Journal of Endocrinological Investigation (2021) 44:2609–2619
https://doi.org/10.1007/s40618-021-01568-7

ORIGINAL ARTICLE

The combination of soluble tumor necrosis factor receptor type 1 and fibroblast growth factor 21 exhibits better prediction of renal outcomes in patients with type 2 diabetes mellitus

L.-H. Chang[1,2] · C.-M. Hwu[3,4] · C.-H. Chu[5,6] · Y.-C. Lin[3,4] · C.-C. Huang[4,7] · J.-Y. You[3] · H.-S. Chen[3,4] · L.-Y. Lin[3,4]

Received: 12 January 2021 / Accepted: 31 March 2021 / Published online: 8 April 2021

Abstract

Purpose Numerous biomarkers of diabetic kidney disease (DKD) are associated with renal prognosis but head-to-head comparisons are lacking. This study aimed to examine the association of soluble tumor necrosis factor receptor type 1 (sTNFR1), fibroblast growth factor 21 (FGF-21), endocan, N-terminal pro-brain natriuretic peptide (NT-pro-BNP), and renal outcomes of patients with or without clinical signs of DKD.

Methods A total of 312 patients were enrolled in a prospective observational study that excluded individuals with estimated glomerular filtration rates (eGFR) < 30 mL/min/1.73 m^2. Composite renal outcomes included either a > 30% decline in eGFR and worsening albuminuria or both from consecutive tests of blood/urine during a 3.5-year follow-up period.

Results Higher sTNFR1 and FGF-21, rather than endocan and NT-pro-BNP, levels were associated with renal outcomes but the significance was lost after adjusting for confounders. However, sTNFR1 levels ≥ 9.79 pg/dL or FGF-21 levels ≥ 1.40 pg/dL were associated with renal outcomes after adjusting for the confounders (hazard ration [HR] 2.76, 95% confidence interval [CI] 1.36–5.60, $p = 0.005$ for sTNFR1 level; HR 1.95, 95% CI 1.03–3.69, $p = 0.03$ for FGF-21 level). The combination of both levels exhibited even better association with renal outcomes than did either one alone (adjusted HR 4.45, 95% CI 1.86–10.65, $p = 0.001$). The results were consistent among patients with preserved renal function and normoalbuminuria.

Conclusion Both sTNFR1 and FGF-21 levels were associated with renal outcomes of in patients with type 2 diabetes, and the combination of the abovementioned markers exhibits better predictability.

Keywords Soluble tumor necrosis factor receptor type 1 · Fibroblast growth factor 21 · Type 2 diabetes mellitus · Renal outcomes

✉ L.-Y. Lin
linly@vghtpe.gov.tw

1 Division of Endocrinology and Metabolism, Department of Medicine, Yeezen General Hospital, Taoyuan, Taiwan

2 Department of Nursing, Hsin Sheng Junior College of Medical Care and Management, Taoyuan, Taiwan

3 Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sec. 2, Shih-Pai Road, Taipei 11217, Taiwan

4 School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

5 Department of Otorhinolaryngology-Head and Neck Surgery, Mackay Memorial Hospital, Taipei, Taiwan

6 Department of Audiology and Speech Language Pathology, Mackay Medical College, New Taipei City, Taiwan

7 Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

Introduction

Type 2 diabetes mellitus (T2D) is highly prevalent globally, and the number of patients is expected to increase to 693 million in 2045 [1]. Diabetic kidney disease (DKD) is a common complication of diabetes, which develops in 20–40% of patients with diabetes. The increasing prevalence of DKD is in parallel with the increasing number of individuals with T2D [2, 3]. DKD is the leading cause of end-stage renal disease and the annual all-cause mortality rate is nearly 20% for subjects with end-stage renal disease caused by DKD [4, 5]. The prediction and prevention of DKD progression in Asian patients are crucial because Asians have higher vulnerability than Caucasians [6].

The early pathological steps of DKD are characterized by hemodynamic dysfunction, such as glomerular hyperfiltration, proximal hyper-reabsorption, and overactivity of the

rein–angiotensin system, and increases in oxidative stress which is the root of the pathogenic molecular mechanisms of DKD [7]. Oxidative stress leads to the over-production of pro-inflammatory cytokine, pro-fibrotic factors, and angiogenetic factors that are relevant to the damage of renal structure and function [8].

Several biomarkers involved in the aforementioned process of DKD have been proven to be associated with the renal prognosis or status of albuminuria in patients with T2D. Soluble tumor necrosis factor receptor type 1 (sTNFR1), a cytokine that may facilitate the inflammatory reaction of the tissue or apoptosis of the targeted cells, was associated with decrease renal function and development of end-stage renal disease in patients with DKD [9–11]. Fibroblast growth factor 21(FGF-21) is one of the members of the FGF superfamily that acts as a factor of cytoprotection, repair, and metabolic regulation [12]. FGF-21 is urinary excretion and FGF-21 levels increased in parallel with the worsening status of albuminuria in patients with T2D without apparent loss of renal function [13–15]. Endocan is a biochemical moiety essential for the proliferation and neovascularization of cells, and plasma endocan levels showed negative correlation with the status of albuminuria in patients with T2D [16, 17]. Natriuretic peptides, which inhibit the renin–angiotensin–aldosterone axis and sympathetic tone, were released as the active hormone B type and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) [18]. The increasing plasma NT-pro-BNP levels were associated with progression to end-stage renal disease from mild to moderate chronic kidney disease (CKD) in patients without T2D and correlated with the albuminuria levels in patients with T2D [19, 20]. However, the prospective evaluation for the predictability of individual markers and head-to-head comparisons among markers has not been well documented. Therefore, this study aimed to test the predictability of plasma sTNFR1, FGF-21, endocan, and NT-pro-BNP levels in patients with T2D.

Materials and methods

Patient population and medical records

Patients with T2D and regular visitors of the outpatient department of the Division of Endocrinology and Metabolism of the Taipei Veterans General Hospital were enrolled in this prospective observational study since June 2014, after exclusion of patients with an estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2 as calculated using the method of Modification of Diet in Renal Disease (MDRD) or signs of acute hepatitis defined by elevated alanine aminotransferase levels twofold higher than the upper normal limit. Ethical approval was provided by the Taipei Veterans General Hospital, Taiwan (TPVGH IRB No: 2014-04-010CC). Informed consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. All research methods follow the Helsinki Declaration. Relevant clinical data of demographic and anthropometric characteristics and history of vascular comorbidity were collected for each patient. The body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Laboratory tests for hemoglobin A1C (HbA1c), lipid profile, and creatinine and spot sampling of urinary albumin and creatinine were conducted every 6 months and the obtained results were verified by the central laboratory of the Department of Clinical Pathology and Laboratory of Taipei Veterans General Hospital. The sTNFR1 levels were measured by a human immunoassay of sTNFR1 (Quantikine ELISA, MN, USA) according to the protocol provided by the manufacturer. The minimal detectable concentration of sTNFR1 was 0.07 pg/dL. The intra-assay coefficient of variation was 3.6–5.0%, while the inter-assay variation was 3.7–8.8%. The FGF-21 levels were measured by immunoassay (BioVendor, Brno, Czech Republic) with the lowest detectable FGF-21 level of 0.07 pg/dL, the intra-assay coefficient of variation of 3.0–4.1%, and the inter-assay variation of 3.6–3.9%. The plasma endocan was measured by immunoassay (Abcam, Cambridge, United Kingdom) with a minimal detectable concentration of 0.3 pg/dL. The intra-assay coefficient of variation was 5.5–7.9%, while the inter-assay variation was 6.3–8.8%. The NT-pro-BNP levels were determined by the assay with the lowest detectable NT-pro-BNP levels of 3.1 pg/dL (R&D Systems, Minneapolis, USA).

Definition of renal outcomes

The renal outcomes were a composite of either the decline in eGFR and or the worsening stage of albuminuria or of both. The decline in eGFR was defined as a loss of > 30% of kidney function compared with the value at baseline, and the decline should be confirmed by the consecutive test after 6 months. Albuminuria status was determined by the urinary albumin–creatinine ratio (UACR) and the patients were categorized according to the following stages: normoalbuminuria (UACR < 30 mg/g creatinine), microalbuminuria (UACR 30–300 mg/g creatinine), and macroalbuminuria (UACR > 300 mg/g creatinine). Progression of albuminuria was defined by progressive shifts in the albuminuria status, i.e., from normoalbuminuria to microalbuminuria, from microalbuminuria to macroalbuminuria, or from normoalbuminuria to macroalbuminuria. Progression of albuminuria was also confirmed by the consecutive result of UACR 6 months after the previous test.

Statistical analyses

All continuous variables were exam by Kolmogorov–Smirnov test. Differences between patients with and without renal events in parametrically continuous variables, which were expressed as mean ± standard deviation, were explored by independent analyses of variance. For nonparametrically continuous variables expressed as median and inter-quarter range, differences between subjects with and without renal events were tested by Mann–Whitney *U* test. Pearson's chi-squared tests were conducted to compare the categorical variables between patients with renal events and those without, which are expressed as a range of numbers and percentages. The subjects were grouped by tertile of sTNFR1, FGF-21, endocan, and NT-pro-BNP. Kaplan–Meier analyses were conducted to estimate the cumulative event-free probabilities of renal composite events, and a log-rank test was used to identify significant differences in the survival probability determined by different sTNFR1, FGF-21, endocan, and NT-pro-BNP levels. The association between sTNFR1, FGF-21, endocan, and NT-pro-BNP levels, or other variables and renal outcome was tested using univariate Cox proportional hazard regression analyses. A two-sided p value < 0.05 was considered significant. Associations were represented by hazard ratio (HR) and 95% confidence interval (95% CI). Three Cox models were constructed sequentially for renal composite events as follows: model 1, which was adjusted for age and sex; model 2, which was adjusted for duration of diabetes in addition to variables in model 1; and model 3, which was adjusted for other factors relevant to the renal outcomes of our study in addition to variables in model 2. A receiver operating characteristic (ROC) curve was used to identify the cutoff levels of sTNFR1, FGF-21, endocan, and NT-pro-BNP which provided the best sensitivity and specificity for the prediction of the effects on composite renal outcome parameters of T2D patients. If the predictive value of the individual biomarker was confirmed by ROC curve, the levels of the biomarker were further transformed to the scores according to the group of percentile. For example, the levels of the biomarker within the 1st percentile were recognized as 1 point of the score. The predictive value of the combination of the biomarkers was also examined by ROC curve. For confirming the improvement of the predictability by the method of combining the biomarkers relevant to the renal events, the categorical net reclassification index (NRI) was used. The subjects were stratified as low, intermediate, or high risk of renal composite events according to the results of logistic regression for the model of the individual biomarker related to the renal outcomes and the combination ones then the categorical NRI was calculated according to the method reported in the literature previously [21]. The statistical analyses except categorical NRI were conducted using the SPSS software package (version 18, IBM Corporation, Armonk, NY, USA) and the analysis of the categorical NRI was conducted using SAS (version 9.4, SAS Institute, Cary, North Carolina, USA).

Results

Baseline characteristics

A total of 312 patients were enrolled in this study, and the last patient was enrolled on April 3, 2019. The median age was 63 years, and the median duration of diabetes was 9 years. Of the patients, 20% had a history of coronary artery disease, while 10% had retinopathy or neuropathy. One-third of the patients had albuminuria, one-fifth had CKD stage 3 or above, and 11% of the patients had both albuminuria and advanced CKD. The metabolic indices relevant to renal outcomes were comparatively optimal. The median HbA1c level was 7.0%, and the median of the mean blood pressure was 115 mmHg. Metformin and sulfonylurea were the most commonly prescribed oral anti-hyperglycemic agents and 17% of patients were treated by subcutaneous insulin injection. Nearly half of the patients received RAS inhibitors, and the coverage of diuretics was 12%. Only three patients were treated with sodium glucose cotransporter type 2 inhibitor (SGLT2i) (two of them had albuminuria), and glucagon-like peptide type 1 (GLP1a) therapy was not evaluated in our cohort (Table 1).

Biomarkers and the renal outcomes

The median follow-up period was 3.5 years, during which 66 renal events occurred (22 events of > 30% decline in eGFR and 44 events of worsening albuminuria) and three subjects died due to the malignancy. In subjects with worsening albuminuria, 21 events progressed from normoalbuminuria to microalbuminuria, 22 events progressed from microalbuminuria to macroalbuminuria. Albuminuria rapidly worsened from normoalbuminuria to macroalbuminuria in one subject. Patients with renal events had several risk factors for worsening nephropathy, including older age, hypertension and microvascular complications of diabetes, higher UACRs, and worse renal function at baseline compared with patients without renal events. Nevertheless, the coverage of RAS blockade in patients with renal events was more extensive (68% vs. 44%; $p < 0.001$) (Table 1).

The tertile groups with the highest sTNFR1 and FGF-21 levels had the highest risk of renal composite events ($p < 0.001$ by log-rank test for sTNFR1 and $p = 0.005$ by log-rank test for FGF-21; Fig. 1a, b). By contrast, the occurrences of renal events were not significantly different among the endocan and NT-pro-BNP tertile groups (Fig. 1c, d). The

Table 1 Baseline characteristics of patients with type 2 diabetes grouped by occurrence of renal composite events

		Renal composite events		
	All ($n = 312$)	Yes ($n = 66$)	No ($n = 246$)	p Value
Age	63.0 (54–70)	65.0 (60.0–72.5)	62.0 (52.0–69.0)	0.003
Male sex (%)	213 (68)	38 (57)	175 (71)	0.03
Smoking (%)	94 (30)	16 (24)	78 (31)	0.40
Coronary artery disease (%)	60 (19)	18 (27)	42 (17)	0.07
Hyperlipidemia (%)	267 (85)	55 (83)	212 (86)	0.55
Hypertension (%)	188 (60)	48 (72)	140 (57)	0.02
Retinopathy (%)	30 (9)	14 (21)	16 (6)	0.001
Neuropathy (%)	32 (10)	11 (16)	21 (8)	0.06
Albuminuria (%)	103 (33)	35 (53)	68 (27)	< 0.001
CKD stage 3(%)	64 (20)	24 (36)	40 (16)	0.001
CKD stage 3 with albuminuria (%)	36(11)	17 (25)	19 (7)	< 0.001
Duration of diabetes (year)	9.0 (6.0–14.0)	13.0 (7.0–16.0)	9.0 (6.0–13.0)	0.001
Body mass index	25.8 (23.5–28.4)	24.7 (23.0–28.3)	26.2 (23.6–28.4)	0.20
Waist-hip ratio	0.92 (0.89–0.96)	0.91 (0.88–0.96)	0.93 (0.89–0.96)	0.39
Systolic blood pressure (mmHg)	133 (123–141)	136 (125–141)	132 (122–141)	0.19
Diastolic blood pressure (mmHg)	78 (72–86)	79 (71–86)	78 (72–86)	0.73
Mean blood pressure (mmHg)	115 (106–122)	117 (109–123)	114 (106–122)	0.23
Urinary albumin–creatinine ratio (mg/g Cr)	15.2 (6.6–51.8)	44.0 (17.7–195.7)	11.2 (5.8–39.5)	< 0.001
HbA1c (%)	7.0 (6.4–7.6)	7.0 (6.6–7.9)	7.0 (6.4–7.6)	0.44
Total cholesterol (mg/dL)	168 ± 27	167 ± 24	168 ± 27	0.75
Creatinine (mg/dL)	0.88 (0.77–1.06)	0.98 (0.77–1.16)	0.87 (0.77–1.05)	0.08
eGFR (mL/min/1.73 m^2)	80 (67–92)	69 (54–81)	81 (70–93)	< 0.001
sTNFR1 (pg/dL)	10.34 (8.31–13.48)	13.31 (9.83–17.69)	9.82 (7.92–12.34)	< 0.001
FGF-21 (pg/dL)	1.64 (0.78–3.21)	2.58 (1.45–4.62)	1.34 (0.67–2.69)	< 0.001
Endocan (pg/dL)	1.28 (0.59–2.18)	0.96 (0.54–1.74)	1.38 (0.61–2.28)	0.07
NT-pro-BNP (pg/dL)	34.74 (22.18–52.46)	32.80 (21.43–50.59)	34.86(22.20–53.59)	0.60
Metformin (%)	239 (76)	48 (72)	191 (77)	0.41
Sulfonylurea (%)	121 (38)	27 (41)	94 (38)	0.77
Dipeptidyl peptidase-4 inhibitor (%)	54 (17)	15 (22)	39 (16)	0.20
SGLT2 inhibitor	3 (0.9)	1 (1)	2 (0.8)	0.51
Insulin (%)	55 (17)	14 (21)	41 (16)	0.37
Renin–angiotensin system blockade (%)	154 (49)	45 (68)	109 (44)	0.001
Diuretics (%)	39 (12)	15 (22)	24 (9)	0.01
eGFR decline > 30% (%)		22 (33)		
Worsening albuminuria (%)		44 (66)		
Normo- to microalbuminuria (%)		21 (32)		
Micro- to macroalbuminuria (%)		22 (33)		
Normo- to macroalbuminuria (%)		1 (2)		

Data are expressed as numbers and percentages in non-continuous variables, as mean ± standard deviation in parametric continuous variables, and as median and interquartile range in nonparametric continuous variables

$p < 0.05$ indicated a significant difference between the two groups

CKD chronic kidney disease; *sTNFR1* soluble tumor necrosis factor receptor type 1; *FGF-21* fibroblast growth factor 21; *NT-pro-BNP* N-terminal pro-brain natriuretic peptide; *SGLT2* sodium glucose cotransporter type 2; *HbA1c* hemoglobin A1c; and *eGFR* estimated glomerular filtration rate

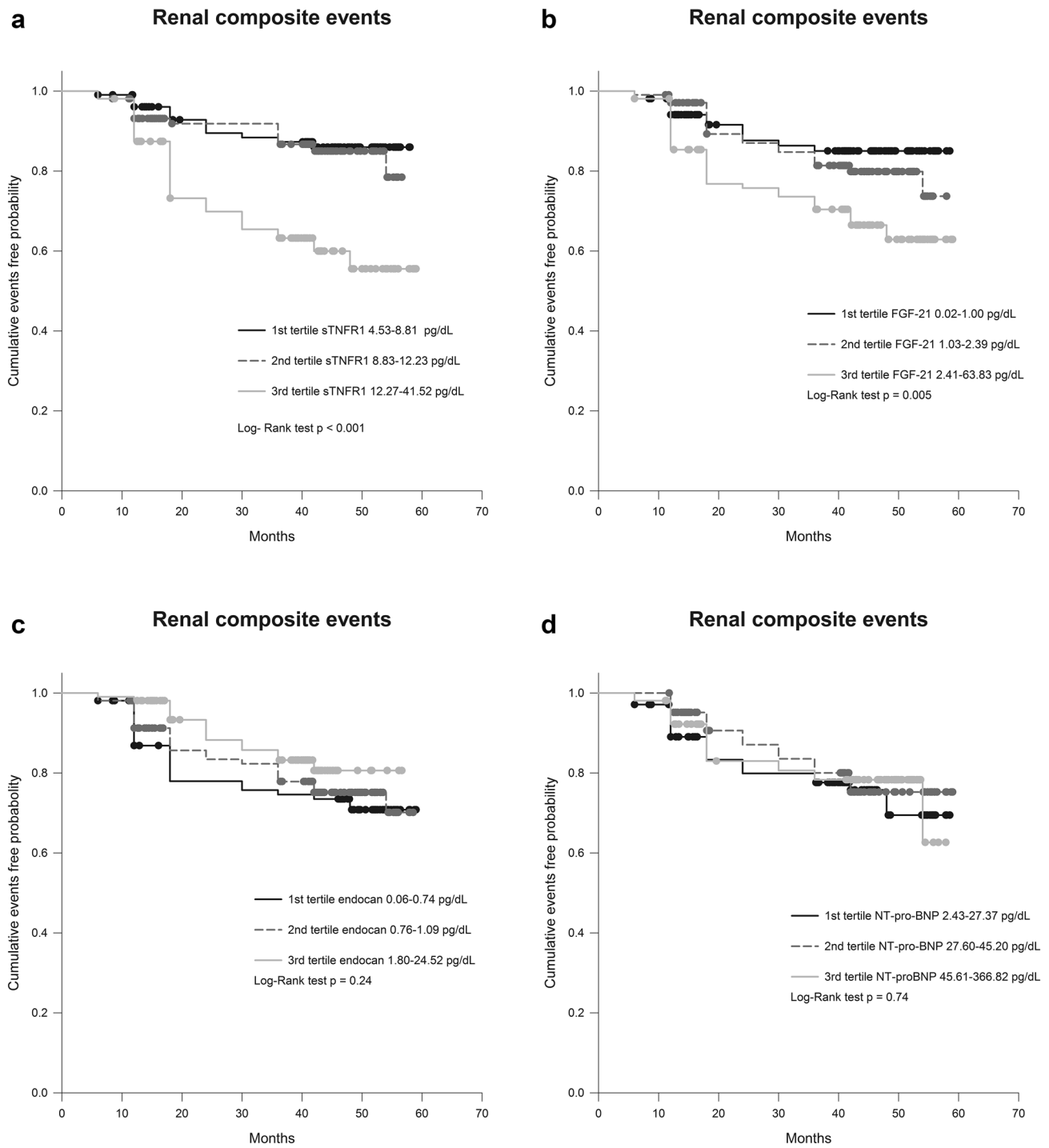

Fig. 1 Kaplan–Meier curve showing the probability of cumulative event-free survival of renal events (either a decline of > 30% in eGFR or worsening stage of albuminuria or both) in patients with type 2 diabetes divided into groups based on concentration tertiles of biomarkers: **a** sTNFR1, **b** FGF-21, **c** endocan, and **d** NT-pro-BNP. *sTNFR1* soluble tumor necrosis factor receptor type 1; *FGF-21* fibroblast growth factor 21; and *NT-pro-BNP* N-terminal pro-brain natriuretic peptide

sTNFR1 or FGF-21 levels were correlated with eGFR and albuminuria levels. Furthermore, the sTNFR1 and FGF-21 levels were highly correlated with each other (correlation efficient 0.423, $p < 0.001$) (Table 2).

Univariate Cox proportional hazard models demonstrated that the sTNFR1 and FGF-21 levels were associated with renal outcomes (sTNFR1, HR 1.07, 95% CI 1.04–1.10, $p < 0.001$; FGF-21, HR 1.04, 95% CI 1.01–1.07, $p = 0.009$).

Table 2 Correlation of sTNFR1, FGF-21, renal function, and urinary albumin excretion

	sTNFR1	*p* Value	FGF-21	*p* Value
FGF-21	$r=0.423$	<0.001	$r=1$	NA
sTNFR1	$r=1$	NA	$r=0.423$	<0.001
eGFR	$r=-0.540$	<0.001	$r=-0.273$	<0.001
UACR	$r=0.376$	<0.001	$r=0.221$	<0.001

r correlation efficient; *sTNFR1* soluble tumor necrosis factor receptor type 1; *FGF-21* fibroblast growth factor 21; *eGFR* estimated glomerular filtration rate; and *UACR* urinary albumin–creatinine ratio

The endocan or NT-pro-BNP levels were not related to the occurrence of renal events in this cohort. The patterns of the associations between aforementioned biomarkers and renal outcomes were consistent across the subgroups who had either normoalbuminuria or preserved renal function. In 176 patients with eGFR ≥ 60 mL/min/1.73 m^2 and normoalbuminuria, sTNFR1 and FGF-21 levels were still associated with renal outcomes in this cohort (sTNFR1, HR 1.13, 95% CI 1.04–1.24, $p=0.003$; FGF-21, HR 1.12, 95% CI 1.02–1.23, $p=0.01$). Moreover, the following risk factors were also associated with renal outcomes: elder age, male sex, longer duration of diabetes, increased UACR or decreased eGFR, presence of retinopathy, and treatment with RAS blockers or diuretics. However, the association between the sTNFR1 or FGF-21 levels and renal outcome did not remain after adjusting for other relevant factors in sequential models (Table 3).

Specific cutoff values of biomarkers and renal outcomes

The appropriate cutoff levels of sTNFR1 and FGF-21 were determined using the ROC curve by mapping the fraction of true-positive results (sensitivity) and false-positive results (1—sensitivity) for specific levels of sTNFR1 and FGF-21, which provided the maximal sensitivity and specificity. The cutoff sTNFR1 level of 9.79 pg/dL yield a sensitivity of 0.783 and specificity of 0.489, and the cutoff FGF-21 level of 1.40 pg/dL demonstrated a sensitivity of 0.773 and specificity of 0.480. The mean areas under the ROC curve (AUC) were 0.68 (95% CI 0.61–0.76, $p<0.001$) for sTNFR1 and 0.66 (95% CI 0.59–0.74, $p<0.001$) for FGF-21 (Fig. 2a). The combination of FGF-21 and sTNFR1 exhibited more extensive AUC than did FGF-21 alone (AUC of sTNFR1 plus FGF-21 versus AUC of FGF-21, $p=0.01$). The trend was consistent but did not reach statistical difference when comparing the AUC of sTNFR1 (AUC of sTNFR1 plus FGF-21 versus AUC of sTNFR1, $p=0.08$) (Fig. 2b). The combination of sTNFR1 and FGF-21 exhibited maximal sensitivity 69.7% as well as the specificity 72.8% and the major contribution of the increment of the AUC should be due to the improvement of the specificity for predicting the renal outcomes. The categorical NRI showed a 23.4% improvement of the accuracy of the prediction of the renal outcomes by the model of the combination of FGF-21 and sTNFR1 versus the model of FGF-21(categorical NRI = 0.234, 95% CI 0.085–0.384, $p=0.002$). The improvement of accuracy of the model of the combination of FGF-21 and sTNFR1 was a 6.8% versus the model of sTNFR1 (categorical NRI = 0.068, 95% CI 0.057–0.194, $p=0.28$).

The univariate Cox proportional analysis showed that sTNFR1 levels ≥ 9.79 pg/dL were associated with a higher incidence of renal events (HR 3.25, 95% CI 1.80–5.87, $p<0.001$). The observed association between high sTNFR1 levels and renal outcomes remained significant after sequential adjustment for other relevant factors in the three different models (HR 3.08, 95% CI 1.69–5.63, $p=0.001$ for model 1; HR 2.77, 95% CI 1.50–5.12, $p=0.001$ for model 2; HR 2.76, 95% CI 1.36–5.60, $p=0.005$ for model 3; Table 3). The levels of FGF-21 ≥ 1.40 pg/dL were also significantly associated with a higher risk of renal events, as demonstrated by the univariate Cox proportional analysis (HR 2.79, 95% CI 1.57–4.97, $p<0.001$). The association was still apparent after adjusting for other covariates in the sequential model (HR 2.55, 95% CI 1.42–4.57, $p=0.002$ for model 1; HR 2.64, 95% CI 1.44–4.81, $p=0.002$ for model 2; HR 1.95, 95% CI 1.03–3.69, $p=0.03$ for model 3; Table 3).

The validated cases in our cohort were further grouped by the cutoff levels of sTNFR1 and FGF-21. There were 76 patients with sTNFR1 level < 9.79 pg/dL and FGF-21 level < 1.40 pg/dL, 69 with sTNFR1 level ≥ 9.79 pg/dL and FGF-21 level < 1.40 pg/dL, 61 with sTNFR1 level < 9.79 pg/dL and FGF-21 level ≥ 1.40 pg/dL, and 106 with sTNFR1 level ≥ 9.79 pg/dL and FGF-21 level ≥ 1.40 pg/dL. The risk of renal events was the highest in patients with sTNFR1 level ≥ 9.79 pg/dL and FGF-21 level ≥ 1.40 pg/dL ($p<0.001$ by log-rank test) (Fig. 3). The combination of sTNFR1 level ≥ 9.79 pg/dL and FGF-21 level ≥ 1.40 pg/dL levels was more strongly associated with the risk of renal events than was sTNFR1 level ≥ 9.79 pg/dL or FGF-21 level ≥ 1.40 pg/dL alone in the overall cohort, and the association was persistent after adjusting for other relevant factors (HR 4.76, 95% CI 1.99–11.35, $p<0.001$ for model 1; HR 4.56, 95% CI 1.90–10.89, $p=0.001$ for model 2; HR 4.45, 95% CI 1.86–10.65, $p=0.001$ for model 3). The better association provided by the combination of the cutoff levels of sTNFR1 and FGF-21 was consistent among patients with preserved renal function or without albuminuria (Table 3).

Table 3 Result of Cox proportional hazard model analysis for the association of factors and renal composite events in the overall cohort, patients with normoalbuminuria, patients with preserved renal function, and patients with normoalbuminuria and preserved renal function

	HR	95% CI	*p* Value
Overall ($n=312$, univariate analysis)			
sTNFR1 (pg/dL)	1.07	1.04–1.10	< 0.001
FGF-21 (pg/dL)	1.04	1.01–1.07	0.009
sTNFR1 ≥ 9.79 pg/dL	3.25	1.80–5.87	< 0.001
FGF-21 ≥ 1.40 pg/dL	2.79	1.57–4.97	< 0.001
sTNFR1 ≥ 9.79 pg/dL + FGF-21 ≥ 1.40 pg/dL	5.28	2.24–12.44	< 0.001
Endocan (pg/dL)	0.87	0.70–1.07	0.20
NT-pro-BNP (pg/dL)	0.99	0.99–1.00	0.53
Age	1.02	1.00–1.04	0.03
Male sex	1.68	1.03–2.74	0.03
Duration of diabetes (years)	1.04	1.01–1.07	0.003
Retinopathy (with versus without)	2.55	1.41–4.60	0.002
eGFR (mL/min/1.73 m^2)	0.97	0.96–0.99	0.001
UACR (per 100 mg/g Cr increase) (mg/g Cr)	1.09	1.04–1.14	< 0.001
RAS inhibitors (with versus without)	2.19	1.30–3.68	0.003
Diuretics (with versus without)	2.13	1.20–3.79	0.01
Overall ($n=312$, multivariate analysis)			
sTNFR1(pg/dL)			
Model 1	1.06	1.02–1.09	< 0.001
Model 2	1.05	1.02–1.09	0.001
Model 3	1.03	0.98–1.08	0.14
sTNFR1 ≥ 9.79 pg/dL			
Model 1	3.08	1.69–5.63	< 0.001
Model 2	2.77	1.50–5.12	0.001
Model 3	2.76	1.36–5.60	0.005
FGF-21 (pg/dL)			
Model 1	1.03	1.00–1.06	0.01
Model 2	1.02	0.99–1.06	0.06
Model 3	1.01	0.97–1.05	0.55
FGF-21 ≥ 1.40 pg/dL			
Model 1	2.55	1.42–4.57	0.002
Model 2	2.64	1.44–4.81	0.002
Model 3	1.95	1.03–3.69	0.03
sTNFR1 ≥ 9.79 pg/dL + FGF-21 ≥ 1.40 pg/dL			
Mode1 1	4.76	1.99–11.35	< 0.001
Mode1 2	4.56	1.90–10.89	0.001
Mode1 3	4.45	1.86–10.65	0.001
UACR < 30 mg/g Cr ($n=200$)			
sTNFR1 (pg/dL)	1.10	1.03–1.17	0.001
FGF-21 (pg/dL)	1.12	1.03–1.23	0.009
sTNFR1 ≥ 9.79 pg/dL	3.73	1.59–8.76	0.002
FGF-21 ≥ 1.40 pg/dL	1.77	082–3.82	0.14
Endocan (pg/dL)	0.65	0.42–1.01	0.05
NT-pro-BNP (pg/dL)	0.99	0.98–1.00	0.71
sTNFR1 ≥ 9.79 pg/dL + FGF-21 ≥ 1.40 pg/dL	4.38	1.46–13.13	0.008
eGFR ≥ 60 mL/min/1.73 m^2 ($n=247$)			
sTNFR1 (pg/dL)	1.13	1.06–1.21	< 0.001
FGF-21 (pg/dL)	1.09	1.02–1.17	0.008
sTNFR1 ≥ 9.79 pg/dL	3.18	1.612–6.22	0.001
FGF-21 ≥ 1.40 pg/dL	2.26	1.17–4.36	0.01
Endocan (pg/dL)	0.79	0.59–1.07	0.13

Table 3 (continued)

	HR	95% CI	*p* Value
NT-pro-BNP (pg/dL)	0.99	0.98–1.00	0.40
sTNFR1 ≥ 9.79 pg/dL + FGF-21 ≥ 1.40 pg/dL	4.83	1.96–11.88	0.001
UACR < 30 mg/g Cr eGFR ≥ 60 mL/min/1.73 m^2 (n = 176)			
sTNFR1 (pg/dL)	1.13	1.04–1.24	0.003
FGF-21 (pg/dL)	1.12	1.02–1.23	0.01
sTNFR1 ≥ 9.79 pg/dL	3.76	1.55–9.12	0.003
FGF-21 ≥ 1.40 pg/dL	1.80	0.78–4.12	0.16
Endocan (pg/dL)	0.58	0.34–0.98	0.04
NT-pro-BNP (pg/dL)	0.99	0.98–1.01	0.66
sTNFR1 ≥ 9.79 pg/dL + FGF-21 ≥ 1.40 pg/dL	4.90	1.57–15.23	0.006

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex, and duration of diabetes

Model 3: adjusted for age, sex, duration of diabetes, retinopathy, eGFR, UACR, treatment with RAS inhibitors, and diuretics

sTNFR1 soluble tumor necrosis factor receptor type 1; *FGF-21* fibroblast growth factor 21; *NT-pro-BNP* N-terminal pro-brain natriuretic peptide; *HR* hazard ratio; *CI* confidence interval; *eGFR* estimated glomerular filtration rate; *UACR* urinary albumin-creatinine ratio; *Cr* creatinine; and *RAS* renin–angiotensin system

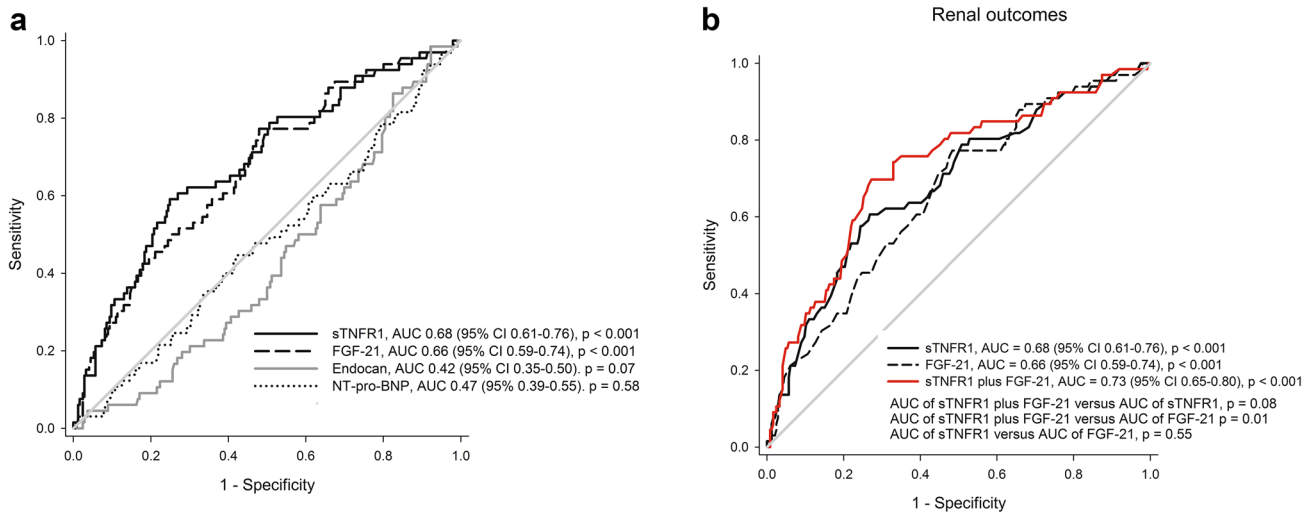

Fig. 2 Receiver operating characteristic curves for prediction of renal events in patients with type 2 diabetes: **a** sTNFR1, FGF-21, endocan, and NT-pro-BNP, **b** sTNFR1 + FGF-21, sTNFR1, and FGF-21. *sTNFR1* soluble tumor necrosis factor receptor type 1; *FGF-21* fibroblast growth factor 21; and *NT-pro-BNP* N-terminal pro-brain natriuretic peptide

Discussion

The cohort of patients with T2D demonstrated that the sTNFR1 and FGF-21 levels, rather than endocan or NT-pro-BNP levels, were associated with renal outcomes and the combinations of sTNFR1 and FGF-21 levels exhibited better predictive value of renal outcomes than either one. The results were consistent in patients without advanced CKD or albuminuria.

Studies on our cohort were not the first to demonstrate an association between sTNFR1 or FGF-21 levels and renal outcomes in patients with T2D, but the design of our study was different from that of other studies. Niewczas et al. highlighted that circulating sTNFR1 and sTNFR2 predicted the development of end-stage renal disease in patients with T2D with or without proteinuria, and Lee et al. reported that circulating FGF-21 levels predicted the presence of progressive kidney disease in patients with T2D without albuminuria [10, 15]. However, the effects of other biomarkers that are

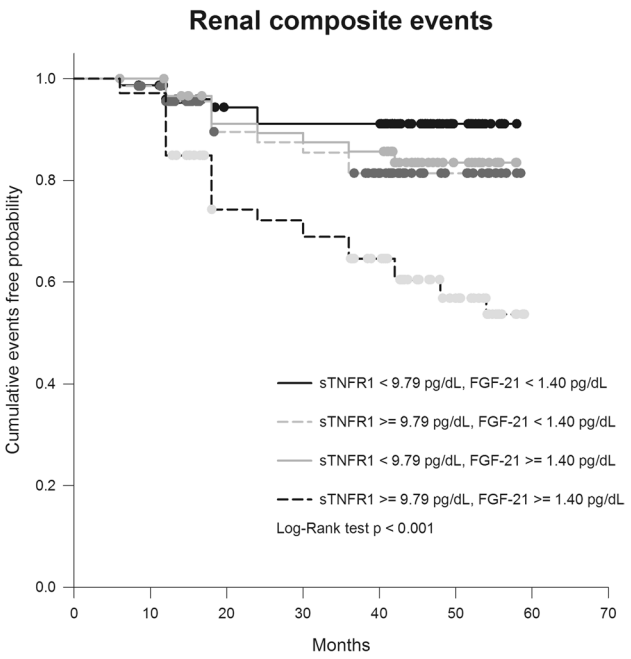

Fig. 3 Kaplan–Meier curve showing the cumulative event-free probability of renal composite events among the diabetic patients grouped according to the sTNFR1 (≥9.79 pg/dL vs. <9.79 pg/dL) combination with the FGF-21 (≥1.4 pg/dL vs. <1.4 pg/dL). *sTNFR1* soluble tumor necrosis factor receptor type 1 and *FGF-21* fibroblast growth factor 21

involved in the different processes of the pathogenesis of DKD were not considered in the abovementioned studies. The endocan or NT-pro-BNP levels were correlated with albuminuria, but whether the endocan or NT-pro-BNP levels were associated with renal outcomes was still inconclusive. Our cohort included four biomarkers representing inflammation, fibrosis, angiogenesis, and accumulation of fluid in DKD in reviewing the association of individual markers and renal outcomes and comparing the predictability across biomarkers in patients with T2D. The association between the sTNFR1 or FGF-21 levels and the decline in renal function or worsening status of albuminuria in our cohort was consistent with those of previous studies but the neutral influences of the plasma endocan or NT-pro-BNP levels on renal outcomes of patients with T2D were novel findings. Moreover, the more accurate predictability exhibited by the combinations of sTNFR1 and FGF-21 levels indicated more validated markers, including a more precise prediction of outcomes. This hypothesis was supported by a study that included 16 biomarkers to predict the renal and cardiovascular outcomes of the patients with T2D [22]. However, the difference of the improvement of the predictability between the comparisons of the combination of FGF-21 and sTNFR1 versus FGF-21 only and the combination of FGF-21 and sTNFR1 versus sTNFR1 only suggested that individual biomarker had impact on the final efficacy of the prediction of the combination. Therefore, further investigation for exploring the best combination of biomarkers was warranted.

Tumor necrosis factor, one of the pro-inflammatory cytokines secreted by infiltrating macrophage or resident renal cells, has been shown to contribute to the pathophysiology of DKD [23, 24]. Since tumor necrosis factor was a crucial marker of infiltrating macrophages, which responses to the oxidative stress induced by hyperglycemia in patients with T2D, and the infiltrating macrophage played the role of the initiator of DKD, it is reasonable to use the sTNFR1 level as a sentinel marker for predicting further structural damage of glomerulus, such as the expansion of mesangial cells or podocytopathy, detected by the presence of albuminuria or decline in eGFR [7]. The association between the increasing FGF-21 levels and renal outcomes was not as strong as that of sTNFR1 level in our study, but the combination of FGF-21 and sTNFR1 levels generated better predictability of renal outcomes, and the FGF-21 levels were highly correlated with the sTNFR1 levels. The increasing plasma FGF-21 levels can be due to decreased urinary excretion or increased stimulation from inflammatory cytokines induced by metabolic and oxidative insult [25, 26]. The latter mechanism was favorable to explain the findings on FGF-21 in our study because two-thirds of patients were free from albuminuria and 80% of patients had preserved renal function. Since the plasma FGF-21 levels were possibly increased by the stimulation of tumor necrosis factor, the FGF-21 levels can be a marker to reflect the activity of inflammation induced by the tumor necrosis factor in the target organ. Therefore, the FGF-21 levels can provide additional predictive value to the sTNFR1 levels. The endothelial cell was one of the targets to be damaged by the oxidative stress of patients with T2D [27]. The real reason for the unremarkable association between the endocan levels and renal outcomes in our study was unclear, but it may be that the defect in neovascularization related to endothelial dysfunction had not yet been apparent in the early stage of DKD. Nevertheless, it is still possible to find the connection between other markers related to endothelial function and renal outcomes in the future. The implicit associations of the NT-pro-BNP levels and renal outcomes in our cohort may be due to the lack of systemic fluid accumulation in patients with preserved renal function. However, the predictive value of the NT-pro-BNP levels was still recognized in patients with hemodialysis for end-stage renal disease [28].

The predictability of renal outcomes by the sTNFR1 and FGF-21 levels in patients with preserved renal function and normoalbuminuria was consistent with the primary cohort. Moreover, 30–50% patients with T2D and eGFR < 60 mL/min/1.73 m^2 did not have albuminuria [29]. The results of our study suggested that FGF-21 and sTNFR1 levels were the alternative choices in estimating the risk of worsening DKD when the sign of albuminuria

was not applicable and sTNFR1 and FGF-21 levels can be the early surrogate markers detected before decline in eGFR or worsening of albuminuria to warn the clinicians to perform the necessary procedures for preventing further renal damage.

There were some limitations to our study. First, causal relationships cannot be concluded from prospective observational studies such as our study. However, the retrospective study showed that biologically anti-inflammatory agents ameliorated the risk of CKD, and the study suggested the activity of tumor necrosis factor was possibly not only a marker but also a risk factor for the pathogenesis of decline in renal function [30]. Second, recent studies have shown that the SGLT2i and GLP1a provide renal protection in patients with T2D, but the minimal number of patients receiving SGLT2i or GLP1a made the influences of SGLT2i or GLP1a on the results of our study unmeasurable [31, 32]. Third, biomarkers that participated in the pathogenesis of DKD were not included in our study. Nevertheless, the rationale for the selection of biomarkers in our study was based on the process of pathophysiology of DKD, and their associations with the albuminuria levels were validated in a clinical scenario. Therefore, the biomarkers in our cohort were representative choices of inflammation, fibrosis, neovascularization, and systemic fluid accumulation in DKD. Lastly, the conclusion of our study was validated in Chinese patients with T2D and should be cautiously applied to different races with T2D.

Conclusion

Circulating sTNFR1 and FGF-21 levels, rather than endocan and NT-pro-BNP levels, were associated with renal outcomes in patients with T2D with or without clinical signs of DKD, and the combination of the TNFR1 and FGF-21 levels exhibited better associations than did either one alone. The results suggested that sTNFR1 and FGF-21 levels could be potential biomarkers in the assessment of DKD.

Acknowledgements This work was supported in part by research grants No. 2021001 to L.H.C. from Yeezen General Hospital and V104E11-004-MY2, V105C-131, V107C-201, V108C-197, V109C-179, and V110C-198 to L.Y.L. from Taipei Veterans General Hospital, Taipei, Taiwan. We also thank the Medical Sciences & Technology Building of Taipei Veterans General Hospital for providing us with an experimental space and facilities. Corresponding author Liang-Yu Lin takes responsibility for the content of the article.

Author contributions Study design and data collection: LHC, CMH, YCL, and LYL. Case contributor: CMH, YCL, CCH, HSC, and LYL. Analysis data: LHC, CHC, JYY, and LYL. Writing the manuscript: LHC, CHC, JYY, and LYL.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Informed consent Informed consent approved by local ethical committee has been obtained from each participant after full explanation of the purpose and nature of all procedures used.

References

1. Cho NH, Shaw JE, Karuranga S et al (2018) IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138:271–281. https://doi.org/10.1016/j.diabres.2018.02.023
2. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J (2011) Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 305:2532–2539. https://doi.org/10.1001/jama.2011.861
3. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T (2016) Diabetic kidney disease: world wide difference of prevalence and risk factors. J Nephropharmacol 5:49–56 (**PMCID: PMC5297507**)
4. Alicic RZ, Rooney MT, Tuttle KR (2017) Diabetic kidney disease: challenges, progress, and possibilities. Clin J Am Soc Nephrol 12:2032–2045. https://doi.org/10.2215/CJN.11491116
5. Bilous R (2008) Microvascular disease: what does the UKPDS tell us about diabetic nephropathy? Diabet Med 25(Suppl 2):25–29. https://doi.org/10.1111/j.1464-5491.2008.02496.x
6. Parving HH, Lewis JB, Ravid M, Remuzzi G, Hunsicker LG, investigators D (2006) Prevalence and risk factors for microalbuminuria in a referred cohort of type II diabetic patients: a global perspective. Kidney Int 69:2057–2063. https://doi.org/10.1038/sj.ki.5000377
7. Muskiet MH, Smits MM, Morsink LM, Diamant M (2014) The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? Nat Rev Nephrol 10:88–103. https://doi.org/10.1038/nrneph.2013.272
8. Navarro-Gonzalez JF, Mora-Fernandez C, Muros de Fuentes M, Garcia-Perez J (2011) Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol 7:327–340. https://doi.org/10.1038/nrneph.2011.51
9. Coca SG, Nadkarni GN, Huang Y et al (2017) Plasma biomarkers and kidney function decline in early and established diabetic kidney disease. J Am Soc Nephrol 28:2786–2793. https://doi.org/10.1681/ASN.2016101101
10. Niewczas MA, Gohda T, Skupien J et al (2012) Circulating TNF receptors 1 and 2 predict ESRD in type 2 diabetes. J Am Soc Nephrol 23:507–515. https://doi.org/10.1681/ASN.2011060627
11. Chang LH, Hwu CM, Lin YC et al (2020) Soluble tumor necrosis factor receptor type 1 levels exhibit the better association with renal outcomes than traditional risk factors in Chinese subjects with type 2 diabetes mellitus. Endocr Pract 26:1115–1124. https://doi.org/10.4158/EP-2020-0114
12. Itoh N, Ornitz DM (2011) Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biochem 149:121–130. https://doi.org/10.1093/jb/mvq121
13. Fisher FM, Maratos-Flier E (2016) Understanding the physiology of FGF21. Annu Rev Physiol 78:223–241. https://doi.org/10.1146/annurev-physiol-021115-105339
14. Salminen A, Kaarniranta K, Kauppinen A (2017) Integrated stress response stimulates FGF21 expression: systemic enhancer

of longevity. Cell Signal 40:10–21. https://doi.org/10.1016/j.cellsig.2017.08.009
15. Lee CH, Hui EY, Woo YC et al (2015) Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. J Clin Endocrinol Metab 100:1368–1375. https://doi.org/10.1210/jc.2014-3465
16. Kali A, Shetty KS (2014) Endocan: a novel circulating proteoglycan. Indian J Pharmacol 46:579–583. https://doi.org/10.4103/0253-7613.144891
17. Cikrikcioglu MA, Erturk Z, Kilic E et al (2016) Endocan and albuminuria in type 2 diabetes mellitus. Ren Fail 38:1647–1653. https://doi.org/10.1080/0886022X.2016.1229966
18. de Lemos JA, McGuire DK, Drazner MH (2003) B-type natriuretic peptide in cardiovascular disease. Lancet 362:316–322. https://doi.org/10.1016/S0140-6736(03)13976-1
19. Spanaus KS, Kronenberg F, Ritz E et al (2007) B-type natriuretic peptide concentrations predict the progression of nondiabetic chronic kidney disease: the mild-to-moderate kidney disease Study. Clin Chem 53:1264–1272. https://doi.org/10.1373/clinchem.2006.083170
20. Zhou L, Cai X, Li M, Han X, Ji L (2016) Plasma NT-proBNP is independently associated with albuminuria in type 2 diabetes. J Diabetes Complicat 30:669–674. https://doi.org/10.1016/j.jdiacomp.2016.01.017
21. Grunkemeier GL, Jin R (2015) Net reclassification index: measuring the incremental value of adding a new risk factor to an existing risk model. Ann Thorac Surg 99:388–392. https://doi.org/10.1016/j.athoracsur.2014.10.084
22. Vistisen D, Andersen GS, Hulman A, Persson F, Rossing P, Jorgensen ME (2019) Progressive decline in estimated glomerular filtration rate in patients with diabetes after moderate loss in kidney function-even without albuminuria. Diabetes Care 42:1886–1894. https://doi.org/10.2337/dc19-0349
23. Hasegawa G, Nakano K, Sawada M et al (1991) Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney Int 40:1007–1012. https://doi.org/10.1038/ki.1991.308
24. Alexandraki K, Piperi C, Kalofoutis C, Singh J, Alaveras A, Kalofoutis A (2006) Inflammatory process in type 2 diabetes: the role of cytokines. Ann N Y Acad Sci 1084:89–117. https://doi.org/10.1196/annals.1372.039
25. Slusher AL, Whitehurst M, Zoeller RF, Mock JT, Maharaj M, Huang CJ (2015) Attenuated fibroblast growth factor 21 response to acute aerobic exercise in obese individuals. Nutr Metab Cardiovasc Dis 25:839–845. https://doi.org/10.1016/j.numecd.2015.06.002
26. Hindricks J, Ebert T, Bachmann A et al (2014) Serum levels of fibroblast growth factor-21 are increased in chronic and acute renal dysfunction. Clin Endocrinol (Oxf) 80:918–924. https://doi.org/10.1111/cen.12380
27. Tan KC, Chow WS, Tam SC, Ai VH, Lam CH, Lam KS (2002) Atorvastatin lowers C-reactive protein and improves endothelium-dependent vasodilation in type 2 diabetes mellitus. J Clin Endocrinol Metab 87:563–568. https://doi.org/10.1210/jcem.87.2.8249
28. Madsen LH, Ladefoged S, Corell P, Schou M, Hildebrandt PR, Atar DN (2007) terminal pro brain natriuretic peptide predicts mortality in patients with end-stage renal disease in hemodialysis. Kidney Int 71:548–554. https://doi.org/10.1038/sj.ki.5002087
29. Halimi JM (2012) The emerging concept of chronic kidney disease without clinical proteinuria in diabetic patients. Diabetes Metab 38:291–297. https://doi.org/10.1016/j.diabet.2012.04.001
30. Sumida K, Molnar MZ, Potukuchi PK et al (2018) Treatment of rheumatoid arthritis with biologic agents lowers the risk of incident chronic kidney disease. Kidney Int 93:1207–1216. https://doi.org/10.1016/j.kint.2017.11.025
31. Perkovic V, Jardine MJ, Neal B et al (2019) Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. N Engl J Med 380:2295–2306. https://doi.org/10.1056/NEJMoa1811744
32. Marso SP, Daniels GH, Brown-Frandsen K et al (2016) Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 375:311–322. https://doi.org/10.1056/NEJMoa1603827