Association of Circulating Biomarkers (Adrenomedullin, TNFR1, and NT-proBNP) With Renal Function Decline in Patients With Type 2 Diabetes: A French Prospective Cohort

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OBJECTIVE

We explored the prognostic value of three circulating candidate biomarkers—midregional-proadrenomedullin (MR-proADM), soluble tumor necrosis factor receptor 1 (sTNFR1), and N-terminal prohormone brain natriuretic peptide (NT-proBNP)—for change in renal function in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Outcomes were defined as renal function loss (RFL), ≥40% decline of estimated glomerular filtration rate (eGFR) from baseline, and rapid renal function decline (RRFD), absolute annual eGFR slope <–5 mL/min/year. We used a proportional hazard model for RFL and a logistic model for RRFD. Adjustments were performed for established risk factors (age, sex, diabetes duration, HbA_{1c}, blood pressure, baseline eGFR, and urinary albumin-to-creatinine ratio [uACR]). C-statistics were used to assess the incremental predictive value of the biomarkers to these risk factors.

RESULTS

Among 1,135 participants (mean eGFR 76 mL/min, median uACR 2.6 mg/mmol, and median GFR slope −1.6 mL/min/year), RFL occurred in 397, RRFD developed in 233, and 292 died during follow-up. Each biomarker predicted RFL and RRFD. When combined, MR-proADM, sTNFR1, and NT-proBNP predicted RFL independently from the established risk factors (adjusted hazard ratio 1.59 [95% CI 1.34–1.89], $P < 0.0001$; 1.33 [1.14–1.55], $P = 0.0003$; and 1.22 [1.07–1.40], $P = 0.004$, respectively) and RRFD (adjusted odds ratio 1.56 [95% CI 1.7–2.09], $P = 0.003$; 1.72 [1.33–2.22], $P < 0.0001$; and 1.28 [1.03–1.59], $P = 0.02$, respectively). The combination of the three biomarkers yielded the highest discrimination (difference in C-statistic = 0.054, $P < 0.0001$; 0.067, $P < 0.0001$ for RFL; and 0.027, $P < 0.0001$ for RRFD).

CONCLUSIONS

In addition to established risk factors, MR-proADM, sTNFR1, and NT-proBNP improve risk prediction of loss of renal function in patients with type 2 diabetes.

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Diabetes is the leading cause of end-stage renal disease (ESRD) in the U.S. and Europe (1). Recent data have confirmed that patients with diabetes have approximately double the risk of ESRD compared with individuals without diabetes (2). However only 20–40% of patients with type 2 diabetes will develop diabetic kidney disease (DKD) in their life span (3).

The estimated glomerular filtration rate (eGFR) and albuminuria are used to stage chronic kidney disease (CKD). Nevertheless, these markers have some limitations, because eGFR may decline in the absence of elevated albuminuria (4) and the structural lesions of DKD may be present in normoalbuminuric patients, including those with normal eGFR (5). Identifying these patients with more sensitive and specific predictive markers of DKD could lead to treatment before irreversible structural injuries occur. This approach could also contribute to more efficient use of medical resources by targeting the patients who could benefit the most from therapeutic intervention.

We performed a literature-based search for independently replicated nontraditional biomarkers of renal outcomes. We selected three of these biomarkers—midregional-proadrenomedullin (MR-proADM), soluble tumor necrosis factor (TNF) receptor 1 (sTNFR1), and N-terminal prohormone brain natriuretic peptide (NT-proBNP)—for evaluation in the current study. MR-proADM concentrations are associated with the doubling of serum creatinine and progression to ESRD (6) and with mortality (7) in patients with type 2 diabetes. Circulating sTNFR is associated in numerous epidemiological studies cross-sectionally with GFR and DKD (8–10) and prospectively with DKD progression and ESRD occurrence (11–16) in patients with type 1 diabetes as well as those with type 2 diabetes. Moreover, sTNFR2 has been associated with GFR variation in patients with type 2 diabetes (17) as well as in patients with type 1 diabetes (18). TNFR2 and TNFR1 have also been associated with DKD structural lesions and especially with early glomerular lesions in type 2 diabetes (19). However, even though TNFR2 and TNFR1 show partially overlapping biological effects, we only had access to an sTNFR1 assay for the current study. NT-proBNP has been reported to be associated with rapid kidney decline in elderly adults (20) and with ESRD in the general population (21). A post hoc analysis of a clinical trial also reported an association of NT-proBNP with ESRD in patients with type 2 diabetes (22). Interestingly, a recent biomarker-panel study showed these three biomarkers were all associated with rapid progression of eGFR in type 2 diabetes and were included in our study to further evaluate their combined value (23). The aim of the current study was to examine the association of these three biomarker candidates with the decline of renal function in a prospective cohort of patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Patients

The SURDIAGENE (SURvie, DIAbete de type 2 et GENEtique) study is a French single-center inception cohort of patients with type 2 diabetes regularly visiting the diabetes department at Poitiers University Hospital in France (24). Patients were consecutively enrolled from 2002 to 2012, and outcome updates were performed every 2 years since 2007. Because this is a referral population, some participants may be more complicated than those in the general population with diabetes. The Poitiers University Hospital Ethics Committee (CPP Ouest III) approved the design. All participants in the study gave their informed written consent.

At baseline, all patients were examined to collect relevant clinical and biological data. A history of cardiovascular disease at baseline was defined as a personal history of myocardial infarction and/or stroke. The present analysis excluded patients with a baseline eGFR <30 mL/min/1.73 m^2 and/or prior renal replacement therapy.

Definition of Outcomes

The primary outcome in the longitudinal analyses was renal function loss (RFL), defined by a decline in eGFR during follow-up of $\geq$40% compared with the baseline value. This end point was recently recommended as an alternative end point for CKD progression (25). The secondary end point was rapid renal function decline (RRFD), defined by an eGFR annual slope ≤ -5 mL/min/1.73 m^2/year, according to The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. Finally, we analyzed determinants of annual eGFR trajectories. The vital status of all study participants was confirmed through 31 December 2013.

Assays

Blood samples and second morning urine samples were obtained in patients after an overnight fast. Serum and urine creatinine and urinary albumin were measured by colorimetry and immunoturbidimetry tests, respectively, on a COBAS System analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The eGFR was calculated using the Chronic Kidney Disease Epidemiology (2009 CKD-EPI) creatinine equation. Glycated hemoglobin was determined using a high-performance liquid chromatography method with a HA-8160 analyzer (Menarini, Florence, Italy).

Remaining samples were processed under standardized conditions and stored at -80°C in the Poitiers Biological Resource Center (BRC BB-0033-00068) undergoing only one prior freeze-thaw cycle before assay. The concentrations of MR-proADM and NT-proBNP were measured in stored plasma-EDTA samples, and sTNFR1 was measured in stored serum.

The MR-proADM concentration was measured using a commercially available automated immunofluorescent sandwich immunoassay (BRAHMS MR-proADM; BRAHMS GmbH, Hennigsdorf, Germany). The limit of detection (LOD) was assessed as 0.05 nmol/L, intraassay coefficient of variations (CV) was 3.5–10%, and the interassay CV was $\leq$20% for 0.2–0.5 nmol/L concentrations and $\leq$11% for 0.5–6 nmol/L concentrations. Serum sTNFR1 concentrations were measured using Human sTNFR1 ELISA (Product #BIO94TNFR1; EKF Diagnostics, Dublin, Ireland) according to the manufacturer's instructions. The LOD was 1.7 pg/mL, the intra-assay CV was 1.8–5.3%, and interassay CV was 3.6–6.8%. NT-proBNP concentration in plasma was measured in a COBAS system by an automated electrochemiluminescence immunoassay. According to the manufacturer's information, the LOD was 5 ng/L, the intraassay CV was 1.2–1.9%, and the interassay CV was 1.7–3.1%.

Statistical Analysis

Quantitative variables are expressed as means $\pm$ SD or medians (25th–75th percentile) for skewed distributions, and qualitative variables are presented as frequencies and percentages. Because of non-Gaussian distribution, concentrations

of biomarkers were log-transformed. Spearman correlations were used to assess the relationship of biomarkers with each other and with clinical variables.

The hazard ratio (HR) of RFL for each biomarker measured at baseline was determined by using Cox proportional hazards regression. We tested each model for log-linearity and proportionality assumptions using Schoenfeld residuals. Results are given with the HR and 95% CI.

To determine whether patients had achieved RRFD, absolute eGFR slopes were individually determined by linear regression in patients with at least three eGFR determinations and 6 months of follow-up between the first and last eGFR. The odds ratio (OR) of RRFD was determined by logistic regression. Three sets of models were used for individual biomarkers: univariate models (model 1), models adjusted for age, sex, diabetes duration, $HbA_{1c,}$ and systolic blood pressure (SBP) (model 2), and models adjusted for the same variables as model 2 plus eGFR and the urinary albumin-to-creatinine ratio (uACR) (model 3) because they represent established key markers associated with renal outcomes (26). The outcome risk associated with the biomarker (HR or OR) was expressed for a 1-SD increase in the distribution of the logarithm of the biomarker concentration. Interactions between sex or antidiabetic drugs and biomarkers for the association between biomarkers and RFL or RRFD were evaluated by the addition of interaction terms into the corresponding regression model. Generalized C-statistics were calculated for model 3 accounting for variable follow-up times (27).

Comparisons of model adequacy were assessed using likelihood ratio χ^2 tests. The relative integrated discrimination improvement (rIDI) index was calculated to assess the improvement in 5-year risk prediction of each biomarker in addition to the traditional risk factors of age, sex, diabetes duration, $HbA_{1c,}$ SBP, eGFR, and uACR (27,28). Five-year risk was selected because it approximates the median follow-up time for RFL or death. The 95% CIs for the changes in the C-statistic and the rIDI were computed from 10,000 bootstrap samples. Receiver operating characteristic curves were also generated for models with traditional risk factors (age, sex, diabetes duration, $HbA_{1c,}$ SBP, eGFR, and uACR) and traditional risk factors plus biomarkers.

The Akaike information criterion (AIC) was used to compare global fit among the models (nested or not nested), and the model with the smallest AIC was considered as the best model. MR-proADM, sTNFR1, and NT-proBNP were used to compute a weighted biomarker risk score that was derived by the following equation:

$$\begin{aligned}\text{score} = 3 \times (&\beta_1 \times \log[\text{MR-proADM}] \\ &+ \beta 2 \times \log[\text{sTNFR1}] \\ &+ \beta 3 \times \log[\text{NT-proBNP}]) \\ &/(\beta_1 + \beta_2 + \beta_3)\end{aligned}$$

β-Coefficients were derived from the model 3 Cox regression model and correspond to the log-HR of the biomarker (β_1 = 4.892; β_2 = 4.279; β_3 = 0.7470).

The time to event was plotted as Kaplan-Meier cumulative incidence curves according to quartiles of biomarkers and biomarker risk score, and comparison was made using the log-rank test.

We also used a linear mixed-effects model to take repeated longitudinal eGFR data into account to test the association between biomarkers and the global pattern of absolute annual eGFR decline (29). The random errors of the linear mixed-effects analyses were defined as a random intercept and slope, assuming that variability between individuals was not identical at baseline and during follow-up. The fixed-effects coefficients were presented with their SE. The validity of the model was demonstrated by the normal distribution of the marginal residuals.

We conducted a series of sensitivity analyses. *1*) We assessed RFL risk in different subgroups by stratifying the study sample by age (<75 vs. ≥75 years), sex, diabetes duration (<12.5 vs. ≥12.5 years), HbA_{1c} (<7 vs. ≥7%), uACR (<30 vs. ≥30 mg/mmol), diuretic use, SBP (<140 vs. ≥140 mmHg), and history of cardiovascular disease. To examine whether the associations between biomarkers and renal outcome were independent of cardiovascular history, we successively *2*) excluded from the analyses patients with prevalent cardiovascular history (defined as prior myocardial infarction or stroke) at baseline *3*) and then patients with incident major cardiovascular event (defined as cardiovascular death or nonfatal myocardial or nonfatal stroke). *4*) In addition, among patients without prevalent cardiovascular history, we examined data by treating nonfatal cardiovascular outcomes that occurred during follow-up as time-varying covariates. *5*) To account for individual changes in HbA_{1c} over time, we used time-dependent Cox regression analyses, including yearly mean of HbA_{1c} as a time-dependent variable. *6*) We then used the competing risk model of Fine and Gray to estimate the subdistribution HRs for RFL while accounting for the competing risk of all-cause deaths (30).

P values <0.05 were considered statistically significant. Statistical analyses were performed with SAS 9.3 software (SAS Institute, Inc., Cary, NC).

RESULTS

Baseline Characteristics

The study population included 1,135 patients with available samples and follow-up data as described in Supplementary Fig. 1. The clinical and biological characteristics of the patients are reported in Table 1. Among the participants, 61 (5%) had a history of stroke at baseline, 171 (15%) had a history of myocardial infarction, and 16 (1.4%) had both. Baseline eGFR was significantly correlated with all three biomarkers (ρ = −0.61 to −0.30, all $P <$ 0.0001) (Supplementary Table 1). MR-proADM, NT-proBNP, and sTNFR1 were significantly intercorrelated (ρ = 0.39–0.72, all $P <$ 0.0001). Compared with women, men had significantly lower median concentrations of MR-proADM (0.7 [0.6–0.9] vs. 0.8 [0.6–0.9] nmol/L, *P* = 0.0004) and similar concentrations of sTNFR1 (1,796 [1,524–2,226] vs. 1,840 [1,566–2,233] pg/mL, *P* = 0.22) and NT-ProBNP (103 [44–276] vs. 100 [49–252] pg/mL, *P* = 0.69). We observed no significant statistical interaction between sex or antidiabetic drugs and biomarkers for the association of biomarkers with outcomes.

Biomarkers and RFL

Patients were monitored for RFL (i.e., ≥40% eGFR drop) for a median of 4.3 years (2.4–7.3 years), during which 397 cases of RFL occurred (incidence rate, 73/1,000 person-years; 95% CI 66–80) and 292 deaths occurred (incidence rate, 40/1,000 person-years; 95% CI 35–44). The cumulative incidence of RFL across quartiles of biomarkers and biomarker risk score is shown in Fig. 1.

Table 1—Clinical and biological characteristics in the SURDIAGENE study

Variables	All (*N* = 1,135)
Male	651 (57)
Age (years)	64 ± 11
BMI (kg/m^2)	31 ± 6
Active smoking	125 (11)
Known diabetes duration (years)	14 ± 10
History of	
Cardiovascular disease	216 (19)
Stroke	61 (5)
Myocardial infarction	171 (15)
Blood pressure (mmHg)	
Systolic	132 ± 18
Diastolic	72 ± 11
Resting heart rate (bpm)	71 ± 13
Therapeutics	
Any antihypertensive drug	950 (84)
Diuretics	504 (44)
RAAS blockers	711 (63)
β-Blockers	388 (34)
Calcium antagonists	418 (37)
Any antidiabetic drug	1,091 (96)
Insulin	683 (60)
Oral antidiabetic agents	740 (65)
Biological determinations	
HbA_{1c} (%)	7.8 ± 1.5
HbA_{1c} (mmol/mol)	62 ± 16.4
eGFR (mL/min/1.73 m^2)	76 ± 21
uACR (mg/mmol)	2.6 (1.0–10.4)
Normoalbuminuria*	455 (45)
Microalbuminuria*	368 (36)
Macroalbuminuria*	187 (19)
MR-proADM (nmol/L)	0.72 (0.59–0.90)
sTNFR1 (pg/mL)	1,818 (1,544–2,231)
NT-proBNP (pg/mL)	101 (47–262)
Biomarker risk score	4.51 ± 0.45

Data are mean ± SD, median (25th–75th percentile), or *n* (%). History of cardiovascular disease was defined as history of stroke and/or myocardial infarction before baseline. RAAS, renin-angiotensin-aldosterone system (angiotensin receptor blocker and/or ACE inhibitor).
*Normoalbuminuria was defined as uACR <30 mg/g, microalbuminuria as uACR 30–299 mg/g, and macroalbuminuria as uACR ≥300 mg/g

All three biomarkers predicted RFL independently in univariate and multivariate models (Table 2). In addition, when considering the combination of the three biomarkers in the adjusted model 3, MR-proADM and NT-proBNP remained independently associated with an increase of RFL risk (adjusted HR per 1-SD 1.59 [95% CI 1.34–1.89], $P < 0.0001$; 1.33 [1.14–1.55], $P = 0.0003$; and 1.22 [1.07–1.40], $P = 0.004$, respectively). This analysis was repeated with diabetes duration and uACR categorized (diabetes duration at its median ≤12.5 or >12.5 years and uACR <30 or ≥30 mg/mmol), after including patients with a baseline eGFR <30 mL/min/1.73 m^2 without the requirement of a renal replacement therapy, and the findings were unchanged.

Biomarkers and RRFD

The prevalence of RRFD (i.e., annualized GFR <–5 mL/min/1.73 m^2/year) was 20% ($n = 233$) in the 1,109 patients with at least three determinations and 6 months between the first and last eGFR. In models 1 to 3, all three biomarkers were individually associated with the risk of RRFD (Table 2). As in the prospective approach for RFL, MR-proADM, sTNFR1, and NT-proBNP remained independently associated with an increased risk of RRFD when considering all three biomarkers with model 3 covariates: adjusted OR for a 1-SD increase 1.56 (95% CI 1.17–2.09), $P = 0.003$; 1.72 (1.33–2.22), $P < 0.0001$; and 1.28 (1.03–1.59), $P = 0.02$, respectively.

Biomarkers and Annual Renal Function Decline (eGFR slope)

Median annual variation of eGFR was −1.6 (−4.1 to 0.03) mL/min/1.73 m^2/year. We registered 24,776 eGFR determinations, corresponding to a median of 14 (7–26) determinations per person, and found a significant negative correlation between baseline concentrations of each biomarker and the annual eGFR slope ($r = -0.16$ for MR-proADM, $r = -0.19$ for sTNFR1, and $r = -0.19$ for NT-proBNP; all $P < 0.0001$).

Considering a mixed-effect model, a significant association was observed between annual eGFR slope and sex, age, and baseline HbA_{1c}, uACR, MR-proADM, sTNFR1, and NT-proBNP (coefficients are reported in Supplementary Table 2).

Discrimination

We assessed improvement in risk discrimination for each candidate biomarker compared with the model with traditional risk factors (model 3). The RFL risk prediction significantly improved when separate biomarkers or any of their combinations were included in the model (Table 3). Inclusion of all three biomarkers in the model yielded the highest discrimination (difference in C-statistic = 0.060, $P < 0.0001$; rIDI = 52.4%, $P < 0.0001$) and also a better overall fit in predicting RFL (smallest AIC).

For RRFD, we found that a combination of MR-proADM, sTNFR1, and NT-proBNP yielded the highest discrimination (difference in C-statistic = 0.068, $P < 0.0001$; rIDI = 70.2%, $P < 0.0001$) as well as the best fit (Table 3).

Supplementary Fig. 2 shows 5-year RFL and RRFD receiver operating characteristic curves for models with traditional risk factor alone and for models with traditional risk factor plus biomarkers.

The biomarker risk score yielded similar results for RFL (difference in C-statistic = 0.060, $P < 0.0001$; rIDI = 56.4%, $P < 0.0001$) and for RRFD (difference in C-statistic = 0.068, $P < 0.0001$; rIDI = 68.9%, $P < 0.0001$).

Sensitivity Analyses

Each biomarker was associated significantly with incident RFL in all subgroups tested, except for NT-proBNP in the subgroup of patients with SBP >140 mmHg, where it conferred a borderline nonsignificant increased risk (Supplementary Fig. 3). In the second sensitivity analysis, after exclusion of 216 participants with

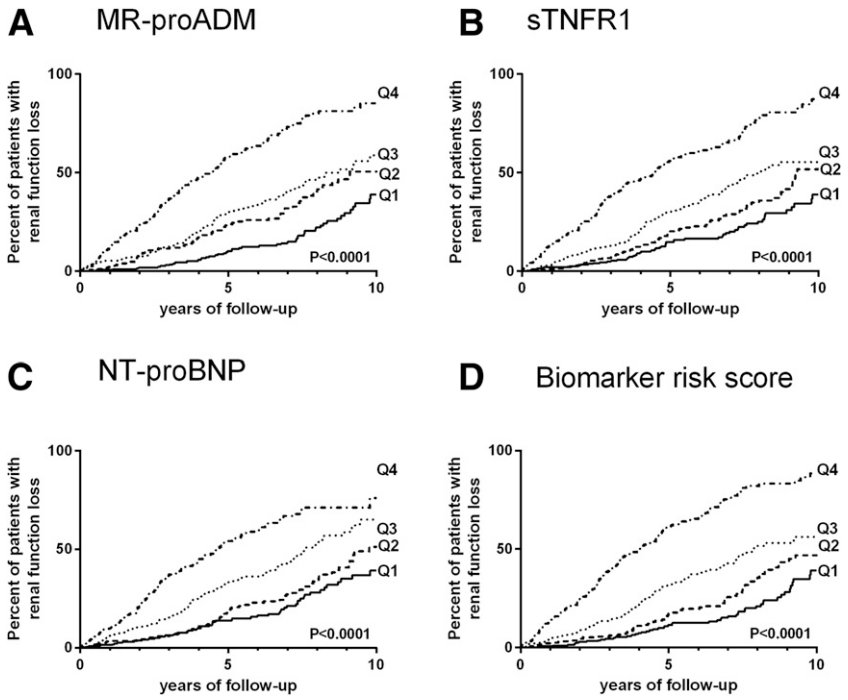

Figure 1—Cumulative percentage of RFL (≥40% GFR drop) according to biomarker quartiles (Q) 1–4, for MR-proADM (*A*), sTNFR1 (*B*), NT-proBNP (*C*), and biomarker risk score quartiles 1–4 (*D*). *P* values were calculated according to the log-rank test.

baseline cardiovascular history, results were unchanged as MR-proADM, sTNFR1, and NT-proBNP remained associated with RFL and RRFD risk (Supplementary Table 3). Accounting for incident cardiovascular disease did not modify the associations (Supplementary Table 4). In the subset 216 participants with baseline cardiovascular history, the three biomarkers together yielded the highest discrimination as well as the best overall fit in predicting RFL and RRFD (Supplementary Table 5). The time-dependent analysis of yearly mean HbA_{1c} values did not change our conclusions. After accounting for the competing risk of all-cause mortality in a Fine and Gray analysis (Table 2), MR-proADM, sTNFR1, and NT-proBNP remained independently associated with an increased risk of RFL.

CONCLUSIONS

In this study, we investigated three biomarkers in a hospital-based sample of 1,135 patients with type 2 diabetes with normal to mildly impaired renal function (eGFR >30 mL/min/1.73 m^2 and no history of RRT) who were monitored for up to 11.8 years. MR-proADM, sTNFR1, and NT-proBNP independently predicted renal outcomes, even when we excluded participants with a history of cardiovascular disease. We also showed that the combination of these three biomarkers improved the prediction of renal complications going beyond traditional risk factors.

MR-proADM (47 amino acids) is a surrogate of adrenomedullin, a short half-life peptide. MR-proADM peptide is formed in equimolar amounts to adrenomedullin during the cleavage of its precursor (31). Adrenomedullin is synthesized by many mammalian tissues, including kidney, adrenal medulla, cardiomyocytes, and endothelial and vascular smooth muscle cells (32). Adrenomedullin exerts pleiotropic actions, such as vasodilation, natriuresis/diuresis, tumor growth, and anti-inflammation (33), and also inhibits the proliferation of mesangial cells (34). Some epidemiological data support the association of adrenomedullin with CKD progression in individuals without diabetes (35) and with cardiorenal syndrome (36) in patients with type 2 diabetes. In the SURDIAGENE cohort, MR-proADM was associated with the risk of doubling of plasma creatinine concentration and/or progression to ESRD, as already shown by Velho et al. (6). That study considered two independent populations with type 2 diabetes: the DIABHYCAR (Non–Insulin-Dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril) patients and the CKD stage 1–4 SURDIAGENE patients (i.e., 149 additional subjects compared with the current study population).

TNFR1 is ubiquitously synthesized and participates in the TNF-α–signaling inflammatory pathway. Circulating sTNFR1 is

Table 2—Risk of RFL (≥40% GFR drop) and RRFD (<−5 mL/min/year) according to biomarkers in the SURDIAGENE patients

Variables	Model 1		Model 2		Model 3	
HR for RFL (≥40% GFR drop)*						
MR-proADM (per 1 SD)	2.05 (1.86–2.25)	<0.0001	2.01 (1.81–2.24)	<0.0001	2.06 (1.81–2.34)	<0.0001
sTNFR1 (per 1 SD)	1.97 (1.78–2.17)	<0.0001	1.81 (1.63–2.01)	<0.0001	1.78 (1.57–2.02)	<0.0001
NT-proBNP (per 1 SD)	1.90 (1.71–2.10)	<0.0001	1.73 (1.54–1.94)	<0.0001	1.52 (1.34–1.72)	<0.0001
Sub-HR of RFL (≥40% GFR drop)†						
MR-proADM (per 1 SD)	1.85 (1.68–2.05)	<0.0001	1.80 (1.6–2.02)	<0.0001	1.84 (1.6–2.12)	<0.0001
sTNFR1 (per 1 SD)	1.84 (1.65–2.04)	<0.0001	1.70 (1.52–1.91)	<0.0001	1.69 (1.47–1.95)	<0.0001
NT-proBNP (per 1 SD)	1.64 (1.47–1.81)	<0.0001	1.51 (1.34–1.71)	<0.0001	1.32 (1.15–1.52)	<0.0001
OR of RRFD (<−5 mL/min/year)‡						
MR-proADM (per 1 SD)	1.74 (1.50–2.02)	<0.0001	1.87 (1.58–2.22)	<0.0001	2.44 (1.94–3.07)	<0.0001
sTNFR1 (per 1 SD)	1.83 (1.57–2.13)	<0.0001	1.83 (1.55–2.15)	<0.0001	2.31 (1.86–2.87)	<0.0001
NT-proBNP (per 1 SD)	1.65 (1.43–1.92)	<0.0001	1.77 (1.48–2.11)	<0.0001	1.64 (1.36–1.99)	<0.0001

Ratios are presented with 95% CI and *P* value. Model 1: univariate. Model 2: age, sex, diabetes duration, SBP, and HbA_{1c}. Model 3: model 2 + eGFR + uACR. *Cox model; †Fine and Gray model (competing risk = all-cause death); ‡logistic model.

Table 3—C-statistics and rIDI using individual biomarkers or their combination for the prediction of RFL (≥40% GFR drop) and of RRFD (<−5 mL/min/year)

Variables	C-statistic with biomarker	Difference in C-statistic (95% CI)	Likelihood ratio *P* value	rIDI (95% CI)	*P* value
Dependent variable: RFL (≥40% GFR drop)					
MR-proADM	0.751	0.050 (0.031–0.069)	<0.0001	0.462 (0.234–0.745)	<0.0001
sTNFR1	0.739	0.038 (0.020–0.057)	<0.0001	0.331 (0.148–0.562)	<0.0001
NT-proBNP	0.731	0.029 (0.014–0.047)	<0.0001	0.159 (0.034–0.332)	0.001
MR-proADM + NT-proBNP	0.757	0.056 (0.036–0.077)	<0.0001	0.486 (0.249–0.774)	<0.0001
MR-proADM + sTNFR1	0.755	0.054 (0.035–0.073)	<0.0001	0.509 (0.200–0.693)	<0.0001
NT-proBNP + sTNFR1	0.753	0.051 (0.032–0.072)	<0.0001	0.428 (0.269–0.812)	<0.0001
MR-proADM + sTNFR1 + NT-proBNP	0.761	0.060 (0.040–0.081)	<0.0001	0.524 (0.281–0.838)	<0.0001
Dependent variable: RRFD (<−5 mL/min/year)					
MR-proADM	0.778	0.052 (0.027–0.078)	<0.0001	0.522 (0.232–0.954)	<0.0001
sTNFR1	0.780	0.053 (0.029–0.079)	<0.0001	0.529 (0.244–0.953)	<0.0001
NT-proBNP	0.755	0.028 (0.009–0.053)	<0.0001	0.186 (0.037–0.451)	0.0003
MR-proADM + NT-proBNP	0.781	0.055 (0.030–0.083)	<0.0001	0.548 (0.247–0.999)	<0.0001
MR-proADM + sTNFR1	0.791	0.065 (0.039–0.091)	<0.0001	0.669 (0.338–1.000)	<0.0001
NT-proBNP + sTNFR1	0.789	0.062 (0.036–0.092)	<0.0001	0.626 (0.295–1.000)	<0.0001
MR-proADM+ sTNFR1 + NT-proBNP	0.794	0.068 (0.042–0.097)	<0.0001	0.702 (0.349–1.000)	<0.0001

Reference model: age, sex, diabetes duration, SBP, HbA_{1c}, eGFR, and uACR. C-statistic reference = 0.702 for RFL and 0.726 for RRFD.

released by proteolytic sheddase-mediated cleavage of the membrane-anchored proteins or by alternative splicing of mRNA transcripts (37). TNFRs are then constitutively released in the circulation where they stabilize circulating TNF (38) or even modify its effect (39). Our results are in accordance with prior epidemiological work that shows circulating TNFR concentrations are robust prognostic factors for progression to advanced CKD or ESRD (11–16). Moreover, eGFR variations were associated with TNFR2 levels in women with type 2 diabetes (40). In the SURDIAGENE study, we found similar results for men and women and found no sex interaction. Pena et al. (41) recently showed that TNFR1 considered in a biomarker panel contributed to improved prediction of eGFR decline in patients with type 2 diabetes.

BNP and its precursors are secreted from myocytes as a reaction to myocytes stretching. BNP and its complementary inactive peptide NT-proBNP (76 amino acids) are secreted in equimolar amounts. BNP has a multitude of actions, including relaxation of vascular smooth muscle cells, natriuresis and diuresis, direct antagonism on the renin-angiotensin-aldosterone system, and lowering of plasma glucose concentrations (42). NT-proBNP is associated with diagnosis and prognosis of chronic heart failure and left ventricular hypertrophy and dysfunction. High plasma concentrations of NT-proBNP are secondary not only to increased myocardial production and myocardial stress but also to impaired kidney function (43). In addition, our findings are consistent with previous reports showing the association of NT-proBNP with progression of CKD in the general population (21) or impaired GFR (44). Finally, we confirmed the renal prognostic value of elevated NT-proBNP for ESRD found in the post hoc analysis of patients with type 2 diabetes selected for a placebo-controlled trial of darbepoetin alfa for the treatment of anemia (22).

Together, our findings support the hypothesis that increased MR-proADM, sTNFR1, and NT-proBNP are independently associated with renal function decline and that they each enhance prediction beyond traditional risk factors. The relationship between these biomarkers and RFL was consistent across the subgroups we explored, including those without cardiovascular disease at baseline. Nevertheless, control of traditional risk factors remains important irrespective of the levels of the new biomarkers.

Physiopathology

The MR-proADM, TNFR, and NT-proBNP pathways are not completely independent of each other. First, there is some evidence linking low-grade inflammation and endothelial dysfunction. MR-proADM actions include vasodilatation, natriuresis, inhibition of ACTH release, and delay of insulin secretion (45). TNF-α can induce adrenomedullin secretion by vascular smooth muscle cells (46). In a mouse model of type 2 diabetes ($Lepr^{db}$), increased TNF expression induced endothelial dysfunction by overproduction of reactive oxidative species (47), and in patients with chronic heart failure, anti-TNF therapies improve systemic endothelial vasodilator capacity (48).

Chronic heart failure is a medical condition in which NT-proBNP and MR-proADM concentrations are elevated. Chronic heart failure could lead to renal impairment not only through chronic activation of the renin angiotensin system, sympathetic activation, and an increase of inflammation and oxidative stress but also through impairment of vascular endothelium (49).

Interestingly, MR-proADM, sTNFR1, and NT-proBNP concentrations are significantly intercorrelated in our data set. That they remained independent predictors of renal complications suggests that the deleterious renal effect of one biomarker could express itself directly on the kidney or might be mediated by more sophisticated interactions between these biological pathways.

Study Strengths and Limitations

Our study had a number of potential limitations. French patients from the SURDIAGENE study were consecutively recruited in a hospital-based single-center cohort, and this design may have caused selection bias. Because our cohort is not population-based, it might not be fully representative of the population of France with type 2 diabetes. Moreover, renal function was estimated

with the CKD-EPI equation rather than by being measured. This study was not designed to assess repeated uACR measurement, and exploration of the association of biomarkers with renal function decline in the absence of albuminuria was therefore beyond our scope. Our study also has a number of strengths, including the large size of the cohort, the standardized procedures for sample collection, processing, and storage, and the storage at −80°C. Furthermore, the results of the study were consistent across several different definitions of declining renal function.

Assays for these biomarkers are commercially available and have good analytical performance. NT-proBNP is currently in routine use for the diagnosis of congestive heart failure. From a clinical perspective, we documented that these biomarkers improve renal risk prediction in addition to traditional risk factors, including eGFR and albuminuria. In addition, the biomarker risk score provides a simple and practical tool to improve the predictive ability of these markers for renal function decline. Nevertheless external validation and cost-benefit studies are needed.

Conclusion

This prospective cohort study found that beyond traditional risk factors, an increased circulating level of MR-proADM, sTNFR1, and NT-proBNP improves risk prediction of renal function alterations in a population with type 2 diabetes. Their value in clinical practice remains to be determined.

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