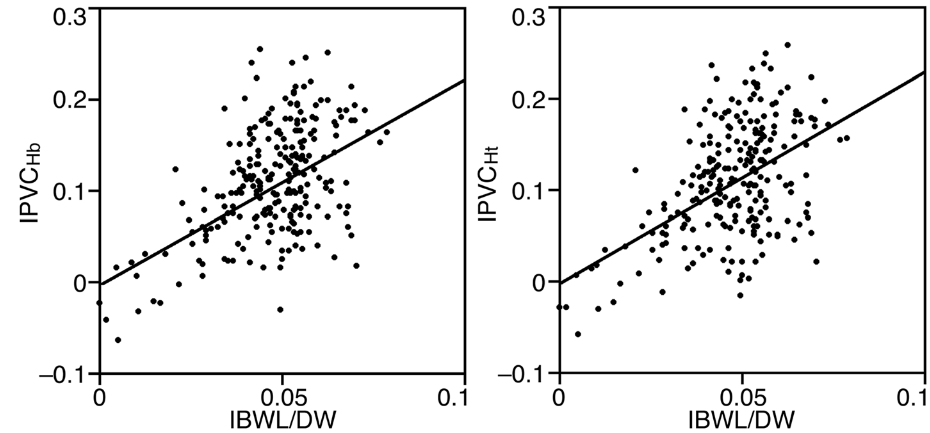

Fig. 2 Scatter plot comparing IBWL with IPVC. There was a positive correlation between IBWL/DW and $IPVC_{Hb}$ with a linear approximation of $y = 2.3\ x–0.003$ (left panel, $R = 0.51$, $P < 0.001$). There was a positive correlation between IBWL/DW and $IPVC_{Ht}$ with a linear approximation of $y = 2.3\ x–0003$ (right panel, $R = 0.50$, $P < 0.001$). *IBWL* intradialytic body weight loss, DW dry weight, $IPVC_{Hb}$ intradialytic plasma volume change based on hemoglobin, $IPVC_{Ht}$ intradialytic plasma volume change based on hematocrit

The present report has several limitations. Firstly, the small sample size is an important limitation. The sample is somewhat skewed, and the study may be limited by the possibility of selection bias. However, variation between the modes would not have affected the present findings significantly; despite the variation, IPVCs and UFR were at the same levels between the modes. Secondly, being a retrospective study, the method of IPVC evaluation was limited. Other methods such as a method using bioimpedance need to be evaluated. Thus, whether the present findings can be generalized needs to be confirmed in prospective studies of large sample sizes and in crossover studies with measurement of bioimpedance.

Conclusions

Online HDF in either push-pull, pre-dilution, or post-dilution mode had the same level of IPVC as std-HD. The findings indicate that ol-HDF has no preventive effect on IPVC irrespective of its modes.

Abbreviations

Alb: Serum albumin concentration; DW: Dry weight; IDH: Intradialytic hypotension; IPVC: Intradialytic plasma volume change; Na: Serum sodium concentration; ol-HDF: Online hemodiafiltration; post-ol-HDF: Post-dilution mode ol-HDF; pp-ol-HDF: Push-pull mode ol-HDF; pre-ol-HDF: Pre-dilution mode ol-HDF; std-HD: Standard hemodialysis

Acknowledgements

We thank Satoshi Ikenaga and Tensho Oka for the excellent technical assistance.

Funding

This study was not supported by any grants or funding.

Availability of data and materials

Please contact the corresponding author for data requests.

Authors' contributions

MT designed the study, performed the data analysis, and wrote the manuscript. YI, YK, and YO collected the clinical data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shin-Kuki General Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 31 May 2018 Accepted: 17 October 2018
Published online: 07 November 2018

References

1. Becker BN, Stone WJ. Options for renal replacement therapy: special considerations. Semin Nephrol. 1997;17:176–87.
2. Movilli E, Cancarini GC, Cassamali S, Camerini C, Brunori G, Maffei C, et al. Inter-dialytic variations in blood volume and total body water in uraemic patients treated by dialysis. Nephrol Dial Transplant. 2004;19:185–9.
3. Lins LE, Hedenborg G, Jacobson SH, Samuelson K, Tedner B, Zetterholm UB, et al. Blood pressure reduction during hemodialysis correlates to intradialytic changes in plasma volume. Clin Nephrol. 1992;37:308–13.
4. Sands JJ, Usvyat LA, Sullivan T, Segal JH, Zabetakis P, Kotanko P, et al. Intradialytic hypotension: frequency, sources of variation and correlation with clinical outcome. Hemodial Int. 2014;18:415–22.
5. Donauer J, Kolblin D, Bek M, Krause A, Bohler J. Ultrafiltration profiling and measurement of relative blood volume as strategies to reduce hemodialysis-related side effects. Am J Kidney Dis. 2000;36:115–23.
6. Colussi G, Frattini G. Quantitative analysis of convective dose in hemofiltration and hemodiafiltration: "predilution" vs. "postdilution" reinfusion. Hemodial Int. 2007;11:76–85.
7. Wang AY, Ninomiya T, Al-Kahwa A, Perkovic V, Gallagher MP, Hawley C, et al. Effect of hemodiafiltration or hemofiltration compared with hemodialysis on mortality and cardiovascular disease in chronic kidney failure: a systematic review and meta-analysis of randomized trials. Am J Kidney Dis. 2014;63:968–78.

8. Locatelli F, Altieri P, Andrulli S, Bolasco P, Sau G, Pedrini LA, et al. Hemofiltration and hemodiafiltration reduce intradialytic hypotension in ESRD. J Am Soc Nephrol. 2010;21:1798–807.
9. Altieri P, Sorba G, Bolasco P, Asproni E, Ledebo I, Cossu M, et al. Predilution haemofiltration—the second Sardinian multicentre study: comparisons between haemofiltration and haemodialysis during identical Kt/V and session times in a long-term cross-over study. Nephrol Dial Transplant. 2001; 16:1207–13.
10. Tattersall JE, Ward RA. Online haemodiafiltration: definition, dose quantification and safety revisited. Nephrol Dial Transplant. 2013;28:542–50.
11. National Kidney Foundation. KDOQI Clinical Practice Guideline for Hemodialysis Adequacy: 2015 update. Am J Kidney Dis. 2015;66:884–930.
12. Masakane I, Nakai S, Ogata S, Kimata N, Hanafusa N, Hamano T, et al. An overview of regular dialysis treatment in Japan (as of 31 December 2013). Ther Apher Dial. 2015;19:540–74.
13. Inagaki H, Kuroda M, Watanabe S, Hamazaki T. Changes in major blood components after adopting the supine position during haemodialysis. Nephrol Dial Transplant. 2001;16:798–802.
14. Daugirdas JT. Dialysis hypotension: a hemodynamic analysis. Kidney Int. 1991;39:233–46.
15. Danziger J, Hoenig MP. The role of the kidney in disorders of volume: core curriculum 2016. Am J Kidney Dis. 2016;68:808–16.
16. Steuer RR, Leypoldt JK, Cheung AK, Harris DH, Conis JM. Hematocrit as an indicator of blood volume and a predictor of intradialytic morbid events. ASAIO J. 1994;40:M691–6.
17. Kron S, Schneditz D, Leimbach T, Aign S, Kron J. Vascular refilling is independent of volume overload in hemodialysis with moderate ultrafiltration requirements. Hemodial Int. 2016;20:484–91.
18. Donauer J, Schweiger C, Rumberger B, Krumme B, Bohler J. Reduction of hypotensive side effects during online-haemodiafiltration and low temperature haemodialysis. Nephrol Dial Transplant. 2003;18:1616–22.
19. Karamperis N, Sloth E, Jensen JD. Predilution hemodiafiltration displays no hemodynamic advantage over low-flux hemodialysis under matched conditions. Kidney Int. 2005;67:1601–8.
20. Jean G, Hurot JM, Deleaval P, Mayor B, Lorriaux C. Online-haemodiafiltration vs. conventional haemodialysis: a cross-over study. BMC Nephrol. 2015;16:70.
21. Santos SF, Peixoto AJ. Sodium balance in maintenance hemodialysis. Semin Dial. 2010;23:549–55.
22. Ok E, Asci G, Toz H, Ok ES, Kircelli F, Yilmaz M, et al. Mortality and cardiovascular events in online haemodiafiltration (OL-HDF) compared with high-flux dialysis: results from the Turkish OL-HDF study. Nephrol Dial Transplant. 2013;28:192–202.