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Mediators between canagliflozin and renoprotection vary depending on patient characteristics: Insights from the CREDENCE trial

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Abstract

Aim: To identify the mediators between canagliflozin and renoprotection in patients with type 2 diabetes at a high risk of end-stage kidney disease (ESKD).

Methods: In this post hoc analysis of the CREDENCE trial, the effect of canagliflozin on potential mediators (42 biomarkers) at 52 weeks and the association between changes in mediators and renal outcomes were evaluated using mixed-effects and Cox models, respectively. The renal outcome was a composite of ESKD, serum creatinine doubling or renal death. The percentage of the mediating effect of each significant mediator was calculated based on changes in the hazard ratios of canagliflozin after additional adjustment of the mediator.

Results: Changes in haematocrit, haemoglobin, red blood cell (RBC) count and urinary albumin-to-creatinine ratio (UACR) at 52 weeks significantly mediated 47%, 41%, 40% and 29% risk reduction with canagliflozin, respectively. Further, 85% mediation was attributed to the combined effect of haematocrit and UACR. A large variation in mediating effects by haematocrit change existed among the subgroups, ranging from 17% in those patients with a UACR of more than 3000 mg/g to 63% in patients with a UACR of 3000 mg/g or less. In the subgroups with a UACR of more than 3000 mg/g, UACR change was the highest mediating factor (37%), driven by the strong association between UACR decline and renal risk reduction.

Conclusions: The renoprotective effects of canagliflozin in patients at a high risk of ESKD can be significantly explained by changes in RBC variables and UACR. The complementary mediating effects of RBC variables and UACR may support the renoprotective effect of canagliflozin in different patient groups.

KEYWORDS

canagliflozin, clinical trial, diabetic nephropathy, drug mechanism, type 2 diabetes

1 | INTRODUCTION

Diabetic kidney disease (DKD) is the most common cause of chronic kidney disease (CKD) and end-stage kidney disease (ESKD) worldwide[1] and is a global public health issue.[1] Emerging data have revealed that sodium-glucose co-transporter-2 inhibitors (SGLT2is) can reduce the risk of kidney disease progression and ESKD in patients with type 2 diabetes (T2D).[2–5] Hence, several sets of guidelines have recommended the use of SGLT2is.[6–8]

SGLT2is have renoprotective effects. Although the underlying mechanisms are incompletely understood, several hypotheses have been proposed, including lowering intraglomerular pressure by activating tubuloglomerular feedback, positive effects on endothelial function, metabolic effects to promote autophagy, as well as mitigation of tubular workload and hypoxia.[9] Clinically, in addition to reducing glucose levels, SGLT2is mitigate various risk factors of CKD progression, such as hypertension, obesity, albuminuria, dyslipidaemia, hyperuricaemia and anaemia.[9,10] The favourable kidney outcomes of SGLT2is may be explained, at least partly, by the positive effects of SGLT2is on these abnormalities, as observed in clinical trials.[3,11,12] Examining the relationship between SGLT2is and kidney outcomes via changes in biomarkers may help in understanding how SGLT2is improve kidney outcomes. Additionally, careful attention to these changes may be a motivator and educational tool to improve patient self-management, including medication adherence.

Previous mediation analyses revealed that changes in red blood cell (RBC) variables (e.g. haematocrit and haemoglobin levels and RBC count) and serum uric acid levels could explain a significant proportion of the renoprotective effects of SGLT2is.[11,12] However, these results were derived from cardiovascular outcome trials including patients with diabetes who presented with a comparatively preserved estimated glomerular filtration rate (eGFR) and low albuminuria. Hence, most individuals who developed renal events did not reach ESKD.[11,12] It remains unknown whether these mediators can be applied to patients at a high risk of ESKD (i.e. a lower eGFR and/or higher albuminuria level). This is because erythropoiesis is impaired with the progression of CKD stages,[13] and the reduction in serum uric acid levels with SGLT2is was attenuated in patients with an eGFR of less than 60 mL/min/1.73m^2.[14]

We hypothesized that the factors mediating the renoprotective effects of SGLT2is or the extent of the contribution of mediating factors can vary based on the eGFR and/or albuminuria level. The CREDENCE trial was the first large-scale placebo-controlled renal primary outcome trial to establish definitive evidence regarding the renoprotective effects of canagliflozin.[4] In this trial, 176 participants underwent dialysis or kidney transplants during the study period, whereas less than 20 patients reached these outcomes in trials in which mediation analyses were conducted.[11,12] In the current study, using data from the CREDENCE trial, the mediators between canagliflozin and robust renal outcomes were explored.

2 | METHODS

2.1 | Patients and study design

This is a post hoc analysis of data from the CREDENCE trial, a multicentre, double-blind, placebo-controlled, randomized trial evaluating the effects of canagliflozin on renal outcomes in T2D and nephropathy.[4] The design and primary results of the CREDENCE trial have been published.[4] Briefly, participants were aged 30 years or older and had T2D, an HbA1c level of 6.5%-12.0%, an eGFR of 30-90 mL/min/1.73m^2, and a urinary albumin-to-creatinine ratio (UACR) of 300-5000 mg/g. All the participants received treatment with the maximum tolerated or labelled dose of renin–angiotensin–aldosterone system inhibitors for 4 weeks or longer before randomization. The protocol was approved by the ethics committees for each trial site. All the participants provided written informed consent. The CREDENCE trial was registered at clinicaltrials.gov (identifier: NCT02065791) and was performed in accordance with the Declaration of Helsinki.

2.2 | Randomization and study treatment

The participants were randomly assigned in a 1:1 ratio to canagliflozin 100 mg or a matching placebo in a double-blind manner. The treatment continued until trial completion, dialysis initiation, kidney transplantation, development of diabetic ketoacidosis, pregnancy or receipt of a prohibited therapy. Other background therapies for cardiovascular risk factors, including glycaemic management, were provided according to the local guidelines.

2.3 | Potential mediators

Face-to-face postrandomization follow-up within the first year was scheduled at 3, 13, 26 and 52 weeks and at 6-month intervals thereafter. A serum chemistry panel and physical examination were performed at each visit. Data on glycaemic indicators, haematology panel, fasting serum lipid profile and urinary results were assessed according to predetermined intervals (Table S1). The aforementioned clinical laboratory tests were performed at the central laboratory. SGLT2is affect multiple biomarkers. Thus, we aimed to identify mediators by extensively testing 42 available biomarkers (Table 1).

2.4 | Outcomes

The primary outcome of interest in this analysis was a composite renal outcome: doubling of creatinine, ESKD (dialysis initiation, kidney transplantation or a sustained eGFR of < 15 mL/min/1.73m^2) or renal death. The secondary outcome was ESKD. All outcomes were adjudicated by independent adjudication committee members, who were unaware of the trial-group assignments.

TABLE 1 Effects of canagliflozin on biomarkers

	Baseline value (median [IQR])[a]		Placebo-adjusted change from baseline (between-group difference [95% CI])[b]	
	Placebo	Canagliflozin	Week 52	During the study period
Vital, weight				
SBP, mmHg	140 (130, 150)	139 (130, 150)	**−3.5 (−4.4, −2.6)**	**−3.2 (−3.8, −2.6)**
DBP, mmHg	80 (72, 85)	79 (72, 85)	**−1.0 (−1.5, −0.5)**	**−0.8 (−1.2, −0.5)**
Pulse rate, bpm	74 (67, 80)	73 (67, 80)	−0.1 (−0.6, 0.5)	0.1 (−0.3, 0.5)
Body weight, kg	84 (73, 98)	84 (73, 98)	**−1.3 (−1.6, −1.1)**	**−1.2 (−1.4, −1.0)**
Serum chemistry panel				
Total protein, g/L	71 (67, 74)	71 (67, 74)	**1.3 (1.1, 1.5)**	**1.2 (1.0, 1.4)**
Albumin, g/L	40 (37, 42)	40 (37, 42)	**1.1 (0.9, 1.3)**	**1.0 (0.8, 1.1)**
AST, U/L	19 (16, 24)	19 (16, 24)	**−1.8 (−3.4, −0.1)**[c]	**−1.3 (−2.5, −0.1)**[c]
ALT, U/L	19 (14, 26)	19 (15, 26)	**−2.2 (−4.3, −0.1)**[c]	**−2.5 (−4.0, −0.9)**[c]
Total bilirubin, μmol/L	7 (5, 9)	7 (5, 9)	**3.5 (1.2, 5.8)**[c]	**3.5 (1.8, 5.2)**[c]
GGT, U/L	26 (18, 42)	26 (18, 42)	**−6.0 (−8.2, −3.7)**[c]	**−5.2 (−7.0, −3.3)**[c]
ALP, U/L	79 (64, 99)	81 (64, 100)	−0.3 (−1.5, 0.9)[c]	**−1.2 (−2.1, −0.2)**[c]
LDH, U/L	181 (160, 206)	179 (158, 205)	**−6.3 (−8.1, −4.5)**	**−5.5 (−6.9, −4.2)**
CPK, U/L	120 (82, 185)	115 (79, 178)	**−6.0 (−8.6, −3.4)**[c]	**−0.1 (−0.1, 0.0)**[c]
BUN, mmol/L	9 (7, 11)	8 (7, 11)	0.1 (−0.1, 0.3)	**0.2 (0.0, 0.3)**
eGFR, mL/min/1.73m^2	54 (41, 69)	55 (42, 70)	−0.1 (−0.8, 0.5)	−0.5 (−1.0, 0.0)
Sodium, mmol/L	138 (137, 140)	138 (136, 140)	0.2 (0.0, 0.3)	**0.2 (0.0, 0.3)**
Potassium, mmol/L	4.5 (4.1, 4.8)	4.5 (4.2, 4.8)	0.01 (−0.02, 0.03)	−0.01 (−0.03, 0.01)
Chloride, mmol/L	101 (99, 103)	101 (99, 103)	0.1 (−0.1, 0.3)	0.0 (−0.1, 0.2)
Bicarbonate, mmol/L	22 (20, 24)	22 (20, 24)	**−0.2 (−0.4, −0.1)**	**−0.3 (−0.5, −0.2)**
Calcium, mmol/L	2.4 (2.3, 2.5)	2.4 (2.3, 2.5)	**0.02 (0.01, 0.03)**	**0.02 (0.01, 0.02)**
Phosphate, mmol/L	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	**0.02 (0.01, 0.03)**	**0.02 (0.02, 0.03)**
Magnesium, mmol/L	0.8 (0.7, 0.9)	0.8 (0.7, 0.9)	**0.07 (0.06, 0.07)**	**0.07 (0.06, 0.07)**
Uric acid, μmol/L	400 (333, 464)	393 (331, 464)	**−13.2 (−17.7, −8.6)**	**−13.1 (−16.5, −9.6)**
Glycaemic indicators				
FPG, mmol/L[d]	8.3 (6.7, 10.7)	8.6 (6.8, 10.8)	**−0.47 (−0.67, −0.26)**	**−0.45 (−0.59, −0.30)**
HbA1c, %	8.0 (7.2, 9.0)	8.1 (7.3, 9.1)	**−0.23 (−0.30, −0.16)**	**−0.19 (−0.25, −0.14)**
Haematology panel				
WBC count, 10^9/L	7.16 (6.02, 8.54)	7.07 (5.96, 8.48)	−0.096 (−0.195, 0.003)	−0.068 (−0.157, 0.021)
Neutrophil, 10^9/L	4.55 (3.71, 5.63)	4.53 (3.69, 5.6)	**−0.088 (−0.171, −0.004)**	−0.063 (−0.136, 0.011)
Lymphocyte, 10^9/L	1.81 (1.47, 2.23)	1.82 (1.45, 2.25)	−0.004 (−0.038, 0.030)	−0.002 (−0.032, 0.028)
Monocyte, 10^9/L	0.38 (0.29, 0.49)	0.38 (0.29, 0.48)	−0.003 (−0.011, 0.005)	−0.003 (−0.010, 0.005)
Eosinophil, 10^9/L	0.18 (0.12, 0.27)	0.18 (0.12, 0.26)	−0.001 (−0.012, 0.010)	−0.002 (−0.011, 0.006)
Basophils, 10^9/L	0.05 (0.03, 0.07)	0.05 (0.03, 0.07)	0.000 (−0.002, 0.003)	**0.002 (0.000, 0.004)**
RBC count, 10^{12}/L	4.4 (4.1, 4.8)	4.5 (4.1, 4.8)	**0.25 (0.22, 0.27)**	**0.25 (0.22, 0.27)**
Haemoglobin, g/L	131 (119, 143)	133 (121, 145)	**6.9 (6.2, 7.6)**	**7.0 (6.3, 7.7)**
Haematocrit, %	40 (37, 44)	40 (37, 44)	**2.4 (2.1, 2.6)**	**2.4 (2.2, 2.6)**
Platelet count, 10^9/L	233 (195, 284)	236 (196, 285)	**−3.8 (−6.7, −0.9)**	−2.4 (−5.1, 0.2)
Fasting lipid profile				
Triglycerides, mmol/L[d]	1.80 (1.29, 2.65)	1.86 (1.34, 2.67)	−0.2 (−2.8, 2.4)[c]	−1.0 (−3.3, 1.4)[c]
Total cholesterol, mmol/L[d]	4.45 (3.75, 5.33)	4.50 (3.71, 5.43)	**0.08 (0.01, 0.15)**	0.05 (−0.01, 0.11)
HDL-C, mmol/L[d]	1.09 (0.92, 1.32)	1.09 (0.91, 1.32)	**0.01 (0.00, 0.03)**	**0.02 (0.00, 0.03)**
LDL-C, mmol/L[d]	2.30 (1.71, 3.04)	2.27 (1.24, 3.10)	**0.05 (0.00, 0.11)**	0.04 (−0.01, 0.09)

(Continues)

TABLE 1 (Continued)

	Baseline value (median [IQR])[a]		Placebo-adjusted change from baseline (between-group difference [95% CI])[b]	
	Placebo	Canagliflozin	Week 52	During the study period
Urinalysis				
UACR, mg/g	914 (469, 1841)	923 (459, 1779)	**−33 (−38, −28)**[c]	**−33 (−37, −28)**[c]
Specific gravity	1.014 (1.011, 1.017)	1.014 (1.011, 1.017)	**0.0015 (0.0012, 0.0018)**	**0.0015 (0.0012, 0.0017)**
pH	5.5 (5.5, 6.0)	5.50 (5.50, 6.00)	**−0.10 (−0.12, −0.08)**	**−0.09 (−0.11, −0.07)**

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; bpm, beats per minute; BUN, blood urea nitrogen; CI, confidence interval; CPK, creatine kinase; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; RBC, red blood cell; SBP, systolic blood pressure; UACR, urinary albumin-to-creatinine ratio; WBC, white blood cell.

[a]These data are for those patients who did not reach the composite renal outcome until the 52-week follow-up and for whom both baseline and 52-week follow-up measurements were available.

[b]Placebo-adjusted changes from baseline were estimated by mixed-effects models with repeated measures using all the data available before the composite renal outcome. The models included the fixed effects of treatment, trial visit, screening eGFR randomization strata, treatment-by-visit interaction, baseline value of each mediator and baseline value of each mediator-by-visit interaction. Values in bold indicate a significant treatment effect ($P < .05$).

[c]Placebo-adjusted changes from baseline values are presented as the geometric mean of the percentage change (95% CI).

[d]Fasting test results.

2.5 | Statistical analysis

First, we considered changes in biomarkers at 52 weeks as potential mediators. Next, the average changes in biomarkers during the study period were analysed as potential mediators. The time point for evaluating mediators was set at 52 weeks, for the following reasons: first, it was the first time point when follow-up of the haematology panel was available, and haematocrit and haemoglobin were reported to be strong mediators between SGLT2is and kidney protection.[11,12] Second, eGFR trajectories in patients receiving canagliflozin and placebo crossed at 52 weeks.[4] Third, the effect of canagliflozin on renal events were mainly observed after 52 weeks.[4] Regarding 42 potential mediators, we evaluated whether canagliflozin affected their values and whether these changes were associated with renal outcomes using mixed-effects models with repeated measures and Cox models, respectively. The mixed-effects models with repeated measures included the fixed effects of treatment, trial visit, screening eGFR randomization strata (30-<45, 45-<60 or 60-<90 mL/min/1.73m^2), treatment-by-visit interaction, baseline value of each mediator and baseline value of each mediator-by-visit interaction. Patients were considered as a random effect (both in slope and intercept). The Cox models were stratified according to the category of eGFR at screening. We calculated the percentages of mediation (i.e. indirect effect) using the following formula[15]: the percentage of mediation = (unadjusted hazard ratio [HR] − adjusted HR)/(unadjusted HR − 1) × 100, where adjusted HR is defined as the HR of canagliflozin after adjusting for the change in the index biomarker, assuming no exposure–mediator, exposure–outcome, mediator–outcome confounding or exposure–mediator interaction. The 95% confidence interval (CI) of the percentage mediation was estimated via a bootstrap procedure using 5000 samplings. The joint mediating effect of multiple mediators was estimated using the same equation. Stratified analyses were conducted by gender, screening eGFR, baseline UACR and haematocrit levels. We did not calculate 95% CIs because of a low number of events in each subgroup. We excluded individuals without measurements of mediators at baseline or 52 weeks or those who reached the composite renal outcome before week 52. Statistical tests were two-tailed and P values less than .05 were considered statistically significant. Analyses were performed using the Stata/IC 15 statistical software package (Stata Corp., College Station, TX).

3 | RESULTS

3.1 | Baseline characteristics

In the CREDENCE trial, the composite renal outcome was observed in 153 of 2202 and 224 of 2199 participants in the canagliflozin and placebo groups, respectively (HR 0.66, 95% CI: 0.53-0.81), with a median follow-up of 2.6 years.[4] Of patients with the composite renal outcome, 75% (281/377) patients reached ESKD. By day 321, the first day of the visit window at week 52, the composite renal outcome was observed in 11 and 18 patients in the canagliflozin and placebo groups, respectively. The baseline characteristics of participants were well balanced after excluding those who achieved the renal outcome by visit at week 52 and those without biomarker measurements at baseline or week 52, with a median baseline eGFR of 55 mL/min/1.73m^2 and a UACR of 921 mg/g (Table 1).

3.2 | Effects of canagliflozin on potential biomarkers

We assessed 42 potential biomarkers comprising clinical laboratory and physiological variables (Table 1). Compared with placebo, canagliflozin significantly increased the levels of 13 biomarkers (serum total

protein, albumin, total bilirubin, calcium, phosphate, magnesium, RBC count, haemoglobin, haematocrit, total cholesterol, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C] and urine specific gravity) and decreased the levels of 16 biomarkers (systolic blood pressure [SBP], diastolic blood pressure, body weight, aspartate aminotransferase, alanine aminotransferase [ALT], gamma-glutamyltransferase [GGT], lactate dehydrogenase [LDH], creatine kinase [CPK], bicarbonate, uric acid, fasting plasma glucose, HbA1c, neutrophil, platelets, UACR and urine pH) at 52 weeks (Table 1). The analyses during the study period yielded similar results (Table 1). No significant difference in terms of eGFR was observed at 52 weeks between the two groups (Table 1).

3.3 | Association of changes in potential mediators and the renal composite outcome

Of 29 biomarkers affected by canagliflozin at 52 weeks, 22 biomarkers (SBP, body weight, serum total protein, albumin, ALT, total bilirubin, GGT, LDH, CPK, bicarbonate, calcium, phosphate, uric acid, HbA1c, RBC count, haemoglobin, haematocrit, total cholesterol, HDL-C, LDL-C, UACR and urine pH) were significantly associated between those changes at 52 weeks and the renal composite outcome (Table S2). Similarly, average changes in 22 of 29 biomarkers (SBP, body weight, serum total protein, albumin, ALT, total bilirubin, alkaline phosphatase, LDH, CPK, blood urea nitrogen, bicarbonate, calcium, phosphate, uric acid, RBC count, haemoglobin, haematocrit, HDL-C, UACR, urine specific gravity and urine pH) were associated with the renal composite outcome (Table S2).

3.4 | Percentage mediation

Of the 22 biomarkers that were eligible for mediators at 52 weeks, four biomarkers (haematocrit [47%, 95% CI: 2%-92%], RBC count [41%, 95% CI: 2%-80%], haemoglobin [40%, 95% CI: 3%-77%] and UACR [29%, 95% CI: 0%-58%]) had statistically significant results (Figure 1). Similarly, haematocrit (55%, 95% CI: 2%-108%), RBC count (47%, 95% CI: 6%-89%), haemoglobin (47%, 95% CI: 7%-87%) and UACR (31%, 95% CI: 2%-60%) were significant mediators in the analysis using the average change as mediators (Figure 1). The point estimates did not alter substantially after replacing the renal composite outcome with ESKD (Table S3). The joint mediating effects of haematocrit and UACR were 85% (95% CI: 4%-167%) and 99% (95% CI: 8%-190%) in the analyses using change at 52 weeks and average change as mediators, respectively. The magnitudes of mediation by changes in haematocrit, RBC count, haemoglobin and UACR at 52 weeks were numerically different, particularly across genders, baseline UACR and haematocrit levels (Figure 2A). Mediations by haematocrit, RBC count and haemoglobin were smaller in females (23%, 22% and 18%, respectively) than in males (65%, 55% and 56%, respectively). Similarly, mediations by these variables at 52 weeks were less prominent in patients with a UACR of more than 3000 mg/g (17%, 17% and 19%, respectively) compared with patients with a UACR of 3000 mg/g or

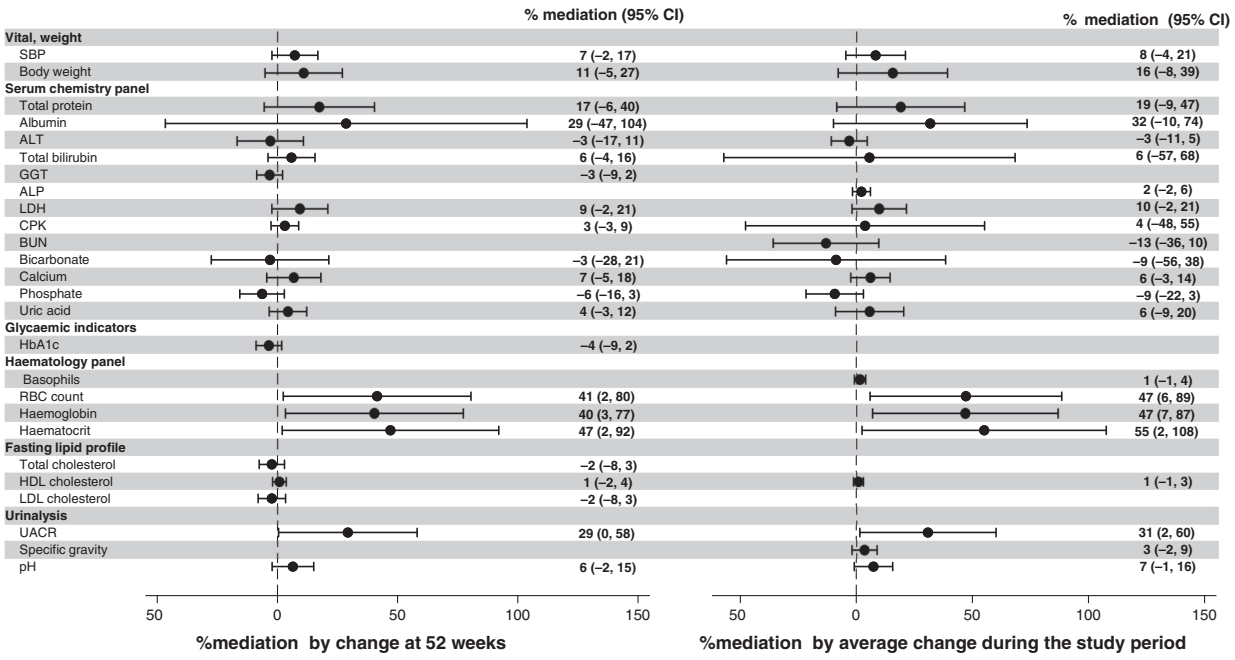


FIGURE 1 Percentages of each biomarker mediated by change at 52 weeks and average change during the study period. ALP, alkaline phosphatase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; CI, confidence interval; CPK, creatine kinase; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; RBC, red blood cell; SBP, systolic blood pressure; UACR, urinary albumin-to-creatinine ratio

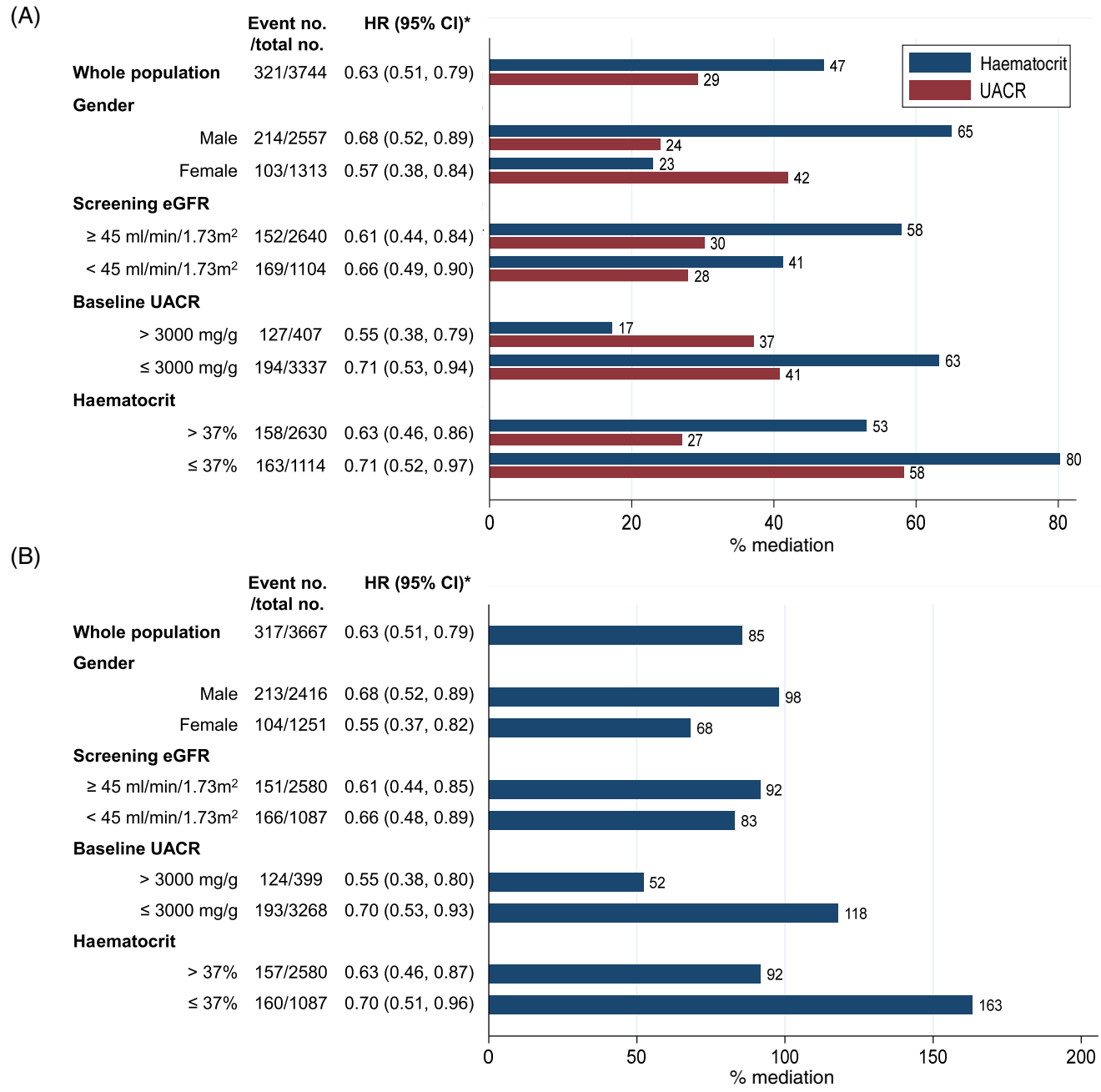


FIGURE 2 Mediating effects of haematocrit, as a representative of RBC variables, and UACR at 52 weeks across patient subgroups. Each mediating effect of A, Haematocrit and UACR, and B, Joint effects of haematocrit and UACR, are shown. CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; RBC, red blood cell; UACR, urinary albumin-to-creatinine ratio. *Unadjusted HRs of canagliflozin in each subgroup

less (63%, 52% and 49%, respectively). By contrast, UACR mediated the benefits of canagliflozin to the same degree across UACR subgroups (Figure 2A). Hence, UACR was found to be the highest mediator (37%) in patients with UACR more than 3000 mg/g. Additionally, UACR change was the highest mediator in females (Figure 2A). In those individuals with a haematocrit of 37% or less, haematocrit, RBC count, haemoglobin and UACR at 52 weeks mediated more substantially (80%, 82%, 72% and 58%, respectively) than in those with a haematocrit of more than 37% (53%, 43%, 45% and 27%, respectively; Figure 2). Screening eGFR did not alter the mediating effects of these variables substantially (Figure 2A). The joint mediating effect of haematocrit and UACR at 52 weeks was particularly low (52%) in patients with a UACR of more than 3000 mg/g (Figure 2B), reflecting the low percentage mediation by haematocrit (17%) in this subgroup (Figure 2A).

To explore the cause of significant variations in the mediating effects across subgroups, we described (1) the effects of canagliflozin on haematocrit (as a representative of haematocrit, RBC count and

		Between-group differences*	Association with kidney outcome; HR (95% CI)	
		Haematocrit (%)	HR for 1% increase in haematocrit	% mediation
Whole population		2.4 (2.1, 2.6)	0.89 (0.86, 0.91)	47
Gender	Male	2.6 (2.3, 2.9)	0.89 (0.86, 0.91)	65
	Female	2.0 (1.7, 2.4)	0.90 (0.85, 0.94)	23
Screening eGFR	≥ 45 ml/min/1.73m^2	2.4 (2.2, 2.7)	0.86 (0.83, 0.90)	58
	< 45 ml/min/1.73m^2	2.2 (1.8, 2.6)	0.90 (0.87, 0.94)	41
Baseline UACR	> 3000 mg/g	1.6 (0.9, 2.3)	0.91 (0.88, 0.95)	17
	≤ 3000 mg/g	2.4 (2.2, 2.7)	0.90 (0.86, 0.93)	63
Haematocrit	> 37%	2.6 (2.3, 2.8)	0.89 (0.85, 0.92)	53
	≤ 37%	1.8 (1.4, 2.2)	0.84 (0.80, 0.88)	80
		UACR (%) †	HR for 30% decrease in UACR	
Whole population		−33 (−38, −28)	0.86 (0.83, 0.89)	29
Gender	Male	−31 (−36, −25)	0.87 (0.83, 0.91)	24
	Female	−38 (−46, −29)	0.84 (0.80, 0.89)	42
Screening eGFR	≥ 45 ml/min/1.73m^2	−33 (−39, −27)	0.86 (0.82, 0.90)	30
	< 45 ml/min/1.73m^2	−33 (−41, −24)	0.86 (0.81, 0.91)	28
Baseline UACR	> 3000 mg/g	−14 (−31, 7)	0.67 (0.60, 0.74)	37
	≤ 3000 mg/g	−35 (−40, −30)	0.85 (0.81, 0.88)	41
Haematocrit	> 37%	−31 (−37, −24)	0.87 (0.83, 0.91)	27
	≤ 37%	−37 (−44, −28)	0.84 (0.79, 0.89)	58

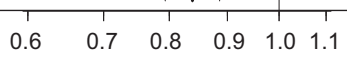


FIGURE 3 Effect of canagliflozin on haematocrit and UACR at 52 weeks and the associations between these changes and the renal composite endpoint across patient subgroups. CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; UACR, urinary albumin-to-creatinine ratio. *Between-group differences from baseline were estimated using mixed-effects models with repeated measures using all the data available before the composite renal outcome. The models included the fixed effects of treatment, trial visit, screening eGFR randomization strata, treatment-by-visit interaction, baseline value of each mediator and baseline value of each mediator-by-visit interaction. †Between-group differences from baseline values are presented as the geometric mean of the percentage change (95% CI)

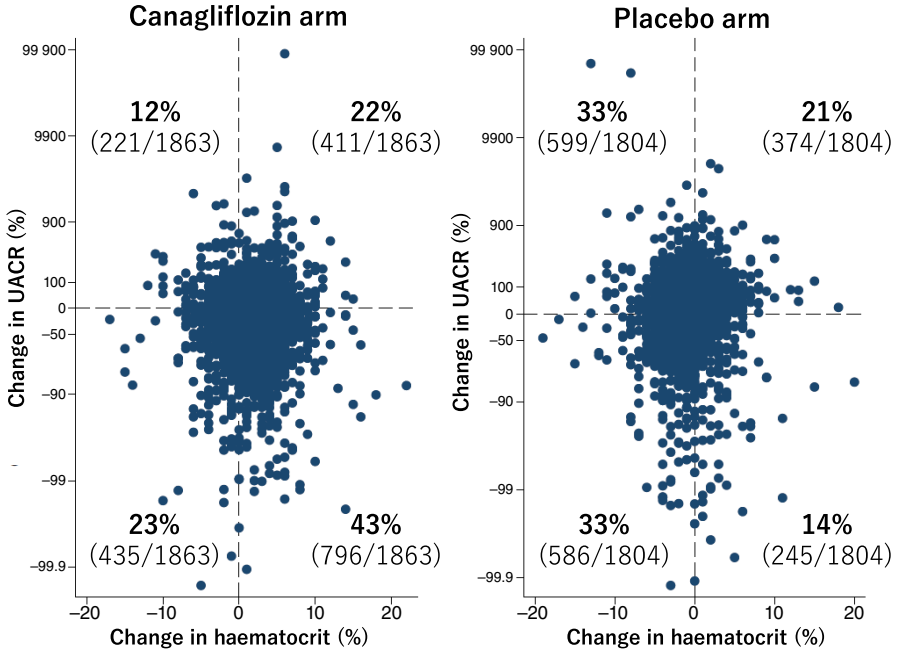
haemoglobin) and UACR; and (2) the associations of these changes with the renal composite outcome. In those subgroups with a UACR of more than 3000 mg/g in which mediation by haematocrit was at its minimum (17%), the benefit of canagliflozin on haematocrit was negligible (0.3%, 95% CI: −0.2% to 0.9%; Figure S1). In fact, the between-group difference in the haematocrit change from baseline to week 52 was at its minimum (1.6%, 95% CI: 0.9%-2.3%) in this subgroup compared with the other subgroups (Figure 3). Moreover, the association between an increase in haematocrit level and the renal outcome was the weakest (HR for 1% increase in haematocrit 0.91, 95% CI: 0.88-0.95; Figure 3) in this subgroup. A reduction in UACR with canagliflozin was also the lowest (−14%, 95% CI: −31% to 7%) in those with a UACR of more than 3000 mg/g (Figures 3 and S2). However, UACR reduction was strongly related to the renal outcome (HR for 30% decrease in UACR 0.67, 95% CI: 0.60-0.74; Figure 3) in this subgroup. In the subgroup with a haematocrit of 37% or less, in which mediation by haematocrit was the largest (80%), the effect size of the association between haematocrit change and the renal outcome was the largest (HR for 1% increase in haematocrit 0.84, 95% CI: 0.80-0.98; Figure 3). Additionally, the association between UACR reduction and the renal outcome was comparatively large (HR for 30% decrease in UACR 0.84, 95% CI: 0.79-0.89, respectively; Figure 3).

Furthermore, by dividing the patients into four categories according to changes in haematocrit and UACR from baseline to week 52, we examined the renal prognosis in each category. The proportion of patients with increased haematocrit (Ht_I) and decreased UACR (UACR_D) was higher in canagliflozin-treated patients than in the placebo group (43% vs. 14%; Figure 4A). Conversely, the percentage of patients with decreased haematocrit (Ht_D) and increased UCAR (UACR_I) was lower in patients with canagliflozin than in those receiving a placebo (12% vs. 33%; Figure 4A). With the placebo group as the reference, the HRs for the renal outcome in the canagliflozin arm were 2.03 (95% CI: 1.46-2.83), 0.74 (95% CI: 0.52-1.07), 0.85 (95% CI: 0.60-1.19) and 0.14 (95% CI: 0.08-0.25) in those with Ht_D/UACR_I, Ht_D/UACR_D, Ht_I/UACR_I and Ht_I/UACR_D, respectively (Figure 4B).

4 | DISCUSSION

In this post hoc mediation analysis of the CREDENCE trial, RBC variables (i.e. haematocrit, RBC count and haemoglobin) and UACR were identified as major mediators between canagliflozin and the composite renal outcome in diabetes patients with overt albuminuria. The proportions of mediation by haematocrit, as a representative of RBC parameters, were substantially different across patient subgroups, ranging from 17% in those with a UACR of more than 3000 mg/g to 63% in patients with a UACR of 3000 mg/g or less. This variable explained 80% of the mediation in individuals with haematocrit of

(A)



(B)

	Haematocrit increase (> 0%)	UACR decrease (< 0%)	Event no. /total no.	HR (95% CI)
Placebo			187/1804	Ref
Canagliflozin	−	−	44/221	2.03 (1.46, 2.83)
	−	+	34/435	0.74 (0.52, 1.07)
	+	−	39/411	0.85 (0.60, 1.19)
	+	+	13/796	0.14 (0.08, 0.25)

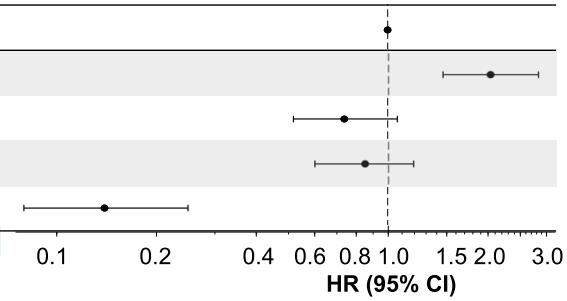


FIGURE 4 A, Changes in haematocrit and UACR from baseline to week 52 for each participant. B, Associations between categories according to changes in UACR and haematocrit at 52 weeks and renal prognosis in the canagliflozin group, with the placebo group as a reference. Values in A represent the percentage and number of patients in the categories classified by increased haematocrit and decreased UACR. CI, confidence interval; HR, hazard ratio; UACR, urinary albumin-to-creatinine ratio

37% or less. In general, UACR change was the second most important mediator after RBC parameters. However, UACR explained the largest proportion of the renoprotective effect of canagliflozin among those with a UACR of more than 3000 mg/g and in females. After the initiation of canagliflozin, the renal prognosis for patients with Ht_I/UACR_D was excellent, while patients with Ht_D/UACR_I had a very poor renal prognosis.

Previous mediation analyses revealed RBC parameters as major mediators between SGLT2is and renal outcomes.[11,12] Our study used robust renal outcomes including ESKD and extended these findings to patients with advanced-stage CKD. It was revealed that increasing RBC parameters do not simply reflect haemoconcentration associated with the diuretic effect of SGLT2is.[16] Rather, these changes reflect erythropoiesis promotion because SGLT2is modulate iron-related proteins such as hepcidin, erythroferrone and erythropoietin, similar to hypoxia-inducible factor prolyl hydroxylase enzyme inhibitors.[17–19] Notably, the change in haematocrit mediated most of the renoprotective effects of canagliflozin (80%) in individuals with a haematocrit of 37% or less. Anaemia is a risk factor for DKD progression.[20] Thus, the current results are consistent with the concept that the amelioration of renal hypoxia by ESA (erythropoiesis stimulating agent) preserves renal function.[21] Unfortunately, the current study could not conclude the causal relationship. However, it is safe to say that RBC parameters can be an early indicator of drug efficacy on renal outcomes. Furthermore, simultaneous evaluation of changes in haematocrit and UACR would provide a simple and practical way of predicting the renal outcomes in canagliflozin-treated patients. Despite the potent renoprotective effects of SGLT2is, a non-negligible residual risk for the

progression of DKD remains,[2–5] especially in those patients with Ht_D/UACR_I. Therefore, future research is needed on the development of effective therapy in this population.

This study showed that the extent of the contribution of the mediating factors may vary based on the characteristics of patients. Surprisingly, the percentage of mediation by haematocrit was low (17%) in the subgroup with a UACR of more than 3000 mg/g. The probable explanation for this is the minor effect of canagliflozin on haematocrit and the weak association between an increase in haematocrit and renal outcomes in this subgroup (Figure 3). Interestingly, the effect size of canagliflozin on UACR reduction in this subgroup was minimal (Figure 3). However, a reduction in UACR in this category was strongly associated with improvements in renal outcomes (Figure 3). Hence, UACR is the most significant mediator in this subgroup (Figure 2A). Consistent with our results, by analysing more than 600 000 participants, the CKD Prognosis Consortium reported that the renal risk reduction attributable to a decrease in UACR was comparatively high in subgroups with higher baseline UACR.[22] Hence, we should take into account not only the degree of change in biomarkers, but also the impact of changes in biomarkers on the outcome.

Contrary to the results from previous mediation analyses, we could not confirm the use of uric acid as a mediator.[11,12] This discrepancy was attributed to the lower therapeutic effect of SGLT2is on uric acid in the CREDENCE trial compared with previous reports, if the association between its change and the renal outcome does not vary significantly. In the mediation analysis of the CANVAS Program, the effect size of canagliflozin on uric acid was −23 μmol/L.[11] Meanwhile, in the current study, the effect size was −13 μmol/L. The CREDENCE trial enrolled patients with more advanced-staged CKD (baseline mean eGFR: 56 mL/min/1.73m^2 in the current study, 76 mL/min/1.73m^2 in the CANVAS Program[3] and 76 mL/min/1.73m^2 in the VERTIS CV trial[23]). A lower eGFR modified changes in uric acid with canagliflozin and ultimately changed mediators.

The current study had several strengths. High-quality data from the CREDENCE trial, which is a large, well-conducted, randomized, double-blind, placebo-controlled trial, were used. All the biomarkers were evaluated in the central laboratory, and renal events were adjudicated by independent adjudication committee members. The current study also had several limitations. Considering the inclusion criteria of the CREDENCE trial, the results cannot be extrapolated to patients without T2D or an eGFR of less than 30 mL/min/1.73m^2. Because this was not a prespecified study, biomarkers were not measured with equal frequency. The haematological panel was first evaluated at 52 weeks postrandomization, which may lead to survivor bias. However, a low number of renal events before 52 weeks minimizes this issue. We sought mediators in a wide range of biomarkers. However, other mediators (e.g. proximal tubular damage markers[24]) may exist. Finally, mediation analysis requires strong assumptions. These include no mediator–outcome confounder affected by exposure and no unmeasured confounding in the exposure–outcome, mediator–outcome or exposure–mediator relationship. Randomizing treatment does not guarantee the absence of confounding factors between the mediator and the outcome. Additionally, for ease of understanding, we used the traditional mediation analysis method, which assumes the absence of an exposure–mediator interaction. Hence, the current results should be considered hypothesis generating.

In summary, this mediation analysis showed that changes in RBC parameters, followed by changes in UACR, account for a major part of canagliflozin-related renal risk reduction in patients who are at a high risk of ESKD. The percentage of mediation varies based on the background characteristics of patients. Nevertheless, further studies should be performed to elucidate the underlying mechanism the renoprotective effects of SGLT2is in patient populations with different renal risk profiles.

AUTHOR CONTRIBUTIONS

Design: YD and SY. Analysis: YD, TH and SY. Conduct/data collection: YD, TH, YS, JYK and YI. Manuscript writing: YD, TH, SY, YS, JYK and YI.

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CONFLICTS OF INTERESTS STATEMENT

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15191.

DATA AVAILABILITY STATEMENT

Data from this study will be made available in the public domain via the Yale University Open Data Access Project.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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