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Associations of Angiopoietin 2 and Vascular Endothelial Growth Factor-A Concentrations with Clinical End Points

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Abstract

Background Angiopoietin 2 regulates endothelial function partially mediated by vascular endothelial growth factor-A (VEGF-A) and may play a role in diabetic kidney disease (DKD). We assessed the association of angiopoietin 2 and VEGF-A with cardiorenal outcomes and investigated the effect of canagliflozin on angiopoietin 2 and VEGF-A concentrations.

Methods Two thousand five hundred sixty-five study participants with DKD and available plasma samples treated with canagliflozin or placebo in the Canagliflozin and Kidney Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial were included. Angiopoietin 2 and VEGF-A concentrations were measured at baseline, year 1, and year 3. The primary composite end point of the trial was a composite of kidney failure, doubling of the serum creatinine level, and kidney or cardiovascular death.

Results Patients with the highest baseline quartile of angiopoietin 2, but not VEGF-A, concentration had the highest risk clinical profile. Treatment with canagliflozin significantly lowered concentrations of angiopoietin 2 (adjusted geometric mean ratio: 0.94; 95% confidence interval, 0.92 to 0.95; $P < 0.001$), but not VEGF-A. In multivariable-adjusted modeling, each 50% increment in log baseline angiopoietin 2 concentrations was associated with a higher risk of primary composite outcome (hazard ratio, 1.27; 95% confidence interval, 1.13 to 1.43). Angiopoietin 2 change at year 1 compared with baseline explained 10% of the effect of canagliflozin on the primary composite outcome. VEGF-A concentrations were not associated with outcomes, alone or in combination with angiopoietin 2.

Conclusions Higher angiopoietin 2 levels were associated with cardiorenal risk among individuals with DKD independent of VEGF-A. Canagliflozin lowered angiopoietin 2 concentrations.

Clinical Trial registry name and registration number Evaluation of the Effects of Canagliflozin on Renal and Cardiovascular Outcomes in Participants With Diabetic Nephropathy, NCT02065791.

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Introduction

Individuals with type 2 diabetes mellitus are at elevated risk of microvascular and macrovascular complications. An important form of microvascular complication of type 2 diabetes mellitus is diabetic kidney disease (DKD), which is the leading cause of kidney failure, and cardiovascular events are the primary cause of mortality among affected individuals.[1] Although glucose control and use of angiotensin-converting enzyme inhibitors,[2] angiotensin receptor blockers,[3] finerenone,[4] and sodium-glucose cotransporter-2 inhibitors (SGLT2is) have been shown to slow declines in GFR and reduce cardiovascular risk among patients with type 2 diabetes mellitus,[5,6] affected individuals are, nonetheless, exposed to considerable residual risk of cardiokidney complications, including progression to kidney failure, heart failure, or other outcomes. Accordingly, further reduction in microvascular and macrovascular complications may require targeting other pathophysiologic pathways activated in the diabetic milieu.

Mounting evidence supports the potential role of endothelial dysfunction in the pathophysiology of type 2 diabetes mellitus complications. Endothelial dysfunction is characterized by an imbalance in vasodilation and vasoconstriction of the vascular wall due to various growth factors and chemokines that alter the permeability of endothelial cells. Angiopoietin 2 has emerged as a biomarker involved in endothelial physiology and cardiac remodeling.[7] Elevated angiopoietin 2 concentrations have been reported in cardiovascular disorders, such as heart failure,[8,9] ischemic heart disease,[10,11] peripheral artery disease,[12] stroke,[13] CKD,[14] and hypertension.[15] It is proposed

that angiopoietin 2 along with angiopoietin 1 and vascular endothelial growth factor-A (VEGF-A) regulate endothelial permeability and angiogenic processes.[7] Accordingly, there is interest in evaluating these biomarkers in DKD.[16]

In this study, using data from study participants with available samples for analysis in the Canagliflozin and Kidney Events in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) trial, we sought to assess the association between angiopoietin 2 and cardiorenal outcomes and further investigate the effect of canagliflozin on angiopoietin 2 concentrations. The direct effect of SGLT2is on angiopoietin 2 levels has not been reported previously.[17] As a secondary goal, we evaluated VEGF-A as a prognostic biomarker either alone or in combination with angiopoietin 2 to prognosticate in individuals with DKD. We hypothesized that higher concentrations of angiopoietin 2 and/or VEGF-A would be associated with adverse cardiokidney risk.

Methods

Study Design and Patient Population

The methods and main results of the CREDENCE trial have been described previously.[16,17] In brief, the CREDENCE study compared the efficacy of canagliflozin 100 mg versus placebo in lowering cardiorenal events among 4401 people with type 2 diabetes mellitus and kidney disease. Participants in the study were to have a minimum glycated hemoglobin level between 6.5% and 12.0%, an eGFR of 30–90 ml/min per 1.73 m^2, and a urine albumin–creatinine ratio of >300–5000 mg/g. Specific to this analysis, owing to local restrictions on sample availability from some regions, only those study participants with available plasma samples were included (N=2565, 58.3% of total population). Plasma samples were collected at baseline, 1 year, and 3 years and stored at −80°C. Plasma levels of angiopoietin 2 and VEGF-A were analyzed on the cobas e 601 analyzer using a prototype Elecsys electrochemiluminescence immunoassay developed by Roche Diagnostics. Both assays use a quantitative sandwich principle, with a first monoclonal antibody specifically binding angiopoietin 2 (or VEGF-A) as the capture antibody and a second monoclonal antibody binding angiopoietin 2 (or VEGF-A) as a detection antibody. Recombinant angiopoietin 2 (or VEGF-A) was used to normalize measurements across runs. For angiopoietin 2 and VEGF-A, the lower limit of detection was 0.03 ng/ml and 0.3 pg/ml, respectively. Local ethics committees approved all study procedures for the CREDENCE trial and subsequent analyses.

Clinical end points examined included the primary composite end point of CREDENCE (a composite of end stage kidney failure, doubling of the serum creatinine level, and kidney or cardiovascular death); the kidney composite end point (a composite of end stage kidney failure, doubling of the serum creatinine level, or kidney death); and the composite of heart failure hospitalization or cardiovascular death, heart failure hospitalization, all-cause death, and cardiovascular death.

Statistical Analysis

Mean (SD)/median (25%–75%) and count (percentage) were used to present continuous and categorical variables, respectively. Comparisons of study population baseline characteristics across angiopoietin 2 quartiles were performed using Kruskal–Wallis, ANOVA, and chi-square tests as appropriate. Multivariable linear regression was used to assess the correlation of biomarkers (angiopoietin 2 and VEGF-A) with baseline variables. To evaluate the effect of canagliflozin on biomarker concentrations, the geometric mean ratios (95% confidence intervals [CIs]) of their year 1 and year 3 ratios to baseline were compared between CREDENCE treatment groups. To calculate a biomarker cut point for the primary composite outcome that provided equal balance between sensitivity and specificity, we used the maximize_metric method within the cutpointr package; metrics were entered as "sum_sens_spec." The Cox proportional hazards regression was used to assess the associations of angiopoietin 2 concentrations with clinical outcomes in models adjusted for selected covariates and including treatment-by-angiopoietin 2 interaction when statistically significant. Simple Cox regression models were fit to each outcome for each variable in Table 1 individually, and variables with P value < 0.05 were entered in the final model (Supplemental Table 1). The mediation package in R was used to assess the relative magnitude of canagliflozin efficacy in lowering primary composite outcome risk associated with its effect on angiopoietin 2 concentrations. The analysis was conducted in three steps: (*1*) producing an M model, (*2*) producing a Y model, and (*3*) conducting a mediation analysis. In the M model using multiple linear regression, angiopoietin 2 was regressed on canagliflozin and baseline covariates to explain change in angiopoietin 2. In the Y model using Weibull regression, the primary composite outcome variable was regressed on change in angiopoietin 2, canagliflozin, and baseline covariates. These two models were grouped with the mediate function, which was run to estimate the direct effect of canagliflozin, its indirect effect through angiopoietin 2, and their 95% CIs by a quasi-Bayesian Monte Carlo method, including 5000 simulations per estimate set. Study participants with events between baseline and year 1 were not included in mediation analysis.

All hypotheses were two-sided, with a P value < 0.05 considered statistically significant. All statistical analyses were performed using the R version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/).

Data and Resource Availability

The data underlying this article can be accessed with a request submitted through the Yale Open Data Access Project site at http://yoda.yale.edu.

Results

Two thousand five hundred sixty-five individuals with DKD had available samples for analysis. Comparisons between those included and excluded from the study are summarized in Supplemental Table 2. Overall, patients included in the study were more likely to be Asian and had lower prevalence of heart failure, coronary artery disease, and cerebrovascular disease; higher peripheral artery disease prevalence, eGFR level, and body mass index; lower diastolic BP and low-density lipoprotein cholesterol; and

Table 1. Baseline characteristics of the participants in the Canagliflozin and Kidney Events in Diabetes with Established Nephropathy Clinical Evaluation trial, stratified by angiopoietin 2 quartile

Characteristic	Q1	Q2	Q3	Q4
n	642	641	641	641
Canagliflozin, *n* (%)	321 (50)	310 (48)	322 (50)	341 (53)
Age, yr	63±9	63±9	63±9	63±9
Male, *n* (%)	491 (77)	436 (68)	409 (64)	366 (57)
Race, *n* (%)				
Asian	167 (26)	80 (13)	50 (8)	32 (5)
Black	26 (4)	39 (6)	34 (5)	42 (7)
White	396 (62)	455 (71)	480 (75)	499 (78)
Comorbidities, *n* (%)				
Heart failure	52 (8)	77 (12)	66 (10)	134 (21)
Smoking	89 (14)	94 (15)	92 (14)	105 (16)
Hypertension	617 (96)	623 (97)	620 (97)	618 (96)
Coronary disease	165 (26)	167 (26)	177 (28)	219 (34)
Cerebrovascular disease	96 (15)	101 (16)	85 (13)	101 (16)
Peripheral artery disease	122 (19)	161 (25)	152 (24)	208 (32)
CKD	594 (95)	590 (95)	602 (96)	611 (97)
Obesity	295 (46)	363 (57)	378 (59)	439 (69)
eGFR, ml/min per 1.73 m^2	58±18	58±18	56±18	55±19
BMI, kg/m^2	30.2±5.2	31.2±5.5	32.4±6.5	33.6±7.2
Systolic BP, mm Hg	139±15	141±16	139±16	141±17
Diastolic BP, mm Hg	78±9	78±9	78±10	77±10
Hemoglobin A1c, %	8.1±1.3	8.3±1.3	8.3±1.3	8.3±1.4
Low-density lipoprotein cholesterol, mg/dl	85 (65–116)	90 (68–121)	89 (66–116)	87 (64–120)
High-density lipoprotein cholesterol, mg/dl	43 (36–53)	43 (36–52)	41 (35–50)	42 (35–50)
Triglycerides, mg/dl[a]	156 (113–218)	159 (115–228)	172 (122–251)	162 (116–237)
Diabetes duration, yr	15.5±8.8	16.1±8.7	16±8.6	16.5±8.8
Albumin/creatinine ratio, mg/g	88 (51–171)	90 (50–184)	112 (52–220)	118 (59–221)
Biomarkers, median (IQR)				
NT-proBNP, pg/ml[a]	103 (56–230)	147 (73–324)	189 (91–399)	403 (167–1006)
Troponin T, pg/ml[a]	16 (11–25)	18 (12–28)	19 (12–28)	22 (15–36)
Angiopoietin 2, ng/ml[a]	1.2 (1.1–1.3)	1.7 (1.6–1.8)	2.2 (2.0–2.4)	3.4 (2.9–4.6)
VEGF-A, pg/ml[a]	61.5 (41.7–110.7)	64.7 (42.6–102.7)	65.0 (43.8–115.0)	69.6 (44.8–117.7)
Medications, *n* (%)				
Diuretic use	260 (41)	296 (46)	322 (50)	387 (60)
Statin use	497 (77)	464 (72)	441 (69)	441 (69)
Antithrombotic use	392 (61)	400 (62)	380 (59)	435 (68)
β-blocker	213 (33)	231 (36)	285 (45)	365 (57)
Metformin	419 (65)	403 (63)	382 (60)	345 (54)
GLP-1	29 (5)	34 (5)	28 (4)	36 (6)
Insulin	382 (60)	414 (65)	439 (69)	473 (74)
Sulfonylureas	198 (31)	184 (29)	152 (24)	152 (24)

BMI, body mass index; GLP-1, glucagon-like peptide 1; IQR, interquartile range; NT-proBNP, *N* terminal pro B-type natriuretic peptide; VEGF-A, vascular endothelial growth factor-A.
[a]Median (interquartile range) was used to describe non-normally distributed variables.

longer type 2 diabetes mellitus duration. In addition, included individuals were more likely to use statin, antithrombotic, β-blocker, metformin, and glucagon-like peptide 1 agonist than those who were excluded.

Angiopoietin 2

Baseline characteristics of the study population according to angiopoietin 2 quartiles are shown in Table 1. Mean age (SD) was 62 (9) years, and 853 individuals (33%) were female. Patients with the highest quartile of angiopoietin 2 concentrations were more likely to be female and White. At baseline, they had a higher prevalence of heart failure, coronary artery disease, peripheral artery disease, and obesity. Moreover, they had lower eGFR levels and higher systolic BP, hemoglobin A1c, triglycerides, albumin/creatinine ratio, and *N* terminal pro B-type natriuretic peptide and high-sensitivity cardiac troponin T concentrations compared with other angiopoietin 2 quartiles. Finally, patients with the highest angiopoietin 2 level had higher use of diuretics, antithrombotic therapies, β-blockers, and insulin and lower use of a statin and sulfonylureas compared with other quartiles. Supplemental Table 3 summarizes the correlation between angiopoietin 2 level and baseline variables. In multivariable linear regression analysis, angiopoietin 2 levels were positively correlated with smoking, history of peripheral artery disease, body mass index, hemoglobin A1c, and *N* terminal pro B-type natriuretic peptide and negatively associated with older age, male, Asian, and high-density lipoprotein concentration.

Table 2 summarizes geometric mean (95% CI) and geometric mean ratio concentrations of angiopoietin 2 at baseline, year 1, and year 3. The unadjusted geometric mean for angiopoietin 2 was lower after canagliflozin treatment versus placebo at year 1 and year 3. To further

Table 2. Geometric mean (95% confidence interval) and adjusted geometric mean ratio concentrations[a] of angiopoietin 2 at baseline, year 1, and year 3

Variable	Baseline	1 yr		Ratio: Canagliflozin/Placebo (95% CI)
	Geometric Mean (95% CI)	Geometric Mean (95% CI)	Ratio: Follow-up/Baseline Geometric Mean (95% CI)	
Canagliflozin	2.03 (1.98 to 2.09)	1.92 (1.86 to 1.98)	0.96 (0.94 to 0.98)	0.92 (0.91 to 0.93)
Placebo	1.96 (1.91 to 2.02)	2.01 (1.95 to 2.08)	1.04 (1.01 to 1.06)	
Variable	Baseline	3 yr		Ratio: Canagliflozin/Placebo (95% CI)
	Geometric Mean (95% CI)	Geometric Mean (95% CI)	Ratio: Follow-up/Baseline Geometric Mean (95% CI)	
Canagliflozin	2.03 (1.98 to 2.09)	1.99 (1.90 to 2.10)	1.04 (1.00 to 1.08)	0.94 (0.92 to 0.95)
Placebo	1.96 (1.91 to 2.02)	2.14 (2.03 to 2.25)	1.11 (1.06 to 1.16)	

CI, confidence interval.
[a]Geometric mean ratio was adjusted for age, GFR, body mass index, systolic BP, hemoglobin A1c, duration of type 2 diabetes mellitus, urine albumin–creatinine ratio, and history of heart failure.

explore the treatment-related effect on biomarker concentrations, adjusted analyses examined the geometric mean ratio of angiopoietin 2 level among the canagliflozin versus placebo group. In these adjusted models, treatment with canagliflozin significantly lowered concentrations of angiopoietin 2 by 8% and 6% at year 1 and year 3, respectively.

Associations between angiopoietin 2 concentrations and adverse clinical outcomes are presented in Table 3. In the multivariable-adjusted model, each 50% increment in log baseline angiopoietin 2 concentrations was associated with a higher risk of all outcomes examined. This includes the primary composite outcome (hazard ratio [HR], 1.27; 95% CI, 1.13 to 1.43) and kidney composite (HR, 1.22; 95% CI, 1.04 to 1.42) along with a significantly higher risk of heart failure hospitalization, cardiovascular death, and the composite of heart failure hospitalization/cardiovascular death and all-cause death. In a similar fashion, a 50% increase in angiopoietin 2 concentrations by 1 year from baseline was associated with a higher risk of all subsequent adverse outcomes (P value < 0.05). Supplemental Table 4 presents the HRs of primary composite outcome for elevated angiopoietin 2 (≥2.29 ng/ml) and VEGF-A (≥79.88 ng/ml) levels according to the optimized cutoff. Elevated angiopoietin level was associated with the primary composite outcome (HR, 1.57; 95% CI, 1.23 to 1.99; P value: 0.001). Figure 1 depicts the efficacy of canagliflozin in lowering risk of primary composite outcome across angiopoietin 2. The treatment-by-angiopoietin 2 interaction was present for primary composite outcome; canagliflozin was at a more effective level relative to the study primary composite outcome among patients with lower angiopoietin 2. Mediation analyses suggested that change in angiopoietin 2 from baseline to year 1 could explain 10.0% (95% CI, 5.0 to 22.0; P value < 0.001) of the effect of canagliflozin on the primary composite outcome.

VEGF-A

Supplemental Table 5 shows the baseline characteristics of the study population across VEGF-A quartiles. Individuals

Table 3. Association between angiopoietin 2 concentration and adverse clinical outcomes

Variable	Baseline Concentration[a]	1-yr Concentration[a]	Per 50% Increase by 1 yr[b]
	HR_{adj} (95% CI)	HR_{adj} (95% CI)	HR_{adj} (95% CI)
Primary composite	1.61 (1.27 to 2.05)	2.01 (1.61 to 2.51)	1.08 (1.03 to 1.13)
Kidney composite	1.48 (1.09 to 2.01)	2.03 (1.54 to 2.68)	1.08 (1.02 to 1.14)
Heart failure/cardiovascular death	1.78 (1.37 to 2.30)	2.10 (1.62 to 2.72)	1.17 (1.09 to 1.25)
Heart failure hospitalization	1.75 (1.24 to 2.48)	2.24 (1.61 to 3.12)	1.20 (1.11 to 1.29)
All-cause death	1.69 (1.28 to 2.25)	1.99 (1.49 to 2.65)	1.11 (1.05 to 1.17)
Cardiovascular death	1.72 (1.21 to 2.45)	2.20 (1.57 to 3.10)	1.11 (1.04 to 1.18)

Primary composite end point: a composite of end stage kidney failure, doubling of the serum creatinine level, and kidney or cardiovascular death. Multivariable model adjusted for age, race, heart failure, peripheral artery disease, CKD, obesity, eGFR, body mass index, systolic and diastolic BP, albumin/creatinine ratio, N terminal pro B-type natriuretic peptide, troponin statin use, antithrombotic use, metformin, insulin, sulfonylureas. CI, confidence interval; HR, hazard ratio; HR_{adj}, adjusted hazard ratio.
[a]Per 1-unit increment in log angiopoietin 2.
[b]$Relative\ increase = \frac{concentration\ at\ year\ 1 - baseline\ concentration}{baseline\ concetration}$.

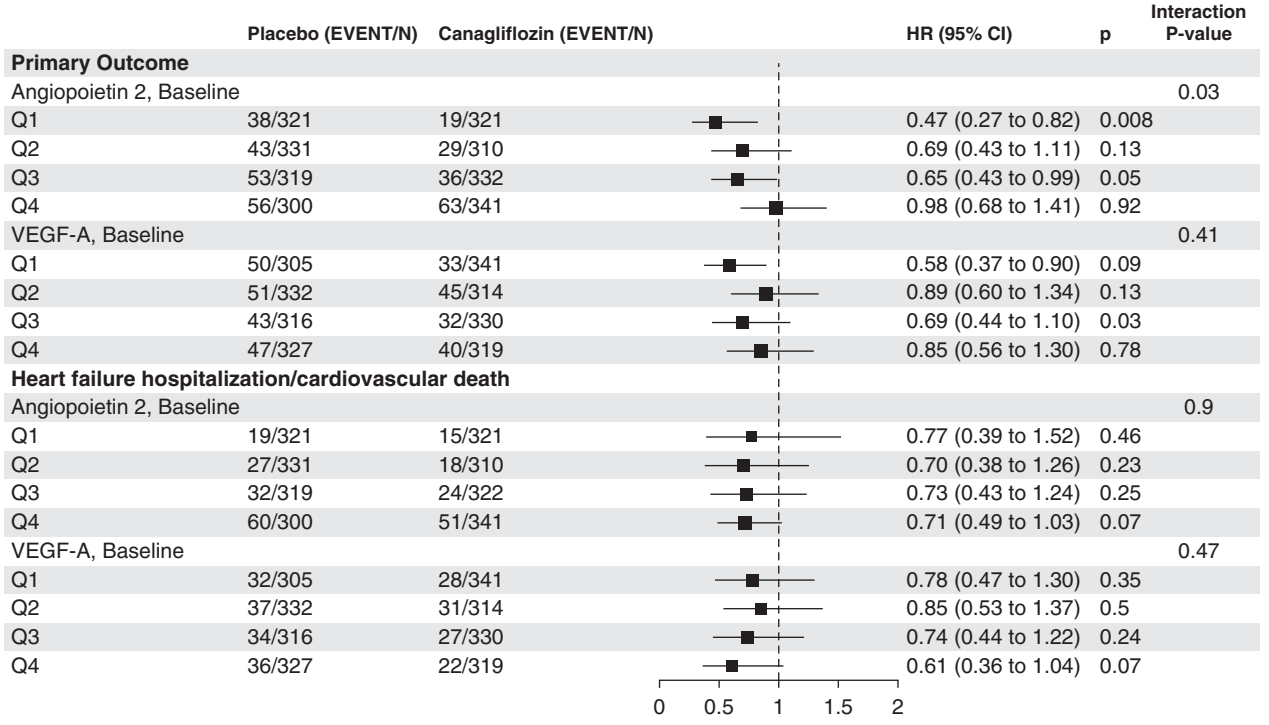

Figure 1. Efficacy of canagliflozin in lowering risk of primary composite outcome and heart failure or cardiovascular death across angiopoietin 2 and VEGF-A. Treatment-by-angiopoietin 2 interaction was present for primary composite outcome; canagliflozin was at a more effective level against the study primary composite outcome among patients with lower angiopoietin 2. CI, confidence interval; HR, hazard ratio; VEGF-A, vascular endothelial growth factor-A. Figure 1 can be viewed in color online at www.cjasn.org.

with the highest VEGF-A levels were more likely to be White and had a higher prevalence of obesity and triglyceride levels than other quartiles. Treatment with canagliflozin did not change VEGF-A concentrations compared with placebo (Supplemental Table 6). Supplemental Table 6 presents the association between baseline VEGF-A and adverse clinical outcomes. In the multivariable-adjusted model, increase in log VEGF-A was not associated with any clinical outcomes (Supplemental Table 7).

Discussion

In the CREDENCE randomized trial of canagliflozin therapy for DKD, elevated baseline angiopoietin 2 concentration was associated with worsened cardiovascular and kidney risk profiles and worse cardiac and kidney disease. Angiopoietin 2 concentrations at baseline were prognostic for adverse clinical outcomes (primary composite outcome, primary kidney outcome, and all-cause mortality) among patients with DKD. Compared with placebo treatment, canagliflozin modestly decreased angiopoietin 2 concentration. This is relevant because an increase in angiopoietin 2 concentration at 1 year from baseline was associated with all adverse clinical outcomes, and in mediation analyses, reduction of angiopoietin 2 explained a percentage of the effects of canagliflozin. By contrast, VEGF-A concentrations were not associated with baseline clinical complexity, nor did they associate with outcomes in the CREDENCE trial either alone or in combination with angiopoietin 2 concentrations. These findings strongly suggest a significant role of angiopoietin 2 in the development and progression of type 2 diabetes mellitus macrovascular complications.

Angiopoietins 1–4 belong to a family of growth factors that activate the endothelial cell-specific tyrosine kinase receptors Tie1 and Tie2. Maintenance of normal functioning vasculature requires a balance between the angiopoietin/Tie system and VEGF-A. Angiopoietin 1 is a potent angiogenic factor that strongly binds to Tie2.[18] It promotes vessel quiescence and structural integrity of the endothelium.[19] Unlike angiopoietin 1, angiopoietin 2 acts as an agonist/antagonist of Tie2 receptor depending on the presence of VEGF-A. In the presence of VEGF-A, angiopoietin 2 potentiates angiopoietin 1, leading to an increased vessel diameter, basal lamina remodeling, and angiogenesis induction. In the absence of VEGF-A, angiopoietin 2 acts as an antagonist of Tie2, leading to vessel regression, instability, and apoptosis. Inflammatory mediators, hypoxia, thrombin, and hyperglycemia can trigger the expression and release of angiopoietin 2 from endothelial cells.[20] Evidence from this study did not support an independent association between VEGF-A and adverse outcomes.

Several studies have reported elevated angiopoietin 2 concentrations in cardiometabolic disorders. Among individuals with type 2 diabetes mellitus, advanced glycation end products upregulate angiopoietin 2 expressions in endothelial cells.[21,22] In a case-control study, Li and colleagues showed that angiopoietin 2 is positively correlated with glycosylated hemoglobin A1c and homeostasis model assessment for insulin resistance. Moreover, persons with type 2 diabetes mellitus and vascular disease had higher angiopoietin 2

concentration but similar angiopoietin 1 concentration compared with those without vascular disease.[23] In line with these findings, Lim and colleagues showed that, regardless of vascular disease, angiopoietin 2, but not angiopoietin 1, was elevated among patients with type 2 diabetes mellitus and associated with endothelial dysfunction as measured by carotid intima-media thickness.[24] Experimental studies suggest that angiopoietin 2 promotes vascular inflammation by enhancing nuclear factor kappa beta–dependent proinflammatory cascades, increasing macrophage infiltration and inhibiting endothelial nitric oxide synthase,[25] ultimately leading to endothelial dysfunction. A cellular study among knockout angiopoietin $2^{-/-}$ mice reaffirmed the role of angiopoietin 2 in endothelial dysfunction because such mice had attenuated atherosclerosis development relative to wild-type mice despite similar lipid levels. On the basis of preclinical studies, it has been observed that using humanized monoclonal antibodies to block angiopoietin 2 can effectively inhibit angiogenesis and tumor growth and cause vascular regression in various tumor tissues.[26] Subsequently, suppression of angiopoietin 2–Tie2 has undergone multiple early clinical trials, showing that it is a safe and potentially effective way to inhibit tumor growth. The effectiveness of inhibiting the growth of new blood vessels has demonstrated promising outcomes in experimental research involving myocardial infarction[10] and vascular retinopathy.[27]

In line with previous findings,[28] this study adds to the current body of evidence underscoring the association between baseline angiopoietin 2 concentrations and risk of developing adverse cardiac and kidney outcomes. It has been discovered that angiopoietin 2 plays a role in several important pathways that influence the onset and progression of heart failure.[29] For instance, angiopoietin 2 promotes oxidative stress and inflammation, both of which are detrimental to heart tissue and compromise cardiac function.[30] In addition, angiopoietin 2 can make blood vessels more permeable, which may result in edema, a common sign of heart failure.[31] In a recent analysis, Harrington and colleagues[32] assessed the association of cardiac structure and function with 25 measured endothelial markers. Among the measured biomarkers, angiopoietin 2 was correlated with higher left ventricular volume and worse cardiac function. Moreover, change in angiopoietin 2 concentrations over time was associated with a parallel change in cardiac function. On the basis of preclinical studies, it has been observed that using humanized monoclonal antibodies to block angiopoietin 2 can effectively inhibit angiogenesis and tumor growth and cause vascular regression in various tumor tissues.[26] Subsequently, suppression of angiopoietin 2–Tie2 has undergone multiple early clinical trials, showing that it is a safe and potentially effective way to inhibit tumor growth. The effectiveness of inhibiting the growth of new blood vessels has demonstrated promising outcomes in experimental research involving myocardial infarction[10] and vascular retinopathy.[27] Finally, it is important to note that prior work from Chu and colleagues showed sex-based differences in the association between angiopoietin 2 level and mortality events: In the study of Chu and colleagues, elevated angiopoietin 2 concentrations were associated with all-cause mortality in male patients, but not in female patients.[33] In the CREDENCE trial, women had higher concentrations of angiopoietin 2 compared with men; however, no statistically significant sex-based differences in associations between angiopoietin 2 and outcomes were noted. The mechanisms for the sex-related differences in circulating growth factors are not fully understood. Previous research has demonstrated that estrogen, a sex hormone, positively affects endothelial dysfunction. Further studies are needed to fully understand the role of sex in relation to endothelial dysfunction.

Mechanistically, the data from this analysis support the concept that unopposed elevated angiopoietin 2 concentration might play a role in the pathophysiology of microvascular and atherosclerotic vascular complications in type 2 diabetes mellitus, partially mediating cardiokidney risk in those with elevated values. Canagliflozin reduced concentrations of angiopoietin 2 in this study (which in turn was associated with mediation of benefit from the drug), although the effect was modest. The direct effect of canagliflozin on angiopoietin 2 level has not been described in the literature.[17] However, improved endothelial function has been proposed as one of the mechanisms associated with cardiokidney benefits of SGLT2is. Several studies have showed that treatment with SGLT2is stimulates vasodilation by activation of nitric oxide synthase, attenuation of inflammation, and reducing oxidative stress.[34–37] Moreover, results from a recent study suggested that improvement in endothelial function associated with SGLT2is might be due to glucotoxicity mediated by upregulation of vascular sodium-glucose cotransporter-2.[38] In this study, we showed that 10% of the protective effect of canagliflozin is mediated through reduction in angiopoietin 2 concentration. An interesting area of future research would be to examine the role of angiopoietin 2 inhibition in the prevention of complications in type 2 diabetes mellitus. A small study has already shown that blocking the angiopoietin 2 pathway prevents cardiac fibrosis and allograft vasculopathy by inhibiting leukocyte infiltration and improving microvascular dysfunction.[39]

Our study findings should be interpreted in light of several limitations. First, ischemic cardiac events during the follow-up period were not ascertained. Hence, we could not assess the association between angiopoietin 2 and atherosclerotic events. Second, angiopoietin 1 concentration was not measured in the CREDENCE trial. It binds to a similar receptor of angiopoietin 2; however, it has the opposite effect by stabilizing endothelial and vascular structures. Hence, the angiopoietin 2/angiopoietin 1 ratio is proposed as a potential predictor in the accelerated development of endothelial dysfunction. Third, we observed disparities in distribution of angiopoietin 2 concentrations across races and ethnicities. Owing to the limited number of non-White individuals enrolled in the study, we could not assess the associations in each race and ethnicity. Fourth, we did not measure other markers of inflammation or endothelial dysfunction such as uric acid, ferritin, and high-sensitivity C-reactive protein in the CREDENCE trial. Association between angiopoietin 2 and risk of cardiorenal events beyond conventional inflammatory markers requires further research. Fifth, by design, study participants with events between baseline and year 1 were not included in mediation analysis; this approach may constitute informative censoring. Finally, the VEGF and angiopoietin families comprise five and four protein members, respectively, with

several soluble receptors. In this study, we measured the most abundant protein in each protein class to decipher the role of their families in progression of kidney disease. Therefore, the interactions between these proteins were not evaluated in this study. Further research is needed to better understand the role of each protein in endothelial function and its association with the risk of kidney disease.

In conclusion, although VEGF-A concentrations were not associated with outcomes in the CREDENCE trial, baseline angiopoietin 2 concentrations were found to be strongly associated with cardiorenal outcomes among patients with type 2 diabetes mellitus. Canagliflozin modestly reduced the angiopoietin 2 concentration at year 1 compared with baseline compared with placebo. Increased angiopoietin concentration at year 1 was further associated with elevated cardiorenal risk. Angiopoietin 2, as a marker (or driver) of endothelial dysfunction and other adverse actions (*e.g.*, inflammation/oxidative stress), may be a future target for reducing type 2 complications.

Disclosures

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Data Sharing Statement

Original data created for the study are or will be available in a persistent repository upon publication. http://yoda.yale.edu.

Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/CJN/B843.

Supplemental Table 1. Association of each variable with primary composite outcome.

Supplemental Table 2. Comparison between those included and excluded in the study.

Supplemental Table 3. Associations of elevated angiopoietin 2 concentration and VEGF-A with clinical outcomes.

Supplemental Table 4. Correlation between angiopoietin 2 level and baseline variables.

Supplemental Table 5. Baseline characteristics of the study population stratified by angiopoietin-2 quartile.

Supplemental Table 6. Geometric mean (95% CI) and geometric mean ratio concentrations of vascular endothelial growth factor at baseline, year 1, and year 3.

Supplemental Table 7. Associations between vascular endothelial growth factor and clinical outcomes.

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