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【 ORIGINAL ARTICLE 】

Roxadustat for Treating Anemia in Patients with Advanced Chronic Kidney Disease Not Undergoing Dialysis: A Retrospective Study

Masanori Tamaki[1,2], Taizo Inagaki[1], Masanori Minato[1], Eriko Shibata[1], Rika Nishioka[2], Satoshi Nishioka[2], Yukiko Matsubara[2], Masamitsu Sasaki[2], Motoyuki Tamaki[2], Masaharu Tamaki[2], Kazuhiro Hasegawa[1], Kojiro Nagai[1,3] and Shu Wakino[1]

Abstract:

Objective Roxadustat, a hypoxia-inducible factor-prolyl hydroxylase inhibitor, increases the hemoglobin (Hb) levels in patients with chronic kidney disease (CKD). To date, limited clinical studies have focused on the excessive increase in the Hb levels in the early weeks after switching from erythropoiesis-stimulating agents (ESA) to roxadustat in adult non-dialysis patients. We conducted a retrospective study to examine whether early overshoot frequently occurs after switching to roxadustat.

Methods This 8-week retrospective pilot study examined patients with anemic, non-dialyzed CKD who switched from ESA (darbepoetin or epoetin beta pegol) to roxadustat or continued ESA. The Hb levels >12.5 g/dL after starting our observation was defined as Hb overshoot.

Patients: Twenty-three patients who switched to roxadustat (roxadustat group) and 63 who continued ESA (ESA group) were included.

Results The baseline median estimated glomerular filtration rate and mean Hb levels were 15.7 mL/min/1.73 m^2 and 10.77 g/dL in roxadustat group and 15.2 mL/min/1.73 m^2 and 10.64 g/dL in ESA group, respectively. Eight patients (34.8%) in the roxadustat group and two patients (3.2%) in the ESA group had Hb overshoot within the 8-week visit [odds ratio: 20.2 (95% confidence interval 3.13-130.0, $p<0.01$) in the background adjusted model]. Among the patients with Hb overshoot in the roxadustat group, the Hb levels were maintained close to baseline 4 weeks after roxadustat discontinuation. A younger age and higher baseline Hb and Hct levels were risk factors for Hb overshoot.

Conclusion Hb overshoot was frequently observed in patients switched to roxadustat. Clinicians should be aware of Hb overshoot and emphasize the importance of early Hb level checks.

Key words: roxadustat, renal anemia, real-world evidence, chronic kidney disease

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Introduction

Anemia is a complication of chronic kidney disease (CKD) and it is associated with an increased risk of death (1-4). Anemia treatment reduces transfusion rates and improves the clinical outcomes (1, 5-8). The standard renal anemia treatment is recombinant human erythropoietin or its analogs [erythropoiesis-stimulating agents (ESA)] with iron supplementation (9). However, treatment with ESA has limitations in that higher doses of ESA are associated with a poor patient prognosis (10, 11). ESA resistance is associated with poor survival rates in CKD patients (11, 12). Patients also often experience pain because ESA is administered via

[1]Department of Nephrology, Tokushima University Hospital, Japan, [2]Department of Internal Medicine, Tamaki Aozora Hospital, Japan and [3]Department of Nephrology, Shizuoka General Hospital, Japan
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Correspondence to Dr. Masanori Tamaki, tamaki.masanori@tokushima-u.ac.jp

injection.

Hypoxia-inducible factor (HIF), a hypoxia-induced transcription factor, increases endogenous erythropoietin production and reduces the circulating hepcidin levels, thereby increasing iron bioavailability (13, 14). Roxadustat, an HIF-prolyl hydroxylase (PH) inhibitor, inhibits PH, stabilizes HIF-α subunits, and increases HIF transcriptional activity. Previous studies have reported that roxadustat increases the hemoglobin (Hb) levels in patients with anemia, dialysis, and non-dialyzed CKD (15-18). However, to date, only a limited number of clinical studies have focused on the excessive increase in Hb levels in the early weeks after switching from ESA to roxadustat in adult non-dialysis patients. One phase 3 trial to date has reported switching from ESA to roxadustat in adult, non-dialyzed patients with renal anemia (18). This study demonstrated the non-inferiority of roxadustat to ESA at 18-24 weeks; however, an increase in the Hb levels was noted in the early weeks, with little mention of this observation. (18). Because maintaining higher Hb levels and a rapid increase in the Hb levels are risk factors for poor outcomes (19, 20), clinicians should be aware of Hb overshoot caused by roxadustat in the early weeks, but this has not yet been studied extensively. Additionally, real-world data on switching treatment from ESA to roxadustat in non-dialyzed patients with CKD is limited. Therefore, we conducted a retrospective study to examine whether early overshoot frequently occurs after switching to roxadustat in patients with anemic, non-dialyzed CKD.

Materials and Methods

Study design and population

In this 8-week, retrospective pilot study, we included patients with anemia and non-dialyzed CKD who switched from ESA to roxadustat or continued ESA between December 1, 2020, and October 31, 2021, at two hospitals in Japan. This study was conducted in accordance with the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of Tokushima University Hospital (#4117). Patient consent was obtained through an opt-out methodology, and informed consent was obtained if necessary.

The inclusion criteria for switching from ESA to roxadustat were: 1) ≥20 years of age, 2) baseline estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2; 3) baseline Hb level ≤12.5 g/dL, 4) patients who had been started on epoetin beta pegol or darbepoetin at least two months prior to switching, 5) the last administration of epoetin beta pegol ≥4 weeks ago or darbepoetin ≥2 weeks ago, and 6) patients who wished to switch from ESA to roxadustat. The exclusion criteria were as follows: 1) patients with proliferative diabetic retinopathy; 2) a history of cancer; 3) hyperkalemia at baseline (≥6.0 mEq/L), 4) severe hypertension (systolic blood pressure ≥180 mmHg and/or diastolic blood pressure ≥120 mmHg), or 5) history of thrombosis (deep vein thrombosis, pulmonary embolism, myocardial infarction, and cerebral infarction). The inclusion and exclusion criteria for patients who continued ESA were the same as those for patients who switched to roxadustat. There were multiple times to start data collection for patients who continued ESA; therefore, data were collected preferentially starting from the time when Hb levels and ferrokinetic parameters were collected simultaneously within the range that met the criteria. For cases in which ferrokinetic parameters were not collected, the earliest date that met the criteria was used as the starting date for the data collection.

Patients with Hb levels >12.5 g/dL after starting observation were treated as patients with Hb overshoot, according to the product information of roxadustat. In patients who switched to roxadustat and had Hb overshoot, roxadustat was discontinued, and they visited the hospital 4 weeks after discontinuation.

The primary endpoint was the Hb overshoot ratio between the patients who switched to roxadustat and those who continued ESA. The secondary endpoints were the Hb levels and the fluctuation range of Hb (ΔHb) levels at 2, 4, and 8 weeks after starting the observation. These parameters were compared with those of patients who continued ESA. The exploratory endpoints were Hb-related parameters such as mean corpuscular volume (MCV), red blood cell distribution width (RDW), and reticulocyte count; ferrokinetic parameters such as transferrin saturation (TSAT), unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), ferritin level, erythropoietin levels, lipid metabolism parameters such as the serum cholesterol and triglyceride levels, and renal parameters, such as serum creatinine levels and eGFR calculated using the Japanese formula [eGFR (mL/min/1.73 m^2)=194× serum creatinine$^{-1.094}$× age$^{-0.287}$ (×0.739 if female)] (21), and urinary protein excretion (g/g creatinine). Additionally, the risk factors for overshot Hb and a rapid increase in Hb levels were explored. The laboratory values of patients who switched to roxadustat were closely monitored, and few missing values were observed. However, because this study was retrospective, significant missing laboratory values other than the Hb levels were observed in patients who continued ESA, especially after 2, 4, and 8 weeks. Therefore, laboratory values could not be compared between the two groups.

The causes of CKD include chronic glomerulonephritis, nephrosis, nephrosclerosis, and diabetic nephropathy. Current smoking and alcohol consumption were defined as smoking and daily alcohol intake, respectively, at the time of observation. The last ESA dose before roxadustat administration was calculated as μg/4 weeks for epoetin beta pegol and as μg/week for darbepoetin. The effects per dose of epoetin beta-pegol and darbepoetin were assumed to be the same. The erythropoietin resistance index (ERI) was calculated using the following formula: dosage of epoetin beta pegol or darbepoetin (per 4 weeks) (μg)/body weight (kg) ×

Hb levels (g/dL). The blood pressure was measured in the examination room. Lower limb edema was defined based examinations performed by each doctor.

The initial dosage of roxadustat was based on product information for almost all patients. This switching regimen was treated at the standard dosage in Japan and was approved by the Pharmaceuticals and Medical Devices Agency. The starting dose for patients treated with ≤100 μg/4 weeks of epoetin beta pegol or <20 μg/week darbepoetin was 70 mg/day for three days weekly, and that for patients treated with >100 μg/4 weeks epoetin beta pegol and ≥20 μg/week darbepoetin was 100 mg/day for 3 days weekly. Several patients were administered 50 mg of roxadustat for three days weekly, following the discretion of the clinician. Dose titration was performed according to the product information.

Statistical analysis

Statistical analyses were performed using the Bell Curve for Excel (Social Survey Research Information, Tokyo, Japan) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). It is a modified version of R commander designed to add statistical functions frequently used in biostatistics (22). The analysis was performed using the full analysis set, defined as patients treated with at least one dose of roxadustat or ESA who had at least one efficacy variable measured after the start of treatment. To evaluate the odds ratio for Hb overshoot between the roxadustat and continued ESA groups, we employed multiple imputations to account for background differences between the two groups as well as the unobserved occurrence of Hb overshoot. This was followed by inverse probability of treatment weighting (IPTW). Subsequently, we conducted a logistic regression analysis to determine the difference in the incidence of overshoot between the two groups. Additionally, we performed best- and worst-case scenario analyses. We imputed the data 100 times (m=100) using the within-subject approach. Missing values were analyzed without imputation in other analyses. Comparisons between two groups were performed using the Mann-Whitney U test and Student's *t*-test. To analyze repeated-measures data, a linear mixed model was used to compare the starting time of roxadustat and the time after roxadustat administration. Single and multiple regression analyses were performed to analyze the explanatory variables for the ΔHb levels after four weeks of roxadustat administration. A natural logarithmic transformation was performed to normalize the parameters. After calculating the logarithmic mean scale, standard deviation, and 95% confidence interval (CI) to return to the original scale, an antilogarithmic transformation was performed if necessary according to a previous study (23). To analyze the cross-table methods, we used Fisher's exact test and the Goodman-Kruskal's gamma test. No formal sample size calculations were performed. A value of $p<0.05$ and $r>0.5$ (for Goodman-Kruskal's gamma) were considered to be statistically significant.

Results

Baseline data

Twenty-three patients who switched from ESA to roxadustat (roxadustat group) and 63 who continued ESA (ESA group) were analyzed in this study. The mean baseline Hb level was 10.77 g/dL for roxadustat group and 10.64 g/dL for ESA group, respectively. The median eGFR was 15.7 mL/min/1.73 m^2 and 15.2 mL/min/1.73 m^2, respectively. Other baseline characteristics are presented in Table 1.

Study process and Hb levels

The number of patients in the roxadustat group who visited the hospital at the time of switching to roxadustat and 2, 4, and 8 weeks after switching was 23, 18, 18, and 15, respectively. Within 8 weeks of switching to roxadustat, two patients experienced adverse effects (one cerebral infarction and one nausea), and one withdrew consent for treatment with roxadustat. The number of patients in the ESA group who visited the hospital at the time of continuing ESA and at 4 and 8 weeks after treatment was 63. Notably, five patients in the roxadustat group and all patients in the ESA group had not been scheduled for a follow-up visit after 2 weeks, and one patient in the ESA group did not undergo a blood examination after 4 weeks. The median dosage of roxadustat and ESA after starting observation are shown in Fig. 1a and b. Eight patients in roxadustat group (34.8%) and two patients in ESA group (3.2%) had Hb overshoot (Hb >12.5 g/dL) within their 8-week visit. A critical elevation of Hb levels >13 g/dL, which is outside the recommended range of the guidelines in Japan (24), was observed in two patients in the roxadustat group and not observed in the ESA group. The cumulative overshoot ratios are shown in Fig. 1c. The odds ratio for overshoot between the two groups was 20.0 (95% CI: 3.77-106.0, $p<0.01$) in the unadjusted model. After adjusting for age, sex, body mass index (BMI), Hb levels, TSAT, ferritin levels, ERI, eGFR, high-density lipoprotein cholesterol (HDL-C), C-reactive protein, and albumin, the ratio was 20.2 (95% CI: 3.13-130.0, $p<0.01$). In the best-case and worst-case scenario analyses, the ratio was 24.5 (95%CI: 3.83-156.0, $p<0.01$) and 9.59 (95% CI: 1.68-54.9, $p<0.05$), respectively. The mean Hb levels and mean changes in Hb levels between baseline and after 2, 4, and 8 weeks are shown in Fig. 1d, e. All patients in the roxadustat group who had Hb overshoot visited the hospital four weeks after discontinuing roxadustat.

Hb-related parameters, ferrokinetic parameters, and erythropoietin levels

The Hb-related parameters, ferrokinetic parameters, and erythropoietin levels in patients who switched to roxadustat are presented in Fig. 2. The MCV, RDW, and reticulocyte counts increased after roxadustat initiation (Fig. 2a-c). Se-

Table 1. Baseline Characteristics of Study Participants.

Baseline characteristics	Roxadustat group (n=23)	ESA group (n=63)	p value
Age median, (IQR)	74, (67-81)	69, (54-76.5)	0.031 (a)
Male sex n, (%)	10, (43.5)	34, (54.0)	0.468 (b)
Primary diagnosis for renal disease n, (%)			
Chronic glomerulonephritis and nephrosis	2, (8.7)	11, (17.5)	0.358 (b)
Nephrosclerosis	13, (56.5)	23, (36.5)	
Diabetic nephropathy	5, (21.7)	13, (20.6)	
Others	3, (13.0)	16, (25.4)	
Renal function			
CKD stage 3 n, (%)	4, (17.4)	6, (9.5)	0.358 (b)
CKD stage 4 n, (%)	10, (43.5)	26, (41.2)	
CKD stage 5 n, (%)	9, (39.1)	31, (49.2)	
Serum creatinine level median, (IQR), mg/dL	2.60, (1.82-4.02)	3.01, (1.96-4.48)	0.246 (a)
eGFR median, (IQR), mL/min/1.73 m^2	15.7, (11.5-22.6)	15.2, (10.0-22.4)	0.435 (a)
Urinary protein excretion median, (IQR), g/gCr	1.40, (0.74-3.47)	1.17, (0.65-2.89)	0.447 (a)
Systolic blood pressure median, (IQR), mmHg	139, (125-147)	134, (120-140)	0.133 (a)
Diastolic blood pressure median, (IQR), mmHg	69, (64-80)	75, (65-80)	0.492 (a)
Antihypertensive drug usage n, (%)	20, (87.0)	54, (85.7)	1.000 (b)
Diabetes n, (%)	11, (47.8)	23, (36.5)	0.455 (b)
Antidiabetic drugs usage n, (%)	10, (43.5)	18, (28.6)	0.204 (b)
SGLT2 inhibitor usage n, (%)	2, (8.7)	4, (6.3)	0.656 (b)
Body weight median, (IQR), kg	53.0, (45.2-60.5)	58.0, (49.7-66.0)	0.151 (a)
BMI median, (IQR), kg/m^2	21.4, (19.0-24.5)	22.3, (19.8-25.6)	0.800 (a)
Lower limb edema n, (%)	5, (21.7)	24, (38.1)	0.201 (b)
Diuretics usage n, (%)	10, (43.5)	28, (44.4)	1.000 (b)
Cardiovascular disease n, (%)	5, (21.7)	12, (19.9)	0.767 (b)
Valvular heart disease n, (%)	4, (17.4)	13, (20.6)	1.000 (b)
Current smoker n, (%)	5, (21.7)	11, (17.5)	0.756 (b)
Alcohol consumption amount			
no drink n, (%)	17, (73.9)	41, (65.1)	0.559 (b)
1-20 g/day n, (%)	5, (21.7)	20, (31.7)	
21-60 g/day n, (%)	0, (0)	1, (1.6)	
>60 g/day n, (%)	1, (4.3)	1, (1.6)	
ESA use			
Darbepoetin n, median dosage, (IQR), μg/week	5, 10.0, (7.5-13.3)	20, 12.5, (6.88-21.3)	0.537 (a)
Epoetin beta pegol n, median dosage, (IQR), μg/4 weeks	18, 87.5, (50.0-143.8)	43, 75.0, (50-137.5)	0.755 (a)
ESA n, median dosage, (IQR), μg/4 weeks	23, 53.3, (35-125)	63, 75.0, (40-110)	0.934 (a)
Erythropoietin resistance index, median, (IQR)	0.096 (0.057-0.215)	0.096, (0.057-0.202)	0.711 (a)
Hemoglobin level mean, (SD), g/dL	10.77, (1.17)	10.64, (0.95)	0.605 (c)
Hematocrit level mean, (SD), (%)	33.23, (3.71)	32.88, (2.92)	0.644 (c)
Serum iron level median, (IQR), μg/dL	80.0, (74.0-103.5)	74.5, (55.5-94.5)	0.246 (a)
Serum UIBC median, (IQR), μg/dL	198.0, (144.0-233.0)	171.5, (148.3-196.8)	0.258 (a)
Serum TIBC median, (IQR), μg/dL	279.0, (237.5-312.0)	249.0, (219.3-271.0)	0.130 (a)
TSAT median, (IQR), (%)	31.1, (23.4-38.4)	29.3, (23.0-37.4)	0.727 (a)
Serum ferritin level median, (IQR), ng/mL	177.0, (70.7-245.7)	122.0, (78.5-218.2)	0.489 (a)
Serum CRP levels median, (IQR), mg/dL	0.12, (0.05-0.39)	0.11, (0.05-0.21)	0.968 (a)
Serum albumin levels median, (IQR), g/dL	4.0, (3.7-4.1)	3.9, (3.5-4.7)	0.478 (a)

(a): Mann-Whitney U test, (b): Fisher's exact test, (c): Student's t test.

IQR: interquartile range, SD: standard deviation, CKD: chronic kidney disease, Cr: creatinine, eGFR: estimated glomerular filtration rate, SGLT2: sodium glucose co-transporter 2, BMI: body mass index, ESA: erythropoiesis-stimulating agents, UIBC: unsaturated iron-binding capacity, TIBC: total iron-binding capacity, TSAT: transferrin saturation, CRP: C-reactive protein

rum iron levels did not change (Fig. 2d), although UIBC and TIBC levels increased significantly after roxadustat initiation (Fig. 2e-f). Ferritin and TSAT levels decreased after roxadustat treatment initiation (Fig. 2g-h). The serum erythropoietin levels increased after roxadustat treatment initiation (Fig. 2i).

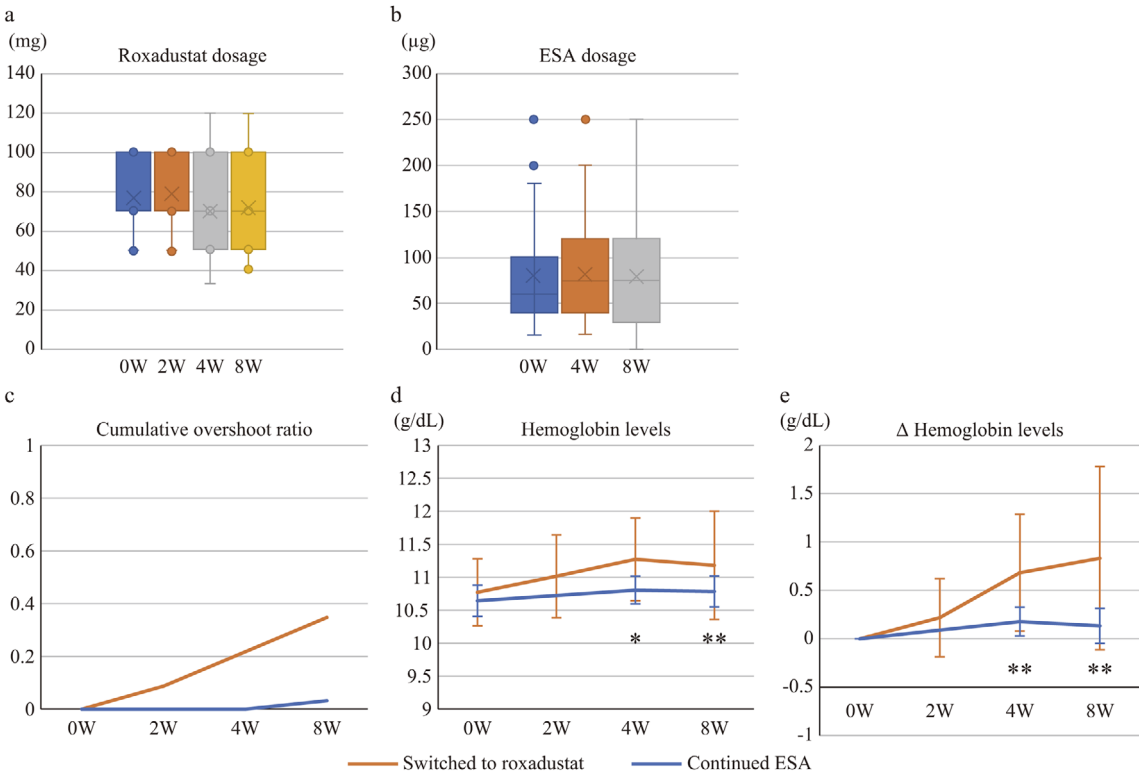

Figure 1. Time courses after starting observation. (a) Median roxadustat dosage per day at 0, 2, 4, and 8 weeks after roxadustat administration. (b) Median ESA dosage per 4 weeks at 0, 4, and 8 weeks after continued ESA. (c) Cumulative overshoot ratio in each group. The denominator of the ratio is the number of patients at the start of observation in each group. (d and e) Mean Hb levels (d) and ΔHb levels in each group (e). These figures include cases of overshoot and data after discontinuation of roxadustat are not included. The Hb and ΔHb levels were analyzed using a linear mixed model. All patients who continued ESA were not visited at 2 weeks after continued ESA. Error bar; 1.5*inter-quartile range for (a) and (b), and 95% confidence interval for (d) and (e). * p<0.05, ** p<0.01 vs. patients who continued ESA. ESA: erythropoiesis-stimulating agents, Hb: hemoglobin

Renal function and lipid metabolism

The renal function and lipid metabolism parameters of patients who switched to roxadustat are shown in Fig. 3. The eGFR and urinary protein excretion did not change after roxadustat administration (Fig. 3a, b). The total cholesterol levels decreased after roxadustat administration (Fig. 3c), accompanied by a decrease in low-density lipoprotein cholesterol (LDL-C) and the HDL-C levels (Fig. 3d, e). The triglyceride levels decreased after roxadustat administration, but this change was not statistically significant (Fig. 3f).

Characteristics of the patients who switched to roxadustat with overshot Hb levels

Among the patients who switched to roxadustat, excluding three patients who discontinued roxadustat owing to side effects and their own decision, we analyzed the characteristics of patients with overshot Hb levels (Table 2). In younger patients and those with higher baseline Hb or hematocrit (Ht) levels, significant Hb overshoot was observed. No significant differences in other factors, including the ERI and iron-related parameters, were observed between the non-overshoot and overshoot groups (Table 2). Details regarding the other characteristics are presented in Table 2. In patients with diabetes, a significant Hb overshoot was observed after switching to roxadustat. However, the baseline Hb levels were higher in patients with diabetes than in those without diabetes (Supplementary material 1). Other characteristics of patients with and without diabetes are presented in Supplementary material 1.

Four weeks after roxadustat administration or at the withdrawal point within 4 weeks (Table 3). The median Hb level in the overshoot group was 12.70 g/dL. Patients exhibiting Hb overshoot demonstrated higher median reticulocyte counts and relatively lower TSAT and ferritin levels.

All patients with overshoot discontinued roxadustat and visited the hospital after 4 weeks. After discontinuing roxadustat, the mean Hb levels remained at 11.7 g/dL, maintained within the recommended range (Fig. 4a, 11.0 ≤Hb ≤13.0 g/dL). The reticulocyte counts and MCV, RDW, UIBC, TIBC, TSAT, and ferritin levels 4 weeks after elevation were similar to the baseline values (Fig. 4b-i). The erythropoietin levels 4 weeks after elevation were lower than the baseline values (Fig. 4j).

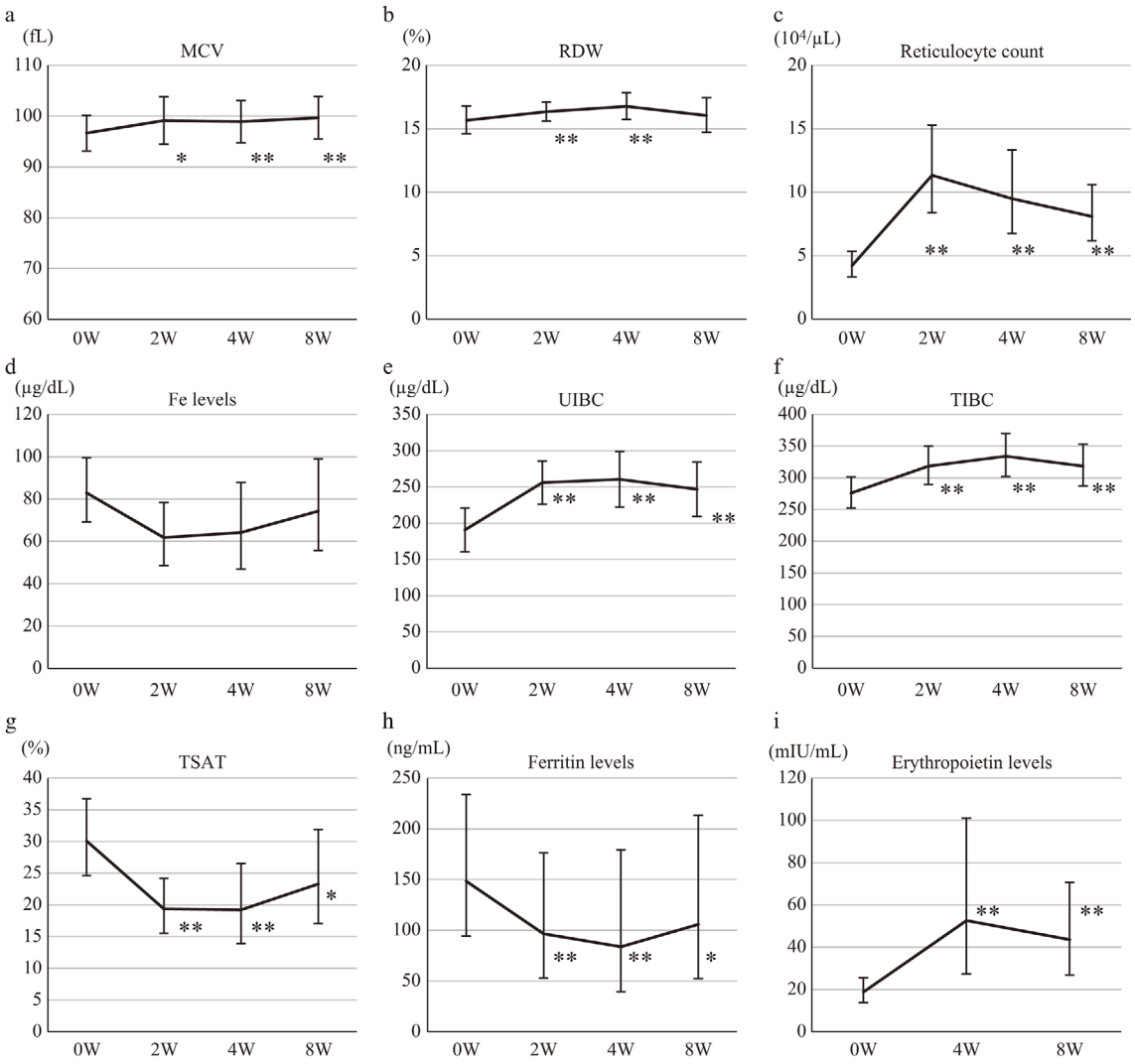

Figure 2. **Time courses after roxadustat administration. Mean MCV (a), RDW (b), reticulocyte count (c), Fe levels (d), UIBC (e), TIBC (f), TSAT (g), ferritin levels (h) and erythropoietin levels (i) at 0, 2, 4, and 8 weeks after roxadustat administration. All data were analyzed using a linear mixed model. Error bar; 95% confidence interval. * p<0.05, ** p<0.01 vs. 0W. MCV: mean corpuscular volume, RDW: red blood cell distribution width, TIBC: total iron-binding capacity, TSAT: transferrin saturation, UIBC: unsaturated iron-binding capacity**

Contributing factors associated with an increase in the Hb levels

The factors contributing to ΔHb levels 4 weeks after roxadustat administration were analyzed. A single regression analysis showed no significant relationship between the ΔHb levels and age, sex, starting dose of roxadustat, ERI, baseline Hb levels, TSAT, ferritin, erythropoietin, eGFR, iron supplementation during treatment, blood pressure, antihypertensive drug use, diabetes, antidiabetic drugs, body weight, BMI, lower-limb edema, diuretic use, serum albumin levels, cardiovascular disease, valvular disease, or iron supplementation. However, HDL-C, but not LDL-C, triglyceride, or total cholesterol, was detected as a statistically significant factor for ΔHb levels. This significance was retained, even after controlling for several factors (Table 4).

Adverse events

Cerebral infarction and nausea were observed in each patient in the roxadustat group. None of the patients experienced any other adverse events, such as thrombosis or thyroid dysfunction. No adverse events were observed in the ESA group.

Discussion

We analyzed the data of patients with anemic, non-dialyzed CKD who switched from ESA to roxadustat or continued ESA. Hb overshoot was observed in eight of 23 patients in the roxadustat group (34.8%) and in two of 63 patients in the ESA group (3.2%) during the study period.

In the roxadustat group, a younger age and higher base-

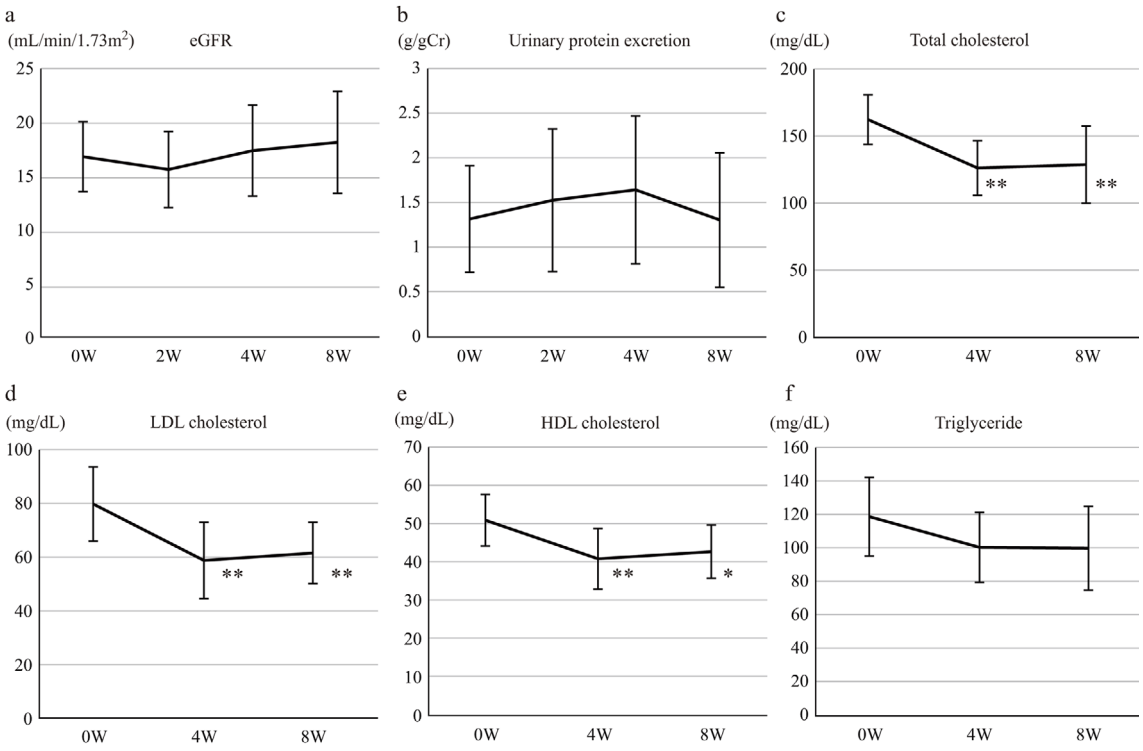

Figure 3. Time courses after roxadustat administration. Mean eGFR (a), urinary protein excretion (b), total cholesterol levels (c), LDL cholesterol levels (d), HDL cholesterol levels (e) and triglyceride levels (f) at 0, 2, 4, and 8 weeks after roxadustat administration. All data were analyzed using a linear mixed model. Error bar; 95% confidence interval. * p<0.05, ** p<0.01 vs. 0W. eGFR: estimated glomerular filtration rate, HDL: high-density lipoprotein, LDL: low-density lipoprotein

line Hb and Ht levels were associated with Hb overshoot. Hb overshoot was also associated with diabetes, but the baseline Hb levels were higher in patients with diabetes; hence, we speculate that the higher baseline Hb levels in patients with diabetes affected the results, although the reason for this elevation in patients with diabetes was unclear. Our speculation is consistent with the findings of a previous large study (25).

In general, erythropoietin resistance and iron utilization disorders are considered risk factors for Hb overshoot. However, the baseline ERI, TSAT, and ferritin levels did not differ significantly between the non-overshoot and overshoot groups. This could be due to the definition of overshoot (Hb >12.5 g/dL), where even a small ΔHb could meet the criteria if the Hb level was high at the time of switching. As shown in Fig. 1e, the stronger Hb-raising effect of roxadustat compared to continuing ESA might explain why ESA resistance and impaired iron utilization were not identified as risk factors. Furthermore, because this study had a small sample size, the statistical power may have been insufficient. Additionally, higher reticulocyte counts and relatively lower TSAT and ferritin levels were observed in the overshoot group 4 weeks after roxadustat administration or withdrawal point within 4 weeks. These findings suggest that more active hematopoiesis and improved iron utilization occurred in these patients; hence, erythropoietin resistance and impaired iron utilization may still be potential risk factors. Further studies are required to confirm these findings.

ΔHb levels after four weeks of roxadustat treatment were negatively correlated with serum HDL-C levels. Several correlations between the HDL-C levels and anemia have been reported. Increased HDL-C level is associated with shortened erythrocyte lifespan (26). High serum adiponectin levels, which positively correlated with the HDL-C levels, are associated with anemia and lower reticulocyte production in patients with CKD (27). Therefore, the HDL-C level is associated with erythrocyte lifespan and reticulocyte production. Roxadustat prolongs erythrocyte lifespan and enhances production (28). In this study, reticulocytes, MCV, and RDW increased after switching to roxadustat, thus indicating enhanced erythrocyte production and the presence of both existing and newly produced erythrocytes of various sizes. This suggests that although roxadustat improves erythrocyte production immediately, it might not immediately affect the lifespan of existing erythrocytes. Therefore, lower HDL-C levels at the time of switching to roxadustat could be related to a larger ΔHb level after 4 weeks of roxadustat treatment. However, the detailed mechanisms remain unclear, and further investigation is thus required.

Our findings suggest a therapeutic shift from ESA to roxadustat. Caution is warranted, especially in younger patients with higher baseline Hb or Ht levels, as they may not

Table 2. Comparison of Baseline Characteristics between Patients with Hemoglobin Overshoot and Those without Overshoot.

	Non-overshoot (n=12)	Overshoot (n=8)	p value or r value
Age median, (IQR)	77.5, (67.0-81.8)	67.0, (64.8-73.3)	0.026 (a)
Male sex n, (%)	6, (50.0)	3, (37.5)	0.670 (b)
Primary diagnosis for renal disease n, (%)			
Chronic glomerulonephritis and nephrosis	2, (16.7)	0, (0)	0.083 (b)
Nephrosclerosis	6, (50.0)	4, (50.0)	
Diabetic nephropathy	1, (8.33)	4, (50.0)	
Others	3, (25.0)	0, (0)	
Renal function			
Serum creatinine level median, (IQR), mg/dL	2.44, (1.67-2.96)	3.38, (2.58-4.26)	0.181 (a)
eGFR median, (IQR), mL/min/1.73 m^2	20.5, (14.5-28.6)	12.9, (10.1-15.8)	0.157 (a)
Urinary protein excretion median, (IQR), g/gCr	1.48, (0.85-3.33)	1.85, (1.20-4.46)	0.606 (a)
Systolic blood pressure median, (IQR), mmHg	138, (124-150)	132, (119-140)	0.110 (a)
Diastolic blood pressure median, (IQR), mmHg	72, (63-82)	63, (60-72)	0.151 (a)
Antihypertensive drug usage n, (%)	10, (83.3)	8, (100)	0.495 (b)
Diabetes n, (%)	2, (16.7)	7, (87.5)	0.005 (b)
Antidiabetic drugs usage n, (%)	2, (16.7)	6, (75.0)	0.019 (b)
SGLT2 inhibitor usage n, (%)	0, (0)	1, (12.5)	0.400 (b)
Body weight median, (IQR), kg	53.0, (50.1-66.1)	56.7, (49.2-59.3)	0.777 (a)
BMI median, (IQR), kg/m^2	23.2, (19.1-25.5)	22.3, (20.4-24.4)	0.970 (a)
Lower limb edema n, (%)	4, (33.3)	1, (12.5)	0.603 (a)
Diuretics usage n, (%)	5, (41.7)	3, (37.5)	1.000 (a)
Cardiovascular disease n, (%)	2, (16.7)	1, (12.5)	1.000 (b)
Valvular heart disease n, (%)	2, (16.7)	1, (12.5)	1.000 (b)
Current smoker n, (%)	2, (16.7)	3, (37.5)	0.348 (b)
Alcohol consumption amount			
no drink n, (%)	9, (75.0)	6, (75.0)	1.000 (b)
1-20 g/day n, (%)	3, (25.0)	2, (25.0)	
ESA use			
Darbepoetin n, median dosage, (IQR), μg/week	3, 10.0, (8.8-11.7)	2, 10.0, (7.5-12.5)	1.000 (a)
Epoetin beta pegol n, median dosage, (IQR), μg/4 weeks	9, 100.0, (50.0-125.0)	6, 62.5, (31.3-131.3)	0.547 (a)
ESA n, median dosage, (IQR), μg/4 weeks	12, 76.7, (37.5-125.0)	8, 55.0, (25.0-93.8)	0.485 (a)
Starting dose for roxadustat			
median, (IQR), mg	70.0, (70.0-100.0)	70.0, (65.0-77.5)	0.499 (a)
100 mg n, (%)	4, (33.3)	2, (25.0)	0.310 (c)
70 mg n, (%)	7, (58.3)	4, (50.0)	
50 mg n, (%)	1, (8.3)	2, (25.0)	
Hemoglobin level median, (IQR), g/dL	10.55, (9.650-11.23)	11.65, (11.10-11.95)	0.029 (a)
Hematocrit level median, (IQR), (%)	32.20, (29.85-33.50)	35.40, (34.10-37.75)	0.039 (a)
Reticulocyte count median, (IQR), 10^4/μL	3.08, (2.63-4.72)	4.61, (3.80-5.07)	0.174 (a)
Serum iron level median, (IQR), μg/dL	83.5, (69.5-100.8)	79.0, (74.8-85.8)	0.610 (a)
Serum UIBC median, (IQR), μg/dL	172.5, (141.8-211.5)	198.5, (166.3-230.8)	0.558 (a)
Serum TIBC median, (IQR), μg/dL	279.0, (227.3-310.8)	263.0, (237.8-316.5)	0.970 (a)
TSAT median, (IQR), (%)	34.3, (25.4-40.6)	29.8, (24.2-31.4)	0.296 (a)
Serum ferritin level median, (IQR), ng/mL	196.0, (128.5-528.7)	121.9, (66.3-178.3)	0.152 (a)
Serum erythropoietin level median, (IQR), mIU/mL	23.0, (15.5-33.8)	15.1, (12.5-18.9)	0.392 (a)
Erythropoietin resistance index, median, (IQR)	0.116, (0.054-0.201)	0.079, (0.043-0.137)	0.305 (a)
Serum CRP levels median, (IQR), mg/dL	0.20, (0.05-0.82)	0.05, (0.05-0.12)	0.866 (a)
Albumin levels median, (IQR), mg/dL	3.8, (3.7-3.9)	4.1, (3.8-4.2)	0.213 (a)
Iron supplementation			
at the start and after of roxadustat n, (%)	5, (41.7)	3, (37.5)	1.000 (b)
at the start and after, and one time before the start of roxadustat n, (%)	7, (58.3)	4, (50.0)	1.000 (b)

(a): Mann-Whitney U test, (b): Fisher's exact test, (c): Goodman-Kruskal's gamma.

IQR: interquartile range, CKD: chronic kidney disease, Cr: creatinine, eGFR: estimated glomerular filtration rate, SGLT2: sodium glucose co-transporter 2, BMI: body mass index, ESA: erythropoiesis-stimulating agents, UIBC: unsaturated iron-binding capacity, TIBC: total iron-binding capacity, TSAT: transferrin saturation, CRP: C-reactive protein

Table 3. Parameters at 4 Weeks after Roxadustat Administration or Withdrawal Point within 4 Weeks.

	Non-overshoot (n=12)	Overshoot (n=8)	
Hemoglobin level median, (IQR), g/dL	10.95, (9.68-11.8)	12.70, (12.45-12.83)	<0.001
Δ hemoglobin levels at four weeks or the withdrawal point within four weeks median, (IQR), g/dL	0.00, (-0.13-1.35)	1.15, (0.90-1.35)	0.229
Reticulocyte count median, (IQR), 10^4/μL	7.15, (5.74-9.08)	12.36, (9.94-18.26)	0.026
Serum iron level median, (IQR), μg/dL	61.0, (42.3-94.3)	61.5, (52.0-90.8)	0.955
Serum UIBC median, (IQR), μg/dL	220.0, (192.0-296.8)	263.0, (247.0-307.8)	0.230
Serum TIBC median, (IQR), μg/dL	327.5, (282.0-354.8)	344.0, (313.3-366.3)	0.305
TSAT median, (IQR), %	22.60, (12.47-33.31)	17.08, (15.33-26.50)	0.624
Serum ferritin level median, (IQR), ng/mL	124.0, (59.7-469.8)	37.1, (26.0-72.8)	0.129

All data were analyzed using the Mann-Whitney U test.

IQR: interquartile range, UIBC: unsaturated iron-binding capacity, TIBC: total iron-binding capacity, TSAT: transferrin saturation

be ideal candidates for this switch. For a trend toward reduced TSAT or ferritin levels, early reduction or discontinuation of roxadustat may have to be considered. Being attentive to rapid increases in the ΔHb levels, especially in patients with lower HDL-C levels Discontinuation of roxadustat appears to be relatively safe in cases of Hb overshoot, as in our study, where the Hb levels remained close to baseline and within the recommended range (11≤Hb≤13 g/dL) 4 weeks after discontinuation. Future large-scale studies are needed to validate this therapeutic strategy.

In our study, one patient with heavy alcohol consumption experienced a cerebral infarction. Despite the thromboembolic risks (29-31), large trials have shown no significant differences in major cardiovascular events, including stroke (32). The patient had no history of iron deficiency, smoking, or hypertension. Given the risk of stroke associated with heavy alcohol consumption (33, 34), it is advisable to use roxadustat cautiously in such patients.

This study is associated with several limitations. First, owing to the small sample size, caution is advised when interpreting the statistical results. However, our findings align with those of previous phase 3 trials, and we believe that our interpretation is largely error-free. Large-scale, real-world studies are required for further validation. Second, because this study was retrospective, several laboratory values could not be compared between the two groups. Furthermore, the retrospective nature of the study introduces potential bias, and a prospective and randomized study is required in the future. Third, a statistical equivalence test between the baseline and post-overshoot weeks was not conducted for patients with elevated Hb levels, considering the challenges in defining the equivalent margin. The descriptive statistics in Fig. 4 indicate that Hb levels, reticulocyte levels, and ferrokinetic parameters were maintained close to baseline after four weeks of roxadustat discontinuation, thus supporting the reversibility of these parameters after discontinuation. Fourth, our focus on the early week effects of roxadustat necessitates further analysis of its long-term impact in future studies. Fifth, few patients received sodium glucose cotransporter 2 inhibitors as they were not approved for CKD treatment by the Japanese health insurance system during the study. Lastly, while the initial dosage and titration followed product information, we acknowledge the possibility that overshooting may be related to the titration strategy of roxadustat.

Despite these limitations, our study contributes significantly to the literature. To date, few clinical studies have focused on the excessive increase in Hb levels in the early weeks after switching from ESA to roxadustat in adult non-dialysis patients. Moreover, large trials, including phase 3 and real-world studies, on switching from ESA to roxadustat in adult, non-dialyzed CKD patients are also limited, with only one phase 3 trial addressing this broader condition without focusing on early Hb overshoot (18). Therefore, we believe that this is an important pilot study. Furthermore, we evaluated the clinical course after four weeks of roxadustat discontinuation in patients with Hb overshoot and determined several risk factors for Hb overshoot. Finally, our findings suggest some of the factors that contribute to the increase in Hb levels. We believe that the results of our study, along with those of previous phase 3 trials, can therefore be applied in clinical practice.

Conclusion

Hb overshoot was observed in eight of 23 patients who switched to roxadustat within 8 weeks and in two of 63 patients who continued ESA. Clinicians should be aware of Hb overshoot and emphasize the importance of early Hb level checks. Further large-scale studies are needed to substantiate these findings.

The authors state that they have no Conflict of Interest (COI).

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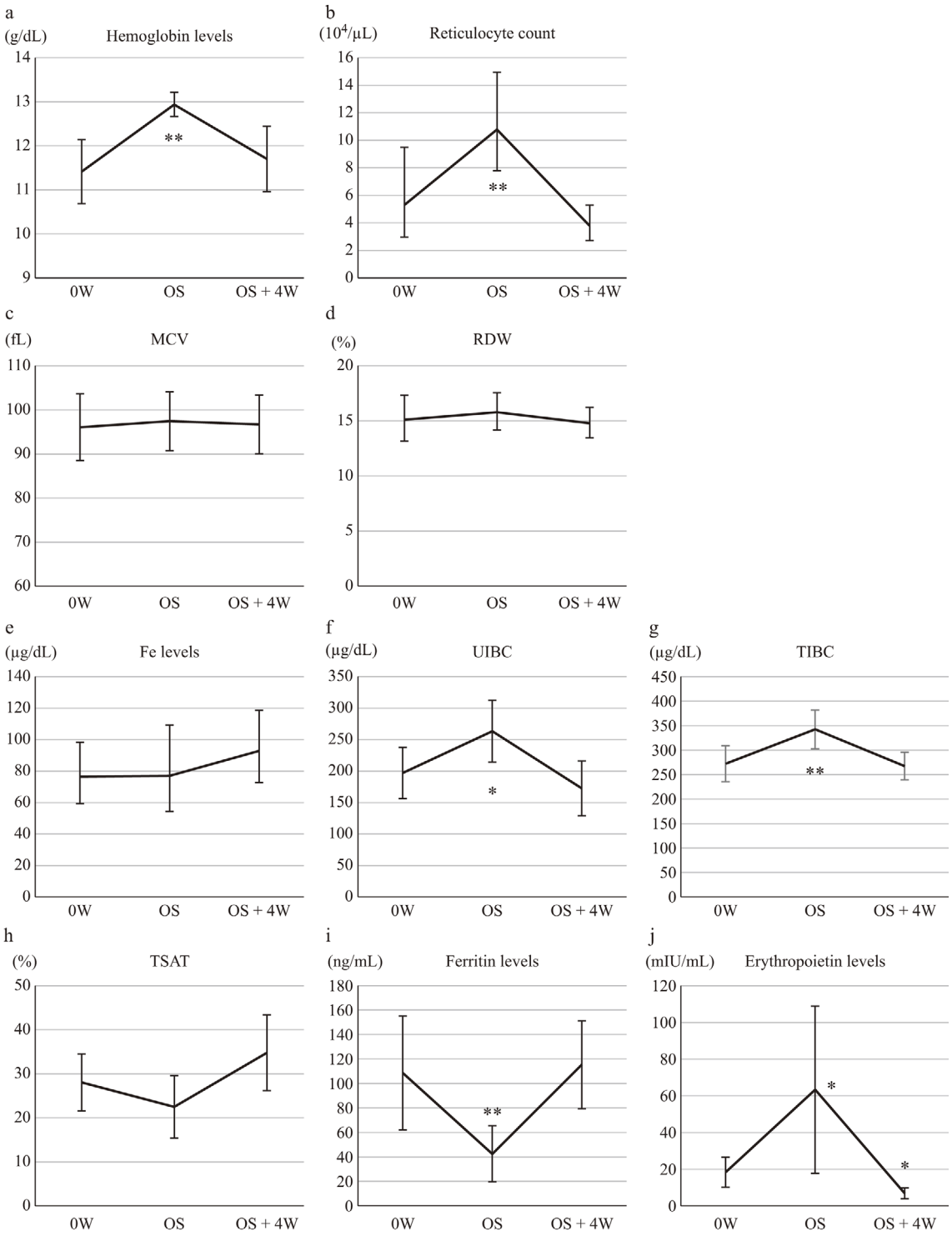

Figure 4. **Time courses after roxadustat administration among patients with overshot hemoglobin levels (n=8). (a–j) Mean hemoglobin levels (a), reticulocyte counts (b), MCV (c), RDW (d), Fe levels (e), UIBC (f), TIBC (g), TSAT (h), ferritin levels (i), and erythropoietin levels (j) at baseline (0W), during overshoot and after 4 weeks of overshoot. All data were analyzed using a linear mixed model. Error bar; 95% confidence interval. * p<0.05, ** p<0.01 vs. 0W. OS: overshoot, OS+4 W: 4 weeks after overshoot, MCV: mean corpuscular volume, RDW: red blood cell distribution width, TIBC: total iron-binding capacity, TSAT: transferrin saturation, UIBC: unsaturated iron-binding capacity**

Table 4. Effect of HDL Cholesterol and Lipid-modifying Drugs on ΔHb Level after 4 Weeks.

Univariate analysis	B (95% CI)	Standard error	p value
HDL-cholesterol (log)	-2.77 (-4.41, -1.13)	0.77	<0.01
Model 1	B (95% CI)	Standard error	p value
HDL-cholesterol(log)	-2.73 (-4.44, -1.02)	0.8	<0.01
Lipid modifying drug	-0.16 (-1.19, 0.87)	0.49	0.74
Model 2	B (95% CI)	Standard error	p value
HDL-cholesterol (log)	-3.25 (-5.07, -1.44)	0.84	<0.01
Lipid modifying drug	-0.36 (-5.07, 0.65)	0.47	0.45
Age	0.03 (-0.02, 0.09)	0.03	0.24
Sex	0.80 (-0.28, 1.88)	0.50	0.14
Model 3	B (95% CI)	Standard error	p value
HDL-cholesterol (log)	-3.61 (-7.04, -0.19)	1.45	0.04
Lipid modifying drug	-0.45 (-2.04, 1.15)	0.68	0.53
Age	0.03 (-0.07, 0.14)	0.04	0.47
Sex	0.66 (-0.98, 2.30)	0.69	0.37
Hemoglobin	0.11 (-0.95, 1.17)	0.45	0.81
TSAT (log)	-0.82 (-3.43, 1.80)	1.11	0.48
Ferritin (log)	0.19 (-1.00, 1.39)	0.5	0.71
eGFR (log)	-0.35 (-2.33, 1.63)	0.84	0.69
Erythropoietin resistance index (log)	0.14 (-0.91, 1.18)	0.44	0.77

A multiple regression analysis was performed. Lipid-modifying drugs included statins, fibrates, and omega-3 fatty acids. B: regression coefficient. HDL: high-density lipoprotein, TSAT: transferrin saturation, eGFR: estimated glomerular filtration rate

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