Original Paper

Angiotensin-2, Renal Deterioration, Major Adverse Cardiovascular Events and All-Cause Mortality in Patients with Diabetic Nephropathy

Yi-Chun Tsai^{a,b,c,e} Chee-Siong Lee^{b,d} Yi-Wen Chiu^{c,e} Jia-Jung Lee^{c,e} Su-Chu Lee^c
Ya-Ling Hsu^f Mei-Chuan Kuo^{a,c,e}

^aGraduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung,

^bDivision of General Medicine, Kaohsiung Medical University Hospital, Kaohsiung, ^cDivision of Nephrology, Kaohsiung Medical University Hospital, Kaohsiung, ^dDivision of Cardiology, Kaohsiung Medical University Hospital, Kaohsiung, ^eFaculty of Renal Care, Kaohsiung Medical University, Kaohsiung, ^fGraduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Key Words

Angiotensin-2 • Renal deterioration • MACEs • Mortality • Diabetic nephropathy

Abstract

Background/Aims: Diabetic nephropathy is the leading cause of end-stage renal disease and accounts for 30~40% of patients requiring maintenance dialysis, thereby increasing the burden on health insurance programs. Diabetic nephropathy is also the strongest predictor of cardiovascular morbidity and mortality. The aim of this study was to examine whether angiotensin-2 (Angpt2), a modulator of endothelial function, affects the clinical outcomes of diabetic patients. **Methods:** This study enrolled 236 patients with diabetes mellitus with estimated glomerular filtration rate (eGFR) < 60ml/min/1.73m² from January 2006 to December 2011, who were followed until June 2017. Clinical outcomes included renal outcomes (commencing dialysis and rapid decline in renal function (eGFR decline > 3 ml/min per 1.73 m²/year)), major adverse cardiovascular events (MACEs), and all-cause mortality. **Results:** Over a mean follow-up period of 3.9±2.7 years, 135 (57.2%) patients commenced dialysis, 106 (44.9%) had rapid decline in renal function, and 50 (21.2%) had MACEs or died from all-causes. Log-formed Angpt2 was significantly associated with increased risks of commencing dialysis (HR: 3.91, 95% CI: 1.56-9.76), rapid renal function decline (OR: 6.81, 95% CI: 1.06-43.88), and MACEs or all-cause mortality (HR: 6.34, 95% CI: 1.18-33.97) in the adjusted analysis. Patients in the highest quartile had hazard ratios of 2.90 and 3.11 for commencing dialysis and rapid

Mei-Chuan Kuo, MD
Ya-Ling Hsu PhD

Division of Nephrology, Department of Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, 100 TzYou 1st Road, Kaohsiung 807 (Taiwan),
Tel. +886 7 3121101-7351-18, E-Mail mechku@cc.kmu.edu.tw, yainghsu@cc.kmu.edu.tw

renal function decline, respectively, compared to those in the lowest quartile after adjustments. Similar significant dose-response results were found in composite outcomes of either MACEs or all-cause mortality. **Conclusion:** Angpt2 is an independent predictor of adverse clinical outcomes in diabetic patients. Further studies are needed to identify the pathogenic role of Angpt2 in renal deterioration and cardiovascular complications of diabetes mellitus.

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Introduction

Diabetes mellitus greatly affects the general population, and its prevalence is expected to increase from 2.89% in 2000 to 4.4% of the global population in 2030 [1]. Moreover, the World Health Organization (WHO) predicts that diabetes mellitus will be the seventh leading cause of death by the year 2030 [2]. Cardiovascular disease is the most common cause of death in diabetic patients. In addition, diabetic nephropathy is the leading cause of chronic kidney disease, accounting for nearly 30~50% of patients starting renal replacement therapy worldwide [3]. Furthermore, diabetic nephropathy is also the strongest predictor of morbidity and mortality in clinical diabetes mellitus. Therefore, diabetic nephropathy and its complications have an enormous impact on the affected individuals and health-care systems.

Diabetes mellitus can cause macrovascular and microvascular co-morbidities, and it can induce abnormal angiogenesis in the microvasculature and endothelial dysfunction [4]. High concentrations of angiogenic markers are easily detected in plasma and urine of diabetic patients, emphasizing the role of angiogenesis and impaired endothelium [3]. The angiotensin (Angpt)/Tie ligand-receptor system tightly controls the endothelial phenotype during angiogenesis and vascular inflammation [5]. Diabetes mellitus increases Angpt2, which then destabilizes vascular walls by competing with Angpt1, and promotes neovascularization when is bound to vascular endothelial growth factor (VEGF) [6]. Angpt2 has been reported to be elevated in diabetic patients and to be associated with indexes of endothelial damage/dysfunction [7]. In diabetes mellitus, chronic hyperglycemia leads to the accumulation of advanced glycation end products (AGEs) and mitochondrial overproduction of reactive oxygen species (ROS). This then causes the up-regulation of Angpt2 mRNA, which has been reported to promote vascular permeability, destabilization and sprouting, further inducing microvascular and macrovascular complications in *in-vitro* and *in-vivo* studies [8-10]. However, the impact of Angpt2 on microvascular and macrovascular complications in clinical patients with diabetic nephropathy is still unclear. The aim of this study was to examine the association between serum Angpt2 and renal outcomes, including commencing dialysis and rapid renal function decline, major adverse cardiovascular events (MACEs), and all-cause mortality in patients with diabetic nephropathy.

Materials and Methods

Study Participants

This observational study was conducted at a tertiary hospital in Southern Taiwan. We enrolled 239 patients with diabetic nephropathy with estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m², who were followed up at our integrated chronic kidney disease (CKD) program, from January 2006 to December 2011. Three patients who had fewer than three serum creatinine examinations during the follow-up period were excluded, and the remaining 236 patients were entered into final analysis. The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-990198). Informed consent was obtained in written form from all of the patients, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Data Collection

Demographic and clinical data were obtained from interviews with the patients and medical records at enrollment. Diabetes was defined as a history of diabetes or the use of anti-diabetes agents. Hypertension was defined as a history of hypertension or the use of antihypertensive drugs. Cardiovascular disease was defined as a history of heart failure, acute or chronic ischemic heart disease, or myocardial infarction. Information on the use of medications including statins, β -blockers, calcium channel blockers, angiotensin converting enzyme inhibitors (ACEIs), and angiotensin II receptor blockers (ARBs) before and after enrollment was obtained from medical records. The patients were asked to fast for at least 12 hours before blood and urine sample collection for biochemistry studies and protein measurement, respectively.

Quantification of Circulating Angiopoietin-2

Plasma Angpt2 was measured in duplicate using commercial enzyme-linked immunosorbent assays (R&D Systems Inc., Minneapolis, MN) according to the instructions of the manufacturer. The sensitivity of the Angpt2 assay was 1.20 pg/ml. The interassay and intraassay coefficients of variation of Angpt2 were 1.2% and 1.8 % respectively.

Clinical Outcomes

The patients were followed up at outpatient clinics at 3-month intervals to ascertain their clinical status. At least three telephone calls were made to the patients at their last known telephone number if they failed to attend follow-up visit. The renal outcomes included entering commencing dialysis (hemodialysis or peritoneal dialysis) and rapid decline in renal function. Commencing dialysis was confirmed by reviewing medical charts or catastrophic illness certificates (issued by the Bureau of National Health Insurance in Taiwan). Rapid decline in renal function was defined as the eGFR decline > 3 ml/min/1.73 m² per year (eGFR slope). The eGFR slope was calculated by using regression coefficient between eGFR and time in units of ml/min per 1.73 m² per year based on all eGFR values available from enrollment to the end of the observation period. At least three eGFR values were required to estimate the eGFR slope. Rapid decline in eGFR was defined as the lowest quartile (eGFR decline > 3 ml/min/1.73 m² per year; an integer near the cutoff point between the lowest two quartiles of the eGFR slope) [11]. MACEs were defined as new onset of acute coronary syndrome (myocardial infarction or unstable angina), acute hemorrhagic or ischemic stroke, or hospitalization related to acute phase of congestive heart failure. We excluded MACEs or death after commencing dialysis. The information obtained by direct contact with the patients and their families was further supplemented by reviewing medical records and the database of the National Mortality Files. Patients were censored at last contact or the end of observation in June 2017.

Statistical Analysis

The baseline characteristics of the patients were stratified by quartiles of Angpt2, with cutoff points at 1573.68, 2076.54, and 2812.26 pg/ml. Continuous variables were expressed as mean $\pm$ SD or median (25th, 75th percentile), as appropriate, and categorical variables were expressed as percentages. Continuous variables with skewed distribution were log-transformed to approximate normal distribution. The significance of differences in continuous variables between groups was tested using one-way analysis of variance (ANOVA) or the Kruskal-Wallis H test, as appropriate. Differences in the distribution of categorical variables were tested using the chi-square test. Kaplan-Meier survival analysis was used to assess the predictive ability of Angpt2 for the risk of commencing dialysis and MACEs. Cox regression models were used to examine the relationship between Angpt2 and either commencing dialysis, MACEs or all-cause mortality. Multivariate logistic regression models were used to evaluate the association between Angpt2 and rapid decline in renal function as the eGFR decline > 3 ml/min/1.73 m² per year. The multivariate models were adjusted for age, sex, smoking status, alcohol consumption, ACEI/ARB usage, cardiovascular disease, eGFR, urine protein-creatinine ratio (PCR) with a cutoff point of 1g/g, serum albumin, hemoglobin and cholesterol levels. Statistical analyses were conducted using SPSS version 18.0 for Windows (SPSS Inc., Chicago, Illinois). Statistical significance was set at a two-sided p-value of < 0.05 .

Table 1. The clinical characteristics of study subjects stratified by angiotensin-2 quartiles. Data are expressed as number (percentage) for categorical variables and mean±SD or median (25th, 75th percentile) for continuous variables, as appropriate. Conversion factors for units: eGFR in mL/min/1.73 m² to mL/s/1.73 m², ×0.01667; hemoglobin in g/dL to g/L, ×10; albumin in g/dL to g/L, ×10; calcium-phosphate product in mg²/dL² to mmol²/L², ×0.0806; cholesterol in mg/dL to mmol/L, ×0.02586; triglyceride in mg/dL to mmol/L, ×0.01129; uric acid in mg/dL to μmol/L, ×59.48. Abbreviations: ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein. ^aAngiotensin-2 quartile cut at 1573.68, 2076.54, and 2812.26pg/ml

Variables	Entire Cohort N=236	Quartile 1 N=60	Angiotensin-2 ^a			P-value
			Quartile 2 N=58	Quartile 3 N=59	Quartile 4 N=59	
Demographics						
Age (year)	65.2±11.3	62.9±13.5	68.2±10.5	66.5±8.9	63.3±11.3	0.030
Sex (male), n(%)	140(59.3)	44(73.3)	36(62.1)	29(49.2)	31(52.5)	0.033
Smoke, n(%)	63(26.8)	19(31.7)	10(17.2)	15(25.9)	19(32.2)	0.228
Alcohol, n(%)	16(6.8)	4(6.7)	4(6.9)	3(5.2)	5(8.5)	0.918
Cardiovascular disease, n(%)	55(23.3)	19(31.7)	12(20.7)	12(20.3)	12(20.3)	0.369
Hypertension, n(%)	227(96.2)	60(100.0)	56(96.6)	55(93.2)	56(94.9)	0.253
Hyperlipidemia, n(%)	142(60.2)	36(60.0)	38(65.5)	32(54.2)	36(61.0)	0.665
Body mass index, kg/m ²	25.4±3.8	25.6±3.5	25.5±4.1	25.2±3.9	25.2±4.0	0.909
Medications						
Calcium channel blocker, n (%)	150(63.6)	33(55.0)	38(65.5)	35(59.3)	44(74.6)	0.136
β-blocker, n (%)	68(28.8)	12(20.0)	15(25.9)	21(35.6)	20(33.9)	0.205
ACEI/ARB, n (%)	160(67.8)	43(71.7)	38(65.5)	37(62.7)	42(71.2)	0.669
Statin, n(%)	94(39.8)	24(40.0)	24(41.4)	17(28.8)	29(49.2)	0.159
Laboratory parameters						
eGFR (mL/min/1.73m ²)	21.3±11.4	22.0±10.6	24.8±11.7	19.6±11.0	19.2±11.7	0.029
Blood urea nitrogen (mg/dl)	41.9(32.5,60.1)	39.6(27.3,53.3)	38.7(29.4,46.9)	44.5(34.8,62.8)	53.0(36.2,65.6)	0.007
Fasting sugar (g/dl)	117(97,149)	112(97,140)	118(98,149)	120(97,136)	120(98,161)	0.587
Glycated hemoglobin (%)	6.8(6.1,8.0)	6.7(6.0,7.7)	6.9(6.3,7.9)	6.5(6.0,7.6)	7.3(6.3,8.8)	0.152
Hemoglobin (g/dl)	10.9±1.9	11.2±2.0	11.4±1.7	10.7±1.9	10.2±1.8	0.005
Albumin (g/dl)	4.0(3.8,4.2)	4.1(3.8,4.3)	4.0(3.8,4.2)	4.0(3.7,4.2)	4.0(3.6,4.2)	0.456
Phosphate (mg/dl)	4.2(3.7,4.9)	4.1(3.7,4.8)	3.9(3.6,4.4)	4.3(3.8,5.0)	4.3(3.9,5.3)	0.017
Calcium (mg/dl)	8.9±0.7	9.1±0.7	9.1±0.5	8.8±0.7	8.7±0.8	0.027
Uric acid (mg/dl)	7.8±1.8	7.9±1.8	7.7±1.7	7.6±1.8	8.0±1.9	0.642
Cholesterol (mg/dl)	183(155,214)	180(155,223)	181(157,212)	181(151,208)	192(159,229)	0.680
Triglyceride (mg/dl)	137(91,200)	124(89,215)	126(88,170)	134(87,185)	156(98,211)	0.415
hsCRP (mg/L)	2.0(0.8,4.8)	1.2(0.7,3.3)	2.0(0.7,3.8)	2.0(0.7,4.3)	2.5(0.9,6.6)	0.178
Urine protein-creatinine ratio > 1g/g, n (%)	128(59.5)	30(52.6)	30(58.8)	29(56.9)	39(69.6)	0.300

Results

Characteristics of the Entire Cohort

Of all 236 patients with diabetic nephropathy, the mean age was 65.2±11.3 years and 59.3% were male. The mean eGFR of 236 patients was 21.8 mL/min/1.73m², including 49 with eGFR < 60mL/min/1.73m² and > 30 mL/min/1.73m², 102 with eGFR < 30mL/min/1.73m² and > 15 mL/min/1.73m², and 85 with eGFR < 15mL/min/1.73m². The median of glycated hemoglobin level was 6.8 % in all patients. Table 1 shows the baseline clinical characteristics stratified by quartiles of Angpt2, at 1573.68, 2076.54, and 2812.26pg/ml. Overall, 85.7 % of the patients had hypertension and 23.3% had cardiovascular disease. The serum blood urea nitrogen and phosphate levels increased and eGFR, serum hemoglobin, and calcium levels decreased with increasing Angpt2 quartile. Serum Angp2 was negatively correlated with urine PCR (Spearman's r: -0.181, p=0.005) and positively correlated with eGFR (Spearman's r: 0.207, p=0.002) in patients with diabetic nephropathy (Fig. 1).

Angiotensin-2 and Renal Outcomes

Over a mean follow-up period of 3.9±2.7 years, 135 patients (57.2%) progressed to commencing dialysis (Table 2). A stepwise increase in the proportion of those commencing dialysis from quartile 1 to quartile 4 was found (p for trend= 0.04). Kaplan-Meier survival curve showed a significant correlation between quartiles of Angpt2 and commencing dialysis (Fig. 2A). In univariate and multivariate Cox-regression analysis, log-formed Angpt2 was significantly associated with the increased risk of commencing dialysis (hazard ratio

Fig. 1. Correlation between serum angiotensin-2 level and renal function in patients with diabetic nephropathy.

Table 2. The adjusted risks for commencing dialysis and rapid progression of renal function according to Angiotensin-2 quartiles. Abbreviations: CI, Confidence Interval; eGFR, estimated glomerular filtration rate. Adjusted model: age, sex, smoke, alcohol, cardiovascular disease, angiotensin converting enzyme inhibitors/angiotensin II receptor blockers use, estimated glomerular filtration rate, hemoglobin, log-form cholesterol and albumin levels, and urine protein-creatinine ratio cut at 1g/g. ^aAngiotensin-2 quartile cut at 1573.68, 2076.54, and 2812.26pg/ml

Angiotensin-2 ^a	Commencing dialysis			eGFR decline > 3 mL/min/1.73 m ² /year			
	Event (n/N)	Hazard ratio (95% CI)	P-value	eGFR decline (mL/min/1.73 m ² /year)	Event (n/N)	Odds ratio (95% CI)	P-value
Log-formed angiotensin-2	135/236	3.91(1.56-9.76)	0.004	-2.78(-5.53,-1.38)	106/236	6.81(1.06-43.88)	0.044
Quartile 1	27/60	Reference		-1.74(-4.09,-0.38)	20/60	Reference	
Quartile 2	30/58	3.07(1.59-5.93)	0.001	-3.12(-6.14,-1.71)	30/58	5.09(1.66-15.59)	0.004
Quartile 3	38/59	3.92(2.02-7.61)	<0.001	-2.74(-4.47,-1.63)	24/59	3.41(1.13-10.28)	0.029
Quartile 4	40/59	2.90(1.60-5.27)	<0.001	-3.51(-6.62,-1.51)	32/59	3.11(1.10-8.81)	0.032

(HR): 4.14, 95% confidence interval (CI): 1.73-9.90, $p=0.001$; HR: 3.91, 95%CI: 1.56-9.76, $p=0.004$). The unadjusted and adjusted risks of commencing dialysis increased by 1.90-fold (95% CI: 1.17-3.10, $p=0.01$) and 2.90-fold (95% CI: 1.60-5.27, $p<0.001$) in the patients in quartile 4 compared to those in quartile 1. There was a longitudinal association between commencing dialysis and a stepwise increase in Angpt2 level (p for trend =0.01).

One hundred and six patients (44.9%) had rapid renal function decline as eGFR decline > 3 mL/min/1.73 m² per year. There was a significant difference in the slope of eGFR decline per year among the Angpt2 quartiles ($p=0.007$), and the patients in Angpt2 quartile 4 had the greatest decline in eGFR. Log-formed Angpt2 was significantly associated with an increased risk of the decline in eGFR > 3 mL/min/1.73 m² per year (odds ratio (OR): 4.78, 95% CI: 1.21-18.93, $p=0.026$; OR: 6.81, 95% CI: 1.06-43.88, $p=0.044$). The unadjusted and adjusted ORs for rapid decline in renal function were 2.37 (95% CI: 1.13-4.97) and 3.11 (95% CI: 1.10-8.81) for of the patients in quartile 4 compared to those in quartile 1 (Table 2).

We performed subgroup analysis (Table 3), and found a consistent association between log-formed Angpt2 and commencing dialysis in the patients with glycated hemoglobin level < 7.5% (HR for every log Angpt2: 3.38, 95%CI: 1.20-9.52), in those with glycated hemoglobin level $\geq 7.5\%$ (HR for every log Angpt2: 5.95, 95%CI: 1.03-34.38), and in those with urine PCR of 1g/g or more (HR for every log Angpt2: 3.79, 95%CI: 1.52-9.44), after adjusting for age, sex, cardiovascular disease and baseline eGFR. In the patients with urine PCR of 1g/g or more, high levels of Angpt2 were correlated with high risk for commencing dialysis independently of glycated hemoglobin.

Angiotensin-2, MACEs and All-cause Mortality

Of all patients, 50 patients (21.2%) had MACEs or died from all-causes (Table 4) before commencing dialysis. Forty-six had MACEs, 26 died, and 14 had both MACEs and then died during the follow-up period. Of the patients who died, sepsis accounted for 27%, MACEs for 23%, malignancy for 12%, and the rest were a mixture of refusing dialysis and other causes. The MACEs included 13 cases of acute myocardial infarction, nine with acute hemorrhagic or ischemic stroke, 23 with hospitalizations due to congestive heart failure, and one with aortic dissection. There were no significant differences in the causes of death and cardiovascular events between the four groups.

Log-formed Angpt2 was significantly associated with composite outcomes of either MACEs or all-cause mortality (HR: 6.34, 95% CI: 1.18-33.97, $p=0.031$) and MACEs alone (HR: 8.11, 95% CI: 1.158-41.52, $p=0.012$) in Cox proportional and multivariate analysis (Table 4). The adjusted HR of composite outcomes of either MACEs or all-cause mortality was 2.69 (95% CI: 1.05-6.90) for the patients in quartile 4 compared to those in quartile 1. The adjusted

Fig. 2. Kaplan-Meier survival curve for commencing dialysis (A) and major advanced cardiovascular events (MACEs) (B) in patients with diabetic nephropathy according to angiotensin-2 quartiles.

Table 3. The adjusted risks for commencing dialysis according to Angiotensin-2 in patients stratified by glycated hemoglobin and urine protein-creatinine ratio. Abbreviations: CI, Confidence Interval; eGFR, estimated glomerular filtration rate, HbA1C, glycated hemoglobin. Adjusted model: age, sex, cardiovascular disease, estimated glomerular filtration rate

Log-formed Angiotensin-2 ^a	Commencing dialysis		
	Event (n/N)	Hazard ratio (95% CI)	P-value
HbA1C $\geq$ 7.5%	41(72)	5.95(1.03-34.39)	0.046
HbA1C<7.5%	89(157)	3.38(1.20-9.52)	0.021
UPCR $\geq$ 1g/g	104(128)	3.79(1.52-9.44)	0.004
UPCR<1g/g	19(87)	1.79(0.18-17.33)	0.618
HbA1C $\geq$ 7.5% & UPCR $\geq$ 1g/g	31(41)	8.40(1.16-60.65)	0.035
HbA1C<7.5% & UPCR $\geq$ 1g/g	70(84)	3.17(1.02-9.86)	0.046
HbA1C $\geq$ 7.5% & UPCR<1g/g	6(23)	1.78(0.01-438.41)	0.838
HbA1C<7.5% & UPCR<1g/g	13(62)	1.56(0.09-27.26)	0.763

Table 4. The adjusted risks for major advanced cardiac events (MACEs) and all-cause mortality according to Angiotensin-2 quartiles. Abbreviations: CI, Confidence Interval; eGFR, estimated glomerular filtration rate. Adjusted model: age, sex, smoke, alcohol, cardiovascular disease, angiotensin converting enzyme inhibitors/angiotensin II receptor blockers use, estimated glomerular filtration rate, hemoglobin, log-form cholesterol and albumin levels, and urine protein-creatinine ratio cut at 1g/g. ^aAngiotensin-2 quartile cut at 1573.68, 2076.54, and 2812.26pg/ml

Angiotensin-2 ^a	MACEs+ all-cause mortality			MACEs			All-cause mortality		
	Event (n/N)	Hazard ratio (95% CI)	P-value	Event (n/N)	Hazard ratio (95% CI)	P-value	Event (n/N)	Hazard ratio (95% CI)	P-value
Log-formed angiotensin-2	50/236	6.34(1.18-33.97)	0.031	46/236	8.11(1.58-41.52)	0.012	26/236	2.37(0.24-23.53)	0.461
Quartile 1	12/60	Reference		13/60	Reference		6/60	Reference	
Quartile 2	14/58	1.29(0.52-5.18)	0.582	10/58	0.95(0.38-2.39)	0.915	8/58	1.04(0.29-3.75)	0.956
Quartile 3	10/59	1.03(0.34-3.10)	0.956	6/59	0.54(0.14-2.05)	0.366	7/59	1.37(0.35-5.36)	0.653
Quartile 4	14/59	2.69(1.05-6.90)	0.039	17/59	2.84(1.20-6.70)	0.017	5/59	1.40(0.37-5.32)	0.622

HR of MACEs was 2.84 (95% CI: 1.20-6.70) for the patients in quartile 4 compared to those in quartile 1. There was a longitudinal association between stepwise increases in Angpt2 levels and MACEs (p for trend= 0.024). Although no significant result was found in Kaplan-Meier survival analysis, the patients in Angpt2 quartile 4 had higher risk for having MACEs than those in the other quartiles (Fig. 2B). There was no significant correlation between Angpt2 level and all-cause mortality in patients with diabetic nephropathy.

Discussion

In this study, high serum Angpt2 levels were associated with the increased risk of poor renal outcomes, including commencing dialysis and rapid decline in renal function in patients with diabetic nephropathy over a follow-up period of nearly 4 years. A significant relationship between serum Angpt2 level and MACEs was also noted in patients with diabetic nephropathy after adjustments for well-known risk factors. Taken together, serum Angpt2 appears to be a potential predictor of adverse clinical outcomes in patients with diabetic nephropathy.

Over-expression of Angpt2 inhibits binding of Angpt1 to Tie-2 receptor, which contributes to the development of diabetic nephropathy. Abnormal alterations in Angpt1/Angpt2 cause excess angiogenesis and inflammation, which play a crucial role in the pathophysiologic mechanism of diabetic nephropathy [12]. Increasing evidence suggests that the upregulation of Angpt2 is harmful for kidney physiology and function. Podocyte-directed Angpt2 transgenic over-expression has been reported to cause glomerular endothelial cell apoptosis, increase albuminuria, and down-regulate the expression of nephrin [13]. In addition, the increased expression of Angpt2 may destabilize glomerular endothelial cells and affect podocytes in the paracrine fashion, inducing the decay of glomerular filtration barrier function [14]. Chen et al. found that serum and urinary Angpt2 levels were increased in normoalbuminuric patients with diabetes mellitus, suggesting that Angpt2 may be an earlier measurable indicator of tubular impairment before the onset of clinical symptoms or signs, such as microalbuminuria [15]. Consistent with their report [15], serum Angpt2 increased stepwisely with urine PCR, and this was inversely correlated with eGFR in our patients with diabetic nephropathy. Our previous study suggested that serum Angpt2 level was an independent risk factor for adverse renal outcomes in patients with CKD stages 3-5 [16]. However, whether serum Angpt2 can predict rapid deterioration in renal function in patients with clinical diabetes mellitus has not been well-explored. We followed diabetic patients over a 4-year period, and found that those with high levels of serum Angpt2 were more likely to commence dialysis and have rapid renal function decline; therefore, Angpt2 may be a reliable marker to detect deterioration in renal function in clinical practice.

Dysfunction in angiogenesis and endothelium has been reported to be a common cause of diabetic vascular complications [17], and Angpt2 involved in various vascular diseases such as arteriosclerosis and hypertension [18-20]. In addition, serum Angpt2 levels has been

correlated with subclinical cardiovascular diseases such as fractional shortening, number of carotid plaques [21] and carotid intima media thickness [22]. In our previous reports, we found that CKD patients with high serum Angpt2 levels were more likely to have high brachial-ankle pulse wave velocity, high left ventricular mass index and left ventricular hypertrophy [23]. Serum Angpt2 was associated with abnormal cardiovascular structure, and increased cardiovascular burden. Furthermore, serum Angpt2 has been considered as a predictor of all-cause mortality and cardiovascular mortality in the general population [24] and in patients with CKD [25]. Nevertheless, the information between serum Angpt2 and MACEs and all-cause mortality is limited in patients with diabetes mellitus. Hyperglycemia has been shown to cause an increase in Angpt2 expression, which can then induce myocardial apoptosis, increase infarction size, impair cardiac angiogenesis and exacerbate cardiac fibrosis in type 1 and type 2 diabetic mice [26, 27]. In the present study, Angpt2 was independently associated with the increased risk for composite outcomes of either MACEs or all-cause mortality, and MACEs alone. The relatively small number of study patients and relatively short observation period may explain the lack of no significant correlation between serum Angpt2 and all-cause mortality. We suggest that serum Angpt2 can potentially be used to predict MACEs in patients with diabetic nephropathy who have high risks of macrovascular complications.

Strict glycemic control has been shown to be very beneficial in preventing the development of microvascular and macrovascular complications in diabetes mellitus [28, 29]. However, some studies have reported that intensive glycemic control may not be enough to stop rapid deterioration in renal function in late CKD [30]. Our diabetic patients had good control of glucose that glycated hemoglobin level was under the recommendation value by American Diabetes Association (ADA) [31]. Therefore, we performed subgroup analysis to examine the effect of glycated hemoglobin on the association between serum Angpt2 and adverse renal outcomes in patients with diabetes mellitus. The results showed a consistent correlation between serum Angpt2 and commencing dialysis in diabetic patients with glycated hemoglobin level of $< 7.5\%$ or $\geq 7.5\%$. Therefore, Angpt2 may have a stronger influence on adverse renal outcomes than glycated hemoglobin level in patients with diabetes mellitus. Additionally, because of the large effect of urine PCR on renal progression, we stratified diabetic patients according to urine PCR of 1g/g, and the association between serum Angpt2 and commencing dialysis only existed in those with urine PCR $\geq 1\text{g/g}$, but not in those with urine PCR $< 1\text{g/g}$. In further analysis, we found that serum Angpt2 was associated with increased risk for commencing dialysis in diabetic patients with urine PCR $\geq 1\text{g/g}$, independently of glycated hemoglobin level. Therefore, serum Angpt2 appears to be an independent predictor of adverse renal outcomes in diabetic patients, especially in those with urine PCR $\geq 1\text{g/g}$.

There are several limitations to this study. First, it is uncertainty whether circulating Angpt2 has a biologically active effect in diabetic patients, and the biological implication of Angpt2 changes in the range observed in our patients is still unclear. Second, Angpt2 was measured once at enrollment, and the effect of variation in Angpt2 levels over time may have been underestimated. Third, the relatively small number of study patients may have limited the statistical analysis of all-cause mortality. However, our results are sufficient to provide strong evidence of the association between Angpt2 and adverse clinical outcomes in patients with diabetic nephropathy. Finally, we did not measure circulating Angpt1 or VEGF levels in this study. From a pathophysiological perspective, it may have been helpful if we had further clarified the potential effects of these markers.

Conclusion

This study demonstrated that elevated levels of serum Angpt2 were associated with high risks of commencing dialysis, rapid renal function decline and MACEs in patients with diabetic nephropathy. Future studies are necessary to evaluate the pathogenic role of

Angpt2 in microvascular and macrovascular complications, and moreover, to investigate the potential benefits in clinical outcomes that may be achieved by targeting Angpt2 in patients with diabetes mellitus.

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Disclosure Statement

The authors declare no conflicts of interest.

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