Original Paper

Impact of the Number of Anti-Thrombosis Agents in Hemodialysis Patients: BOREAS-HD2 Study

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Key Words

Hemodialysis • Mortality • Hemorrhagic event • Anti-thrombosis agent.

Abstract

Background/Aims: Relationships between the number of anti-thrombosis agents, clinical benefits and adverse events in hemodialysis (HD) patients are unclear. **Methods:** All patients on HD in 22 institutes (n = 1,071) were enrolled and followed up for 3 years. After exclusion of patients with missing data, kidney transplantation or retraction of consent during the follow-up period (n = 204), mortality rate and ischemic and hemorrhagic events were compared between different regimens of anti-thrombosis agents. **Results:** The use of dual or triple antiplatelet (AP) agents (HR:2.03, 95% CI:1.01-4.13, p = 0.04) and the combination of an AP agent and warfarin (WF) (HR:4.84, 95%CI 1.96-11.96, p < 0.001) were associated with an increase in hemorrhagic events compared with no use of anti-thrombosis agents. No anti-thrombosis regimen was associated with a significant change in risk of ischemic stroke. The use of dual or triple AP agents, but not WF, was associated with an increase in cardiovascular mortality (HR:2.48, 95% CI:1.24-4.76, p = 0.01). **Conclusion:** A significant increase in hemorrhagic events by the use of dual or more AP agents and by co-administration of an AP agent and WF in patients on HD should be considered in planning their anti-thrombosis regimen.

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Introduction

Among patients with chronic kidney diseases (CKDs), the cardiovascular event rate and cardiovascular mortality rate are 5~30-fold higher in patients with end-stage renal disease (ESRD), including those on hemodialysis (HD), than in those without ESRD [1-3]. Accumulation of multiple risk factors, including endothelial dysfunction and hypertension, underlies the high cardiovascular event rate in patients with CKD. Thus, comprehensive management of risk factors is currently recommended for reduction of mortality in CKD patients [4]. However, patients on HD have characteristic risk factors in addition to those in CKD in general, including hemostatic complications [5]. There are apparently opposing hemostatic problems in patients on HD: a pro-thrombotic state due to turbulent flow via the arterio-venous shunt, endothelial injury and hypercoagulability and a pro-hemorrhagic state due to platelet dysfunction and vascular fragility. Thus, the dilemma between use and non-use of an anti-platelet agent or anti-coagulant is a frequent problem in management of patients with atherosclerotic diseases on HD [6].

Clinical evidence regarding benefits of anti-platelet (AP) and anti-coagulant therapies in patients with ESRD is very limited because few placebo-controlled randomized trials regarding this issue have been performed [7]. Because of substantial differences between the clinical profiles of patients with ESRD and those without ESRD [8], the benefit-risk ratios are presumably different in these two groups. However, there have been only a few studies in which relationships between the number of anti-thrombosis (AP and/or anti-coagulant) agents, clinical benefits and adverse events including major hemorrhage were examined [9, 10]. Thus, in the present study, we analyzed data in a registry for a cohort for patients on HD ($n = 1,071$), BOREAS-HD Registry [11], to clarify the relationships between mortality, ischemic events, hemorrhagic events and number of anti-thrombosis agents in HD patients.

Materials and Methods

This study was a multicenter prospective cohort study, BOREAS-HD2. The study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the institutional ethical committee of Sapporo Medical University (Number: 21-29). Written informed consent was obtained from all of the subjects.

Study subjects and clinical outcomes

All patients on HD in 22 institutes affiliated with Sapporo Medical University in the first week of January 2009 ($n = 1,071$) were initially enrolled in the BOREAS HD registry [11]. Prespecified exclusion criteria were transient HD and history of kidney transplantation. The primary outcomes were all-cause and cardiovascular mortalities, and the secondary outcomes were ischemic stroke and major hemorrhagic events during a 3-year follow-up period.

Collection of data

Data for baseline clinical characteristics were collected and recorded in an entry sheet at enrollment. A follow-up sheet for each patient was filled out annually for endpoint information. Cause of death was diagnosed clinically with or without autopsy by attending physicians. Ischemic stroke was diagnosed clinically with or without image modalities by attending physicians, and a major hemorrhagic event was defined as hemorrhage requiring clinical treatment. Attending physicians who participated in the present study are board-certified members of the Japanese Circulation Society and fellows of the Japanese Society for Dialysis Therapy. They were responsible for collection and confirmation of data in data sheets. All of the entry and follow-up sheets were sent from each institute to Sapporo Medical University for statistical analyses.

Statistical analysis

Statistical analysis was performed on the basis of an intention-to-treat policy. Numeric variables are expressed as means $\pm$ SD or medians (interquartile ranges). Pearson's chi-squared test was used to compare groups for categorical data. For multiple comparisons of continuous data, Dunnett' test and Steel' test were used for examination of intergroup differences. Intergroup differences in survival rates were examined by log-rank tests of Kaplan-Meier curves. The hazard ratio (HR) and 95% confidence interval

Table 1. Basal characteristics of hemodialysis patients divided into groups on different anti-thrombosis regimens (n = 867)

	All patients	0-WF/AP group	1-AP group	2-AP group	3-AP group	WF-AP group	WF therapy (+)
n	867	433					34
Age (year)	65.5 $\pm$ 12.5	63.8 $\pm$ 13.2	66.8 $\pm$ 13.2 **	66.8 $\pm$ 11.9	67.2 $\pm$ 6.9	67.0 $\pm$ 12.0	69.8 $\pm$ 8.2 **
Male / Female	499 / 368	230 / 203	171 / 99 **	54 / 35	9 / 2 *	20 / 10	19 / 15
Duration of hemodialysis (month) ^a	56 (25-108)	54.0 (23.0-99.5)	54.0 (27.8-115.3)	67.0 (21.0-111.5)	112.0 (42.0-158.0)	52.5 (27.8-110.5)	86.5 (41.3-145.3)
Systolic blood pressure (mmHg)	153.7 $\pm$ 25.6	153.1 $\pm$ 25.0	155.4 $\pm$ 26.1	155.6 $\pm$ 27.1	150.3 $\pm$ 29.8	147.7 $\pm$ 21.8	148.4 $\pm$ 25.8
Pre dialysis	136.8 $\pm$ 25.1	137.1 $\pm$ 24.9	136.6 $\pm$ 24.9	135.4 $\pm$ 26.8	134.7 $\pm$ 21.8	138.0 $\pm$ 29.3	138.8 $\pm$ 23.4
Post dialysis							
Diastolic blood pressure (mmHg)	76.8 $\pm$ 14.5	77.5 $\pm$ 14.2	75.7 $\pm$ 15.7	77.6 $\pm$ 13.2	78.1 $\pm$ 15.8	76.5 $\pm$ 14.2	74.2 $\pm$ 12.3
Pre dialysis	71.6 $\pm$ 14.1	72.5 $\pm$ 13.6	70.5 $\pm$ 13.8	71.0 $\pm$ 13.5	72.5 $\pm$ 11.4	72.3 $\pm$ 16.7	69.7 $\pm$ 13.2
Post dialysis							
Body weight (kg)	56.7 $\pm$ 11.3	55.7 $\pm$ 11.2	57.7 $\pm$ 11.4 *	56.7 $\pm$ 10.9	55.3 $\pm$ 8.3	59.9 $\pm$ 14.9	57.5 $\pm$ 9.7
Pre dialysis	54.2 $\pm$ 10.9	53.3 $\pm$ 10.9	55.3 $\pm$ 11.0 *	54.2 $\pm$ 10.4	52.7 $\pm$ 7.7	57.3 $\pm$ 14.4	54.9 $\pm$ 9.3
Post dialysis							
Laboratory data							
Hemoglobin (g/dL)	10.6 $\pm$ 1.3	10.6 $\pm$ 1.3	10.5 $\pm$ 1.3	10.5 $\pm$ 1.2	10.6 $\pm$ 1.2	10.9 $\pm$ 0.9	10.8 $\pm$ 1.6
Serum Calcium (mg/dL)	8.8 $\pm$ 0.9	8.8 $\pm$ 0.9	8.9 $\pm$ 0.8	8.8 $\pm$ 0.8	9.2 $\pm$ 0.8	8.8 $\pm$ 0.7	9.0 $\pm$ 1.1
Serum Phosphorus (mg/dL)	5.5 $\pm$ 1.6	5.6 $\pm$ 1.7	5.6 $\pm$ 1.4	5.5 $\pm$ 1.6	4.8 $\pm$ 1.5	5.1 $\pm$ 1.7	5.3 $\pm$ 1.3
Diabetic nephropathy ^b	345 (39.8)	129 (30.0)	126 (46.7) **	57 (64.0) **	7 (63.6) **	17 (56.7) **	9 (26.5)
Cardiovascular complications ^b	462 (53.3)	157 (36.3)	166 (61.4) **	74 (83.2) **	11 (100) **	25 (83.3) **	29 (85.3) *
Ischemic heart disease	152 (17.5)	28 (6.5)	54 (20.0) **	43 (48.3) **	10 (90.9) **	12 (40.0) **	5 (14.7)
Atrial fibrillation	123 (14.2)	43 (9.9)	34 (12.6)	10 (11.2)	3 (27.2) *	14 (46.6) **	21 (61.8) **
Aortic valve disease	95 (11.0)	36 (8.3)	46 (15.1)	12 (11.1)	0 (0)	3 (10.0)	12 (35.3) *
Mitral valve disease	48 (5.5)	19 (4.4)	15 (5.5)	7 (7.8)	1 (9.1)	4 (13.3)	2 (5.9)
Peripheral arterial disease	144 (16.6)	31 (7.2)	53 (19.6) **	37 (41.5) **	9 (81.8) **	10 (33.3) **	4 (11.8)
Cerebral infarction	136 (15.7)	41 (9.5)	57 (21.1) **	17 (19.1) **	5 (45.5) **	10 (33.3) **	6 (17.5)
Intracranial hemorrhage	40 (4.6)	26 (6.0)	9 (3.3)	5 (5.6)	0 (0)	0 (0)	0 (0)
Other cardiovascular disease	89 (10.2)	38 (8.8)	34 (12.6)	6 (6.7)	0 (0)	7 (23.3)	4 (11.8)
History of hemorrhagic events ^b	205 (23.6)	110 (25.4)	58 (21.4)	25 (28.1)	0 (0)	5 (16.7)	7 (20.5)
Gastrointestinal bleeding	115 (13.3)	67 (15.5)	29 (10.7)	9 (10.1)	0 (0)	4 (13.3)	6 (17.6)
Intracranial hemorrhage	40 (4.6)	26 (6.0)	9 (3.3)	5 (5.6)	0 (0)	0 (0)	0 (0)
Retinal hemorrhage	57 (6.1)	21 (4.8)	25 (9.3) *	9 (10.1)	0 (0)	1 (0.03)	1 (2.9)
Other bleeding disease	19 (2.2)	8 (1.9)	6 (2.2)	3 (3.4)	0 (0)	0 (0)	2 (5.9)
Drugs ^b							
Angiotensin II receptor blocker	395 (45.6)	186 (42.9)	128 (47.4)	50 (56.2)	4 (36.4)	17 (56.7)	10 (29.4)
Angiotensin-converting enzyme	58 (6.7)	22 (5.1)	27 (10.0)	7 (7.9)	1 (9.1)	0 (0)	1 (2.9)
Beta blocker	71 (8.1)	25 (5.8)	28 (10.4)	10 (11.2)	2 (18.2)	5 (16.8)	1 (2.9)

* p < 0.05, ** p < 0.01 with 0-WF/AP group as the referent. Variables are expressed as means $\pm$ SD, ^a medians (interquartile ranges), ^b number (%). Intracranial hemorrhage belongs to both cardiovascular complications and history of hemorrhagic events. AP, anti-platelet; WF, warfarin; WF/AP, warfarin and/or anti-platelet agents; WF-AP, warfarin and anti-platelet agents.

(CI) were calculated using the Cox proportional hazard model. In multivariate analyses, we made a multivariate logistic regression model in which adjustment factors were selected by a stepwise regression method. A p value of less than 0.05 was considered statistically significant. All data were analyzed by using JMP 9.0.2 for Windows (SAS Institute, Cary, NC) and EZR [12].

Results

Clinical characteristics of the study subjects

Of 1,071 HD patients initially enrolled in this study, 204 patients were excluded: 196 patients were excluded due to incomplete data description in entry sheets or follow-up data sheets, 4 patients were excluded due to incomplete written informed consent and 4 patients were excluded due to kidney transplantation. Basal clinical characteristics of the patients are shown in Table 1. Warfarin (WF) was the only anticoagulant used in the study subjects (n = 64), and their INR was 2.08 ± 0.70 . Of the 867 finally enrolled patients, 433 patients (49.9%) received no anti-thrombosis agents (WF and/or AP agents) (0-WF/AP group), while 400 (46.1%) and 64 (7.4%) were treated with AP agents and WF, respectively. Of the 400 patients on AP agents, 370 received only AP agents (i.e., without WF); the numbers of patients treated with a single AP agent (1-AP group), dual AP agents (2-AP group) and triple AP agents (3-AP group) were 270 (31.1%), 89 (10.3%) and 11 (1.3%), respectively. Thirty patients (3.4%) were treated with both AP agents and warfarin (WF-AP group); the numbers of patients treated with a single AP agent, dual AP agents and triple AP agents were 19, 10 and 1, respectively. INR in the WF-AP group was 2.15 ± 0.81 . The types of AP agents used in the study subjects are shown in Table 2. The total number of AP agents used in the study subjects was 560. Thirty-four patients (3.9%) received WF alone (WF alone group). INR in the WF alone group was 2.00 ± 0.54 . The rates of cardiovascular complications and diabetic nephropathy at baseline were significantly

Table 2. Type of AP agents

Total number of used AP agents	560
Aspirin	279 (49.8)
Cilostazol	79 (14.1)
Ticlopidine	74 (13.2)
Clopidogrel	37 (6.6)
Beraprost Sodium	36 (6.4)
Sarpogrelate Hydrochloride	35 (6.3)
Dipyridamole	12 (2.1)
Limaprost Alfadex	8 (1.4)

Variables are expressed as number (%). AP, anti-platelet

Fig. 1. Numbers of cardiovascular complications in groups with different regimens of anti-thrombosis agents. WF, warfarin; AP, anti-platelet; WF/AP, warfarin and/or anti-platelet agents; WF-AP, warfarin and anti-platelet agents. *P<0.05, **P<0.01 vs. 0-WF/AP group.

Table 3. Cox regression analysis for all-cause mortality, cardiovascular mortality, ischemic stroke and hemorrhagic events by different numbers of AP agents in hemodialysis patients after exclusion of patients on WF (n = 803)

	All-cause mortality		Cardiovascular mortality		Ischemic stroke		Hemorrhagic events	
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
Unadjusted model								
0-WF/AP group (n = 433)	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-
1-AP group (n = 270)	1.36 (0.97-1.93)	0.073	1.74 (0.98-3.11)	0.058	0.77 (0.32-1.88)	0.578	1.06 (0.61-1.87)	0.823
2-AP group (n = 89)	1.79 (1.12-2.85)	0.013	3.56 (1.84-6.89)	<0.001	0.35 (0.04-2.63)	0.309	1.69 (0.90-3.70)	0.093
3-AP group (n = 11)	4.43 (1.93-10.18)	<0.001	7.29 (2.19-24.24)	<0.001	3.33 (0.44-25.06)	0.242	1.83 (0.23-12.4)	0.601
Adjusted model								
0-WF/AP group (n = 433)	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-
1-AP group (n = 270)	1.15 (0.79-1.68)	0.445	1.49 (0.80-2.79)	0.204	0.52 (0.21-1.31)	0.169	1.22 (0.67-2.21)	0.514
2-AP group (n = 89)	1.28 (0.77-2.12)	0.328	2.54 (1.22-5.29)	0.012	0.21 (0.03-1.57)	0.127	1.99 (0.96-4.16)	0.064
3-AP group (n = 11)	1.93 (0.80-4.68)	0.142	3.61 (0.97-13.3)	0.053	1.20 (0.14-9.85)	0.862	2.34 (0.31-17.64)	0.408
Age (per 5 years)	1.42 (1.30-1.54)	<0.001	1.43 (1.25-1.63)	<0.001	1.12 (0.94-1.33)	0.203	1.26 (1.11-1.42)	<0.001
Sex (Male)	1.06 (0.75-1.48)	0.747	0.97 (0.57-1.67)	0.940	0.95 (0.42-2.15)	0.913	1.01 (0.59-1.73)	0.966
Diabetic nephropathy	1.51 (1.07-2.12)	0.017	1.82 (1.05-3.14)	0.031	-	-	-	-
Number of cardiovascular complications (per number)	1.57 (1.34-1.75)	<0.001	1.28 (1.01-1.63)	0.399	1.63 (1.15-2.30)	0.005	-	-
Angiotensin II receptor blocker	0.62 (0.43-0.88)	0.008	0.56 (0.32-0.99)	0.046	-	-	-	-
History of hemorrhagic events	-	-	-	-	-	-	1.91 (1.10-3.31)	0.019

AP, anti-platelet; WF, warfarin; WF/AP, warfarin and/or anti-platelet agents; HR, hazard ratio; CI, confidence interval

lower in the 0-WF/AP group than in other groups, though the rates of history of hemorrhagic events were similar (Table 1). As shown in Figure 1, the 0-WF/AP group had a smaller number of cardiovascular complications per patient than the numbers in other groups. The clinical characteristics in groups divided by the number of anti-thrombosis agents (WF and/or AP agents) are shown in Supplementary Table 1. For all supplemental material see www.karger.com/doi/10.1159/000480487.

Clinical profiles in the patients excluded from the present analyses were similar to those included for the analyses except for serum phosphorus level and percentage of patients treated with a beta blocker (Supplementary Table 2).

Primary and secondary endpoints during the follow-up period

The mean follow-up period was 31.4 months (range: 1 to 36 months), and follow-up summation was 32,048 patients-months. Of the 867 patients, 163 (18.8%) died during the follow-up period. Cardiovascular death, infectious death, neoplastic death and others were responsible for 27.0%, 26.3%, 7.4% and 18.4% of the deaths, respectively. Causes of death were unclear in 20.7% of the patients.

All-cause mortality and cardiovascular mortality were 6.54 (CI 5.63-7.33) and 2.70 (CI 2.05-3.34) per 100 person years, respectively. Ischemic

stroke and a hemorrhagic event occurred developed in 26 patients (3.0%) and 64 patients (7.4%), respectively, during the follow-up period. The hemorrhagic events included gastrointestinal bleeding, intracranial hemorrhage, and retinal hemorrhage in 39 patients (60.9%), 14 patients (21.9%), and 4 patients (6.5%), respectively, and 7 patients (10.9%) had hemorrhage in other locations (pulmonary hemorrhage, intramuscular hemorrhage, massive nasal bleeding and urinary tract hemorrhage). Kaplan-Meier survival curves in patient groups divided by the number of AP agents and in groups divided by the number of anti-thrombosis agents (WF and/or AP agents) are shown in Figure 2 and Supplementary Figure 1, respectively.

Impacts of different AT regimens on clinical endpoints during follow-up

We examined four Cox regression models to assess relationships between different combinations of anti-thrombosis agents and clinical endpoints during the follow-up period. First, we excluded patients on WF (n = 64) and examined the difference in the outcomes by different numbers of AP agents prescribed to the patients (n = 803) (Table 3). In the unadjusted models, HRs of all-cause mortality and cardiovascular mortality were significantly larger in the 2-AP and 3-AP groups than in the 0-WF/AP group. After adjustment of age, sex, diabetic nephropathy, number of cardiovascular complications and angiotensin II receptor blocker (ARB), HR of cardiovascular mortality in the 2-AP group was significantly larger than that in the 0-WF/AP group (HR:2.54, CI:1.22-5.29, p = 0.012) (Table 3).

Next, we compared the impact of an AP agent alone and that of an AP agent plus WF on the outcome after exclusion of patients on WF alone (n = 34) (Table 4). In this analysis (n = 833), we divided patients into a 0-WF/AP group, 1-AP group, 2/3-AP group (consisting of patients receiving dual or triple AP agents), and WF-AP group. In unadjusted models, there were differences between the groups in HR of all-cause mortality, HR of cardiovascular mortality and HR of hemorrhagic events. However, after adjustment of age, sex, diabetic nephropathy, number of cardiovascular complications and ARB, HRs of all-cause mortality in the four groups were not significantly different. On the other hand, HR of cardiovascular

Fig. 2. Kaplan-Meier survival curves according to the number of AP agents. All-cause mortality (n = 803). (B) Cardiovascular mortality (n = 803). AP, anti-platelet; WF/AP, warfarin and/or anti-platelet agents. *P<0.05, **P<0.01 vs. 0-WF/AP group.

Table 4. Cox regression analysis for all-cause mortality, cardiovascular mortality, ischemic stroke and hemorrhagic events by different anti-thrombosis regimens in hemodialysis patients after exclusion of patients on WF alone (n = 833)

	All-cause mortality HR (95%CI)	p value	Cardiovascular mortality HR (95%CI)	p value	Ischemic stroke HR (95%CI)	p value	Hemorrhagic events HR (95%CI)	p value
Unadjusted model								
0-WF/AP group (n = 433)	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-
1-AP group (n = 270)	1.51 (1.06-2.16)	0.021	1.91 (1.04-3.45)	0.032	0.78 (0.32-1.91)	0.597	1.23 (0.69-2.21)	0.471
2/3-AP group (n = 100) [†]	2.26 (1.46-3.51)	<0.001	4.26 (2.24-8.08)	<0.001	0.64 (0.14-2.79)	0.554	2.11 (1.05-4.24)	0.035
WF-AP group (n = 30)	3.22 (1.70-6.10)	<0.001	2.81 (0.83-9.43)	0.093	1.21 (0.16-9.17)	0.849	4.71 (1.95-11.39)	<0.001
Adjusted model								
0-WF/AP group (n = 433)	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-	1 [Reference]	-
1-AP group (n = 270)	1.17 (0.80-1.70)	0.398	1.48 (0.79-2.77)	0.210	0.53 (0.21-1.33)	0.179	1.23 (0.68-2.24)	0.487
2/3-AP group (n = 100) [†]	1.41 (0.88-2.27)	0.146	2.65 (1.31-5.34)	0.006	0.35 (0.08-1.60)	0.178	2.03 (1.01-4.13)	0.049
WF-AP group (n = 30)	1.47 (0.79-3.12)	0.197	1.55 (0.43-0.55)	0.497	0.48 (0.05-3.97)	0.502	4.84 (1.96-11.96)	<0.001
Age (per 5 years)	1.40 (1.29-1.51)	<0.001	1.43 (1.26-1.63)	<0.001	1.12 (0.94-1.32)	0.181	1.23 (1.10-1.38)	<0.001
Sex (Male)	0.99 (0.71-1.38)	0.985	0.91 (0.53-1.54)	0.717	1.04 (0.47-2.30)	0.909	1.06 (0.64-1.77)	0.803
Diabetic nephropathy	1.44 (1.04-2.01)	0.027	1.83 (1.07-3.13)	0.025	-	-	-	-
Number of cardiovascular complications (per number)	1.48 (1.28-1.70)	<0.001	1.28 (1.02-1.61)	0.031	1.61 (1.15-2.26)	0.004	-	-
Angiotensin II receptor blocker	0.59 (0.42-0.83)	0.003	0.56 (0.33-0.97)	0.031	-	-	-	-
History of hemorrhagic events	-	-	-	-	-	-	2.07 (1.22-3.49)	0.006

[†]The 2/3-AP group (n = 100) was constituted by the integration of the 2-AP (n = 89) and 3-AP (n = 11) groups. AP, anti-platelet; WF, warfarin; WF/AP, warfarin and/or anti-platelet agents; WF-AP, warfarin and anti-platelet agents; HR, hazard ratio; CI, confidence interval.

mortality after adjustment with the five variables was significantly larger in the 2/3-AP group (HR:2.65, CI:1.31-5.34, $p = 0.006$) than in the 0-WF/AP group (Table 4). HR of hemorrhagic events after adjustment of age, sex and history of hemorrhagic events was significantly larger in the 2/3-AP group (HR:2.03, CI:1.01-4.13, $p = 0.049$) and WF-AP group (HR:4.84, CI:1.96-11.96, $p < 0.001$) than in the 0-WF/AP group.

In the third analysis, we did not separate patients on WF and simply examined relationships between the number of any anti-thrombosis agents (WF and/or AP agents; WF/AP) and clinical endpoints in the enrolled patients ($n = 867$). In unadjusted models, HRs of all-cause mortality, cardiovascular mortality and hemorrhagic events in the 2-WF/AP and 3/4-WF/AP groups were significantly larger than that in the 0-WF/AP group. HRs of all-cause and cardiovascular mortalities after adjustment of age, sex, diabetic nephropathy, number of cardiovascular complications and ARB were significantly larger in the 3/4-WF/AP group than in the 0-WF/AP group (HR:2.31, CI:1.15-4.60, $p = 0.034$; HR:3.01, CI:1.24-9.71, $p = 0.009$). Furthermore, HRs of hemorrhagic events after adjustment of age, sex and history of hemorrhagic events were larger in the 2-WF/AP and 3/4-WF/AP groups than in the 0-WF/AP group (HR:2.06, CI:1.03-4.09, $p = 0.039$; HR:6.20, CI:2.29-16.76, $p < 0.001$) (Supplementary Table 3). Finally, we compared the endpoints between patients on WF alone and those on different anti-thrombosis regimens (Supplementary Table 4). HRs of all-cause mortality, cardiovascular mortality and hemorrhagic events in the WF alone group were similar to those in the 0-WF/AP group before and after adjustment. No anti-thrombosis regimen was associated with significant change in the risk of ischemic stroke (Tables 3 and 4, Supplementary Tables 3 and 4).

Patients received primarily heparin as an anticoagulant during dialysis in all of the institutes that participated in this study, though low-molecular-weight heparin and nafamostat mesilate were also used depending on the condition of each patient. Heparin, low-molecular-weight heparin and nafamostat mesilate were administered during dialysis in 757 patients (87%), 100 patients (12%) and 10 patients (1%), respectively. HRs of the clinical outcomes after adjustments with variables including anticoagulants during dialysis (Supplementary Tables 5 and 6) were similar to those in Tables 3 and 4. For exclusion of a centre effect of the results, we performed Cox regression analysis adjusted with the influence among each institutes. HRs of the clinical outcomes (Supplementary Tables 7 and 8) were similar to those in Tables 3 and 4.

Discussion

The effects of anti-thrombosis agents on all-cause mortality or cardiovascular mortality in HD patients have not been examined by randomized clinical trials. However, this issue was addressed in several prospective observational studies. In the Dialysis Outcomes and Practice Patterns Study (DOPPS), a multi-national prospective study, 48, 144 patients on HD were enrolled from 12 countries and followed up for 12 years [13]. The study subjects in DOPPS were divided into a No-anti-thrombosis agent group, an Aspirin group (a group treated with aspirin alone), an AP group (a group primarily treated with a non-aspirin AP agent) and a WF group. Twenty-six percent of the patients in the AP group were treated with both a non-aspirin AP agent and aspirin, and 16% and 7% of the patients in the WF group received aspirin and non-aspirin AP agents, respectively. The group with multiple AP agents and the group with WF therapy showed significantly higher cardiovascular mortality and all-cause mortality than did the No-anti-thrombosis group. Similarly, a retrospective cohort study ($n = 41, 425$) by Chan et al [14]. showed that all-cause mortality was significantly higher in a group treated with WF and a group treated with clopidogrel than in a group without anti-thrombosis therapy, although the mortality in a group treated with aspirin alone was not significantly higher. Recently, Hayashi et al [15]. reported that AP agents, but not WF, were associated with increased all-cause mortality in a prospective analysis of 1, 057 HD patients.

These previous findings suggest that increase in the number of anti-thrombosis agents is associated with increases in risks of all-cause mortality and cardiovascular mortality. However, the relationship between the number of AP agents or combination of an AP agent and WF and change in mortality has not been characterized.

In the present study, the number of AP agents was a larger in patients with larger number of cardiovascular complications (Figure 1), and HRs of all-cause and cardiovascular mortalities tended to be higher in groups treated with larger numbers of AP agents (Figure 2, Table 3). Similar relationships between HRs and number of anti-thrombosis agents were observed for different combinations of AP agents and WF (Table 4, Supplementary Figure 1, Supplementary Tables 3 and 4). Cox proportional hazard models showed that use of multiple AP agents was significantly associated with increased cardiovascular mortality (Tables 3 and 4). Interestingly, adjusted HRs of cardiovascular mortality in patients on a combination of WF with an AP agent were similar to those in patients on a single AP agent, though the number of cardiovascular complications was larger in the WF-AP group (Table 4, Figure 1). These results indicate that use of multiple AP agents, but not WF, were significantly associated with an increase in cardiovascular mortality independently of age, sex, diabetic nephropathy, number of cardiovascular complications and ARB. The association is likely to be explained by multiple atherosclerotic vascular diseases that require use of multiple AP agents for disease control.

In contrast to earlier reports [13-15], all-cause mortality was not significantly different between any groups on different anti-thrombosis regimens in the present study. This discrepancy might be explained by differences in clinical profiles and/or overall management of patients, as suggested by lower all-cause mortality and lower cardiovascular mortality in the present cohort than in the earlier studies (6.5% per year vs. 8%~25% per year and 2.7% per year vs. 6~11% per year, respectively [13-15]).

Whether AP agents increase hemorrhagic events in patients on HD is still controversial [9]. The impact of anti-thrombosis agents on risk of hemorrhage in HD patients has been examined in only a few prospective randomized trials [16-18]. Kaufmann et al [16]. randomized patients with an arterio-venous shunt using graft materials (n = 200) to an aspirin plus clopidogrel group or to a placebo group and showed that risk of hemorrhagic events was approximately 2-fold higher in the aspirin plus clopidogrel group than in the placebo group. In addition, they pointed out that bleeding risk associated with use of antiplatelet agents was much higher in their study subjects, i.e., HD patients, than in non-uremic patients enrolled in the CAPRIE trial [19] and the CURE trial [20]. In contrast, Sreedhara et al [17]. reported that the incidence of gastrointestinal hemorrhage was not significantly different among four antiplatelet regimens (dipyridamole, aspirin, dipyridamole plus aspirin, no drug control) in HD patients with a graft arterio-venous shunt (n = 107). Coli et al [18]. randomized 144 HD patients into a ticlopidine group and a WF plus ticlopidine group and found no significant difference between the two groups in hemorrhagic events during 12 months of follow-up. However, because of the lack of placebo controls, change in the risk of hemorrhage by ticlopidine was unclear in their study. Although the number of enrolled HD patients was not large (n = 255), interesting observations were reported by Holden et al [21]. In their study, the risks of new hemorrhagic events during 43.2 months of follow-up were 5.2-fold higher in an aspirin-treated group and 6.2-fold higher in a group treated with both aspirin and WF than in a no anti-thrombosis agent group [21]. We largely confirmed the findings by Holden et al [21]. using a larger number of patients; HR of new hemorrhagic events tended to increase with an increase in the number of AP agents (Table 3) and the combination of an AP agent with WF was associated with even higher bleeding risk (HR:4.84, p < 0.001, Table 4). Additionally, we found that the use of dual or triple AP agents was associated with a significant increase in the risk of hemorrhagic events compared with no use of an anti-thrombosis agent (HR:2.03, p = 0.049, Table 4). The results of randomized trials [16-18] and those of an earlier study [21] and the present observational study are not easily reconciled, presumably due to differences in clinical profiles of the study subjects. However, the results

of the present study support the notion that clinical benefits of a combination of AP agents and those of the combination of an AP agent with WF are partly cancelled by an increase in hemorrhagic events in HD patients.

The effect of anti-thrombosis agents on the incidence of ischemic stroke in HD patients is controversial [22]. A notable difference between a study by Olesen et al [23], showing a reduction in the incidence of ischemic stroke by WF and studies showing negative results [24-29] is clinical characteristics of atrial fibrillation (AF) in enrolled subjects. Olesen et al [23], enrolled only patients with "lone AF" (i.e., AF without structural cardiac diseases), but subjects with any type of AF were enrolled in the other studies [24-29]. In the present cohort, only 14.2% of the patients had AF, and the number of cardiovascular complications, but not the use of any anti-thrombosis regimen, was a predictor of ischemic stroke (Table 3 and 4, Supplementary Table 3 and 4). It is possible that an increase in stroke risk by accumulated cardiovascular diseases overwhelmed the preventive effects of anti-thrombosis agents in HD patients. However, this possibility needs to be examined in further studies.

The present study has several limitations. First, the time-weighted impact of anti-thrombosis regimens on clinical outcomes in HD patients remains unclear since data for duration of treatment with anti-thrombosis agents were not collected in this study. Second, we were not able to obtain laboratory data regarding nutritional status (such as serum albumin) from all study subjects, though nutrition has a significant impact on the mortality of patients on HD therapy. However, in a *post-hoc* analysis of subgroups of patients in whom serum albumin, total cholesterol and/or triglyceride data were available, there was no significant difference in serum albumin or lipid level between patients on different anti-thrombosis regimens (Supplementary Table 9). HRs of the clinical outcomes after adjustments with variables including serum albumin (Supplementary Tables 10 and 11) were similar to those in the entire study subjects (i.e., HRs without adjustment with serum albumin) (Table 4 and Supplementary Table 4). Thus, it is unlikely that the lack of indices of nutritional status in the adjustment of HRs affected the results for relationships between clinical outcomes and anti-thrombosis regimens in the present study. Third, adjustment of HRs might not have been sufficient because severity of cardiovascular diseases and therapies were not incorporated into the adjustment. Fourth, we did not collect data for use of intravenous prostaglandin agents, which could have influenced the results. Finally, study subjects consisted of only Japanese patients receiving treatments according to the Japanese Guidelines of HD therapy and management of cardiovascular diseases. Thus, selection of antiplatelet agents (for example, cilostazol) for cardiovascular diseases is not the same as those in other countries, and the present results may not be directly extrapolated to other ethnic populations.

Conclusion

HD patients on multiple AP agents, but not WF, had significantly increased cardiovascular mortality. The use of dual or triple AP agents was associated with a two-fold increase in the risk of hemorrhagic events compared with no use of an AP agent in patients on HD, and the combination of an AP agent and WF increased the hemorrhage risk by almost five-fold. Although patients with ESRD are at very high risk of cardiovascular events and are often treated with multiple anti-thrombosis agents, the substantial increase in hemorrhagic events by the use of dual or more AP agents and by co-administration of an AP agent and WF should be taken into consideration in planning the anti-thrombosis regimen and duration of treatment for patients on HD.

Disclosure Statement

The authors have declared that no conflict of interest exists.

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