Risk Factors for Thromboembolic Events in Patients With Dialysis-Dependent CKD: Pooled Analysis of Phase 3 Roxadustat Trials in Japan

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Supplementary Material:

Methods: Nested Case-Control Analysis

A nested case-control analysis was conducted to infer the possible causal role of potential risk factors in the development of thromboembolic events. An advantage of a nested case-control analysis is the flexibility to control for confounding effects via matching techniques, and thus relatively high statistical power is expected even in studies of a small number of events where naive model adjustment for multiple confounding factors may be challenging [1-4].

Case Definition

A patient was defined as a case at the first recorded incidence of an event. Patients were only included as a case once; subsequent events in the same patient were not counted.

Definition of Patients at Risk

Identifying appropriate patients at risk from which controls are chosen allows the analysis to incorporate time-dependent data of potential risk factors and adjust for known confounders. A matched nested case-control design was used to match a patient with an event to patients with similar characteristics with respect to important confounding variables who have not experienced any events at the onset time of the case. Each time an event occurred (case), patients who were still at risk were eligible to be selected as a control. A matching algorithm was used to select controls with similar characteristics from these patients at risk. Patients selected as controls could go on to become a case themselves if they experienced the event of interest

subsequently and could also be included in the patients at risk for other cases before they experienced the event. **Figure S1b** is a schematic diagram of this process, where potential controls corresponding to a case are defined as all patients at risk (eg, case subject S002 has 5 potential control subjects: S003, S004, S007 S008, and S009).

Selection of Matching Variables

An important consideration is the appropriate selection of matching variables, as well as the optimum mechanism for matching. We selected the matching variables based on the results from the Cox regression analysis. The matching variables used for the analysis of events with onset before Week 12 included:

- Type of dialysis: hemodialysis, peritoneal dialysis
- Hemodialysis/peritoneal dialysis vintage category (months): <4, ≥4
- Age category: +/− 2 years
- Ferritin at Week 0
- Transferrin saturation at Week 0

The matching variables used for the analysis of events with onset after Week 12 included:

- Type of dialysis: hemodialysis, peritoneal dialysis
- History of thromboembolism: no, yes
- Age category: +/− 2 years
- History of cardiovascular: no, yes

- Pre-treated ESA monthly dose, calculated by converting to darbepoetin alfa unit with the following conversion rate: darbepoetin alfa (ug/wk) : rHuEPO (IU/wk) = 1:200, darbepoetin alfa (ug/4 wk) : epoetin beta pegol (ug/4 wk) = 1:0.8.

Selection of Controls

Another important consideration is the method used to select controls from patients at risk for each case. We used the combination of exact matching and nearest neighbor matching, where a case was first matched to patients with the same levels of binary matching variables (exact matching), and among such patients, the case was matched to patients with the smallest Mahalanobis distance of continuous matching variables (nearest neighbor matching). Patients could be selected more than once as a control. We also included future cases of developing a thromboembolic event as controls, as their exclusion could also lead to biased estimates of relative risk [5].

Number of Controls

In standard case-control studies, it has been shown that there is little statistical efficiency gained from having more than four matched controls relative to each case [6,7]. There is also a concern that increasing the number of controls sampled per case would lead to an increase in repeated sampling, resulting in a larger number of duplicates present in the overall matched control population. However, it is also true that the present pooled data included the limited number of patients experiencing

thromboembolic events. Therefore, to preserve statistical accuracy, we limited the number of matched controls to 10 per case in principle (eg, cases were matched to 10 controls with the same level of binary matching variables and the smallest Mahalanobis distance of continuous matching variables). As an exception, if more than 10 patients per case had the smallest Mahalanobis distance, caused due to tied distance, all these patients were selected as controls.

Statistical Analysis

The potential risk factors of patients experiencing thromboembolic events and the matched controls were compared. By case and matched control group, numbers and percentages of patients were calculated for binary and categorical factors. A conditional logistic regression model was used to calculate an odds ratio for cases compared with matched controls with 95% confidence intervals and *P* values. The conditional logistic regression allowed incorporation of matching by having different constant terms for each paired case-control, which is given by

$$Y_{ij} \sim Bernoulli(p_{ij})$$

$$p_{ij} = \frac{\exp(\alpha_i + \beta X_{ij})}{1 + \exp(\alpha_i + \beta X_{ij})}$$

where Y_{ij} is a binary outcome ($Y_{ij} = 1$ for event and $Y_{ij} = 0$ for no event) for i-th case ($j = 0$) or his j-th control, and X_{ij} is a factor for i-th case ($j = 0$) or his j-th control.

References

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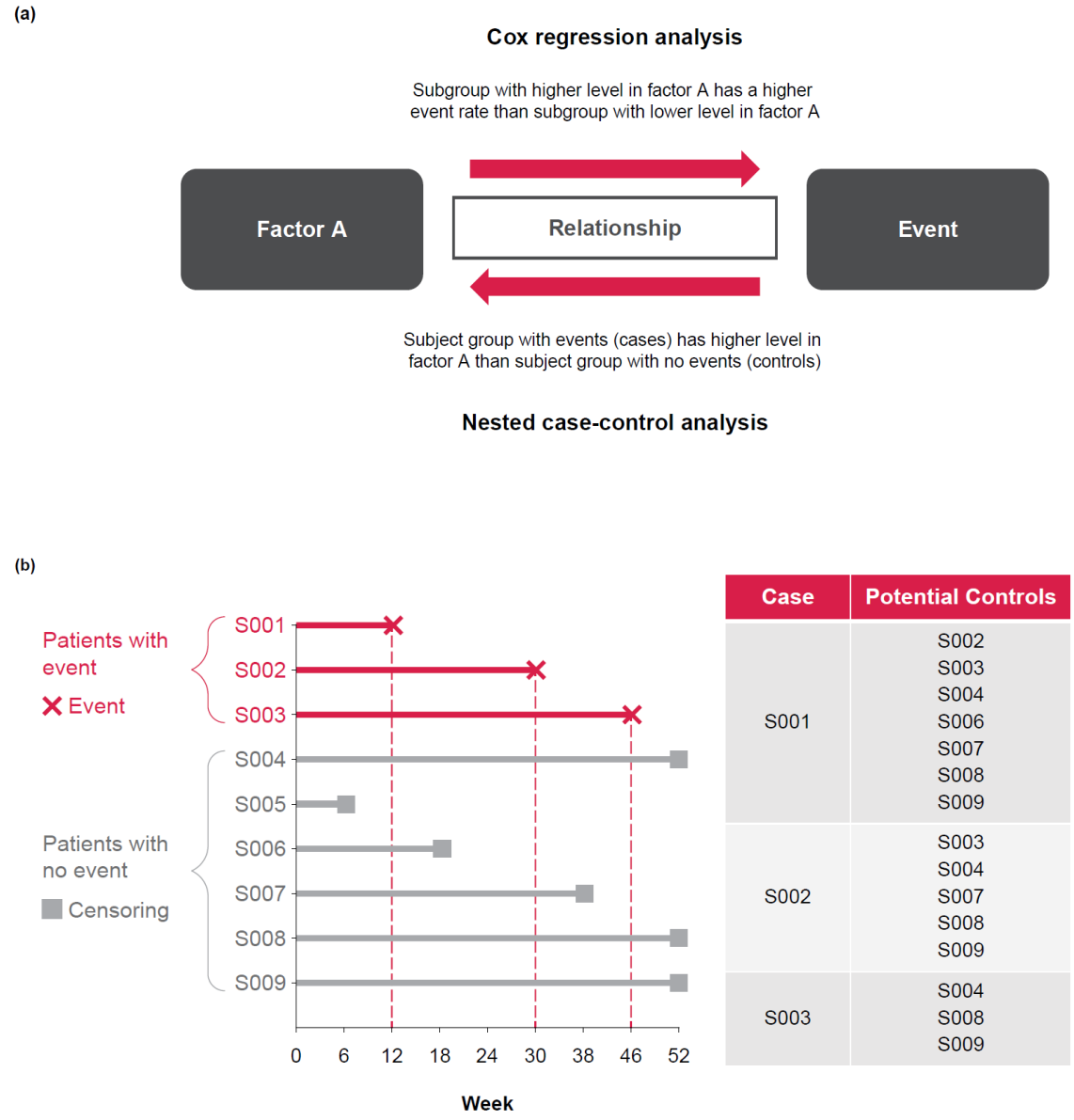
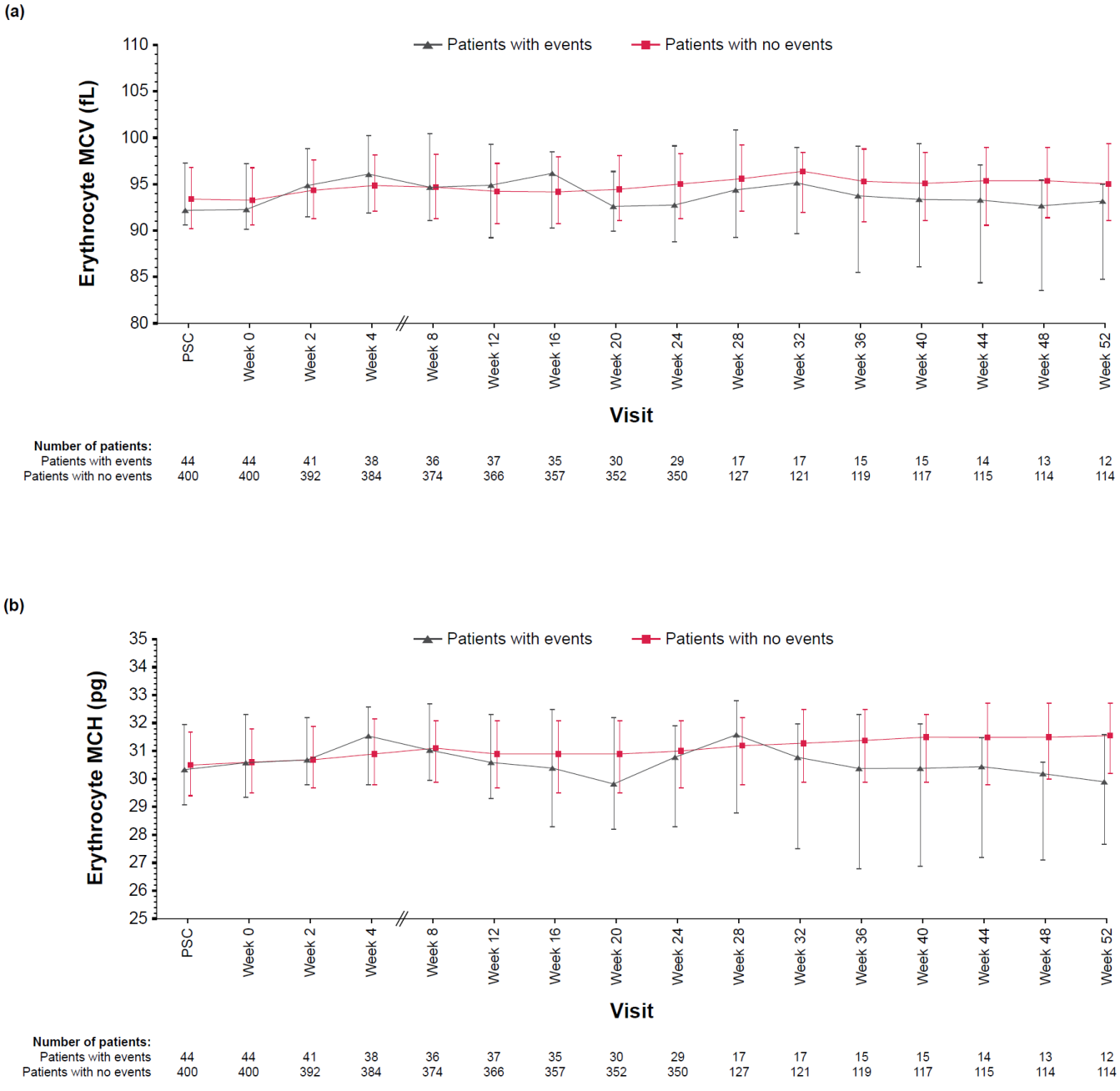
Figure S1. Illustration of Methodology: (a) Cox Regression Analysis and Nested Case-Control Analysis; (b) Schematic Diagram Illustrating Potential Controls for Each Patient With Event (Case)



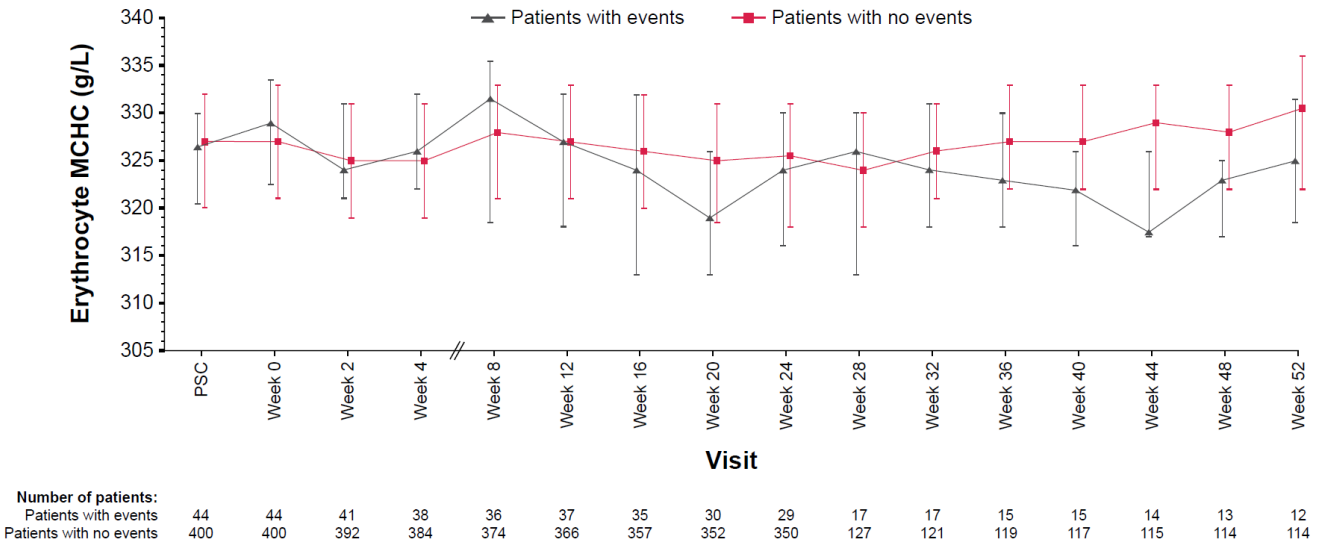
Case	Potential Controls
S001	S002 S003 S004 S006 S007 S008 S009
S002	S003 S004 S007 S008 S009
S003	S004 S008 S009

Figure S2. Medians ± Interquartile Ranges Plot of (a) MCV, (b) MCH, and (c) MCHC in Patient Subgroups With and Without Thromboembolic Events

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PSC, prescreening.



(c)



Number of patients:	PSC	Week 0	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 52
Patients with events	44	44	41	38	36	37	35	30	29	17	17	15	15	14	13	12
Patients with no events	400	400	392	384	374	366	357	352	350	127	121	119	117	115	114	114

Figure S3. Medians ± Interquartile Ranges Plot of Platelets in Patient Subgroups With and Without Thromboembolic Events

PSC, prescreening.

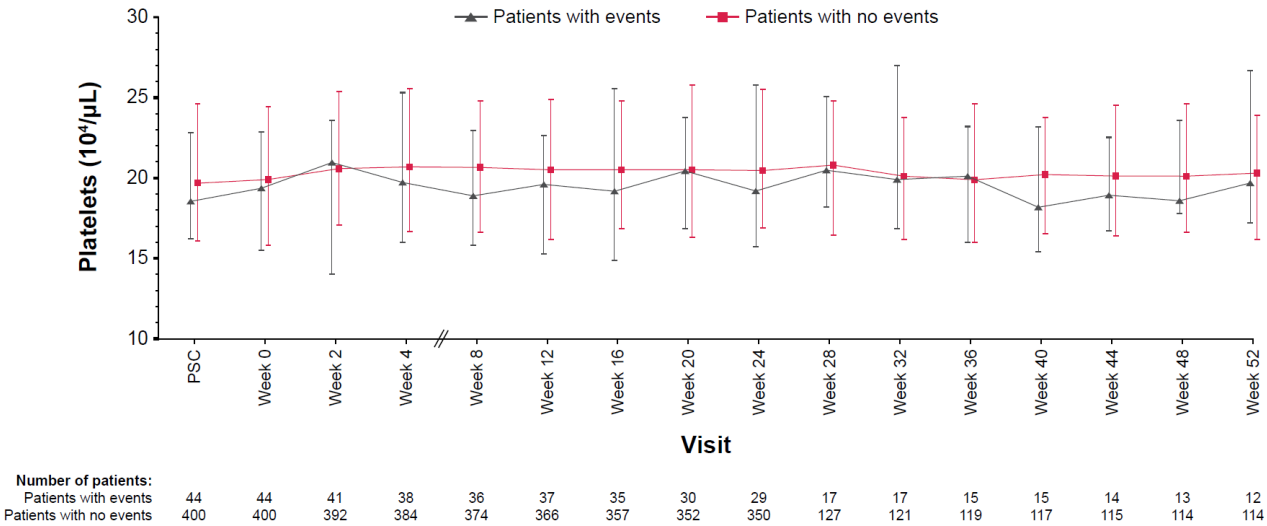


Table S1. Dose Conversion Between Average Doses of ESA Before Study Registration and Roxadustat Doses

Study 1517-CL-0302, Study 1517-CL-0312				
	Epoetin Beta Pegol (μg/4 wk)	**rHuEPO (IU/wk)**	**Roxadustat (mg/dose)**	**Roxadustat (mg/wk)**
DA (μg/wk)				
<20	≤100	<4500	70	210
≥20	>100	≥4500	100	300
Study 1517-CL-0307				
		rHuEPO (IU/wk)	**Roxadustat (mg/dose)**	**Roxadustat (mg/wk)**
DA (μg/wk)				
<20	—	<4500	70	210
≥20	—	≥4500	100	300

Patients in study 1517-CL-0308 were ESA-naive and received a starting dose of either 50 mg or 70 mg of roxadustat three times weekly.
DA, darbepoetin alfa; ESA, erythropoiesis-stimulating agent; rHuEPO, recombinant human erythropoietin.

Table S2. Univariate Cox Regression Analysis for Thromboembolic Events With Onset Before Week 12: Other Factors

Category	n	No. of Events (%)	Hazard Ratio (95% CI)[a]	*P* Value[b]
Sex				
Male	291	18 (6.2)	Ref	0.312
Female	153	6 (3.9)	0.62 (0.25–1.57)	
Primary disease of CKD				
Chronic glomerular nephritis	151	10 (6.6)	0.92 (0.33–2.52)	0.529
Diabetic nephropathy	145	5 (3.4)	0.47 (0.14–1.53)	
Nephrosclerosis	67	3 (4.5)	0.62 (0.15–2.46)	
Other	81	6 (7.4)	Ref	
History of diabetes				
No	278	16 (5.8)	Ref	0.652
Yes	166	8 (4.8)	0.82 (0.35–1.92)	
Roxadustat starting dose (mg)				
50	43	3 (7.0)	Ref	0.781
70	251	12 (4.8)	0.68 (0.19–2.42)	
100	150	9 (6.0)	0.87 (0.23–3.20)	
Concomitant anticoagulant use				
No	426	23 (5.4)	Ref	0.978
Yes	18	1 (5.6)	1.03 (0.14–7.61)	
Concomitant antiplatelet agent use				
No	242	15 (6.2)	Ref	0.411
Yes	202	9 (4.5)	0.71 (0.31–1.62)	

CI, confidence interval; CKD, chronic kidney disease; Ref, reference.
[a]Estimated using Cox proportional hazards model.
[b]*P* values based on log-rank test to test the null hypothesis of no difference in incidence across subgroup categories.

Table S3. Matching Variables and Other Baseline Characteristics in Nested Case-Control Analysis for Thromboembolic Events with Onset Before Week 12

Variable	Statistics/Category	Cases (N=24)	Controls (N=223)[a]
Age	Mean (SD), years	66.7 (9.3)	67.5 (8.6)
	≥65 years, n (%)	17 (70.8)	156 (70.0)
Type of dialysis, n (%)	Hemodialysis	24 (100.0)	223 (100.0)
	Peritoneal dialysis	0 (0.0)	0 (0.0)
Dialysis vintage, months	Mean (SD)	55.4 (75.4)	60.2 (83.0)
	Median (Q1, Q3)	19.6 (0.6, 69.7)	27.9 (0.9, 75.5)
	≥4 months, n (%)	15 (62.5)	150 (67.3)
Ferritin at Week 0	Median (Q1, Q3), ng/mL	86.2 (53.5, 154.8)	80.3 (46.0, 113.5)
	<100 ng/mL, n (%)	13 (54.2)	152 (68.2)
TSAT at Week 0	Mean (SD), %	29.12 (11.33)	26.83 (7.79)
	<20%, n (%)	4 (16.7)	32 (14.3)

Q, quartile; SD, standard deviation; TSAT, transferrin saturation.
[a]Number of unique controls, N=158.

Table S4. Cox Regression Analysis for Thromboembolic Events With Onset After Week 12: Other Factors

Category	n	No. of Events (%)	Hazard Ratio (95% CI)[a]	*P* Value[b]
Sex				
Male	275	15 (5.5)	Ref	0.890
Female	148	8 (5.4)	0.94 (0.40–2.22)	
Primary disease of CKD				
Chronic glomerular nephritis	142	8 (5.6)	0.99 (0.30–3.30)	0.894
Diabetic nephropathy	141	9 (6.4)	1.17 (0.36–3.81)	
Nephrosclerosis	64	2 (3.1)	0.65 (0.12–3.54)	
Other	76	4 (5.3)	Ref	
History of diabetes				
No	264	12 (4.5)	Ref	0.280
Yes	159	11 (6.9)	1.56 (0.69–3.55)	
Roxadustat starting dose (mg)				
50	40	1 (2.5)	Ref	0.352
70	240	11 (4.6)	1.03 (0.13–8.22)	
100	143	11 (7.7)	1.86 (0.23–14.91)	
Concomitant anticoagulant use				
No	406	22 (5.4)	Ref	0.989
Yes	17	1 (5.9)	1.01 (0.14–7.53)	
Concomitant antiplatelet agent use				
No	228	8 (3.5)	Ref	0.082
Yes	195	15 (7.7)	2.11 (0.89–4.97)	

CI, confidence interval; CKD, chronic kidney disease; Ref, reference.
[a]Estimated using Cox proportional hazards model.
[b]*P* values based on log-rank test to test the null hypothesis of no difference in incidence across subgroup categories.

Table S5. Matching Variables and Other Baseline Characteristics in Nested Case-Control Analysis for Thromboembolic Events With Onset After Week 12

Variable	Statistics/Category	Cases (N=23)	Controls (N=164)[a]
Age	Mean (SD), years	67.2 (10.2)	66.8 (8.1)
	≥65 years, n (%)	17 (73.9)	123 (75.0)
Type of dialysis, n (%)	Hemodialysis	23 (100.0)	164 (100.0)
	Peritoneal dialysis	0 (0.0)	0 (0.0)
History of thromboembolism, n (%)	Yes	11 (47.8)	51 (31.1)
History of cardiovascular disease, n (%)	Yes	6 (26.1)	20 (12.2)
Pre-treated ESA monthly dose (µg/mo)	Mean (SD)	117.23 (127.31)	87.13 (64.46)
	Median (Q1, Q3)	80.00 (40.00, 143.75)	80.0 (40.00, 120.00)
Pre-treated ESA monthly dose group (µg/mo), n (%)	<40.0	5 (21.7)	25 (15.2)
	40.0 to <160.0	12 (52.2)	118 (72.0)
	≥160.0	6 (26.1)	21 (12.8)

ESA, erythropoiesis-stimulating agent; Q, quartile; SD, standard deviation.
[a]Number of unique controls, N=110.