# Association of Serum Concentration of TNFR1 With All-Cause Mortality in Patients With Type 2 Diabetes and Chronic Kidney Disease: Follow-up of the SURDIAGENE Cohort

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*Pierre-Jean Saulnier,[1,2,3] Elise Gand,[4] Stéphanie Ragot,[1,2,3,5] Grégory Ducrocq,[6,7,8] Jean-Michel Halimi,[9,10] Charlotte Hulin-Delmotte,[11,12] Pierre Llaty,[11,12] David Montaigne,[13,14] Vincent Rigalleau,[15,16] Ronan Roussel,[17,18,19,20] Gilberto Velho,[21,22] Philippe Sosner,[11,12] Philippe Zaoui,[21,22] and Samy Hadjadj,[1,2,3,5,23] for the SURDIAGENE Study Group*

[1]*Université de Poitiers, UFR Médecine Pharmacie, Centre d'Investigation Clinique, Poitiers, France*
[2]*CHU de Poitiers, Centre d'Investigation Clinique, Poitiers, France*
[3]*INSERM, Centre d'Investigation Clinique 1402, Poitiers, France*
[4]*CHU de Poitiers, Pole DUNE, Poitiers, France*
[5]*Université de Poitiers, UFR Médecine Pharmacie, Poitiers, France*
[6]*INSERM U698, Paris, France*
[7]*UFR Medecine, Université Paris 7 Denis Diderot, Paris, France*
[8]*Département de Cardiologie, Groupe Hospitalier Bichat Claude Bernard, Assistance Public-Hôpitaux de Paris (AP-HP), Paris, France*
[9]*CHU de Tours, Service Néphrologie, Dialyse et Transplantation, Tours, France*
[10]*INSERM, Centre d'Investigation Clinique 0202, Tours, France*
[11]*CHU de Poitiers, Service de Cardiologie, Poitiers, France*
[12]*Université de Poitiers, Laboratoire MOVE (EA 6314), Poitiers, France*
[13]*Université de Lille 2, EA 4484, Faculté de Médecine, Lille, France*
[14]*CHRU Lille, Hôpital Cardiologique, Lille, France*
[15]*CHU de Bordeaux, Service Endocrinologie, Diabétologie Maladies Métaboliques et Nutrition, Bordeaux, France*
[16]*Université Victor Segalen, Faculté de Médecine, Bordeaux, France*
[17]*UFR Medecine, Université Paris-Diderot, Sorbonne Paris-Cité, France*
[18]*Diabetologie, AP-HP, Hôpital Bichat, Paris, France*
[19]*Département Hospitalo-Universitaire FIRE, Paris, France*
[20]*INSERM UMR-S 1138, Paris, France*
[21]*CHU de Grenoble, Service Néphrologie, Dialyse et Transplantation, Grenoble, France*
[22]*Université Joseph Fournier, Faculté de Médecine, Grenoble, France*
[23]*CHU de Poitiers, Service d'Endocrinologie, Poitiers, France*

*Corresponding author: Samy Hadjadj, samy.hadjadj@gmail.com.*

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## OBJECTIVE

**Renal dysfunction is a key risk factor for all-cause mortality in patients with type 2 diabetes (T2D). Circulating tumor necrosis factor receptor 1 (TNFR1) was recently suggested as a strong biomarker for end-stage renal failure in T2D. However, its relevance regarding all-cause death has yet to be conclusively established. We aimed to assess the prognostic value of serum TNFR1 concentration for all-cause death in T2D and diabetic kidney disease (DKD) from the SURDIAGENE (Survie, Diabete de type 2 et Genetique) study.**

## RESEARCH DESIGN AND METHODS

**A total of 522 T2D patients with DKD (estimated glomerular filtration rate [eGFR] <60 and/or urinary albumin-to-creatinine ratio [uACR] >30 mg/mmol) were followed for a median duration of 48 months, and 196 deaths occurred.**

## RESULTS

**Incidence rate (95% CI) for death increased as quartiles of TNFR1 concentration increased (first quartile: 4.7% patient-years [3.0–6.3%]; second quartile: 7.7% [5.4–10.0%]; third quartile: 9.3% [6.7–11.9%]; fourth quartile: 15.9% [12.2–19.5%]). In multivariate analysis taking age, diabetes duration, HbA1c, uACR, and eGFR into account, compared with the first quartile, patients from the fourth quartile had an adjusted hazard ratio for death of 2.98 (95% CI 1.70–5.23). The integrated discrimination improvement index was statistically significant when adding TNFR1 concentration to the UK Prospective Diabetes Study outcome equation ($P$ = 0.031).**

## CONCLUSIONS

**TNFR1 is a strong prognostic factor for all-cause mortality in T2D with renal dysfunction, and its clinical utility is suggested in addition to established risk factors for all-cause mortality.**

Individuals with diabetes mellitus represent a large and growing population at increased risk of cardiovascular events and mortality. Recent meta-analyses have confirmed that diabetes approximately doubles the risk of mortality and a wide range of vascular diseases compared with individuals without diabetes (1). As type 2 diabetes (T2D) incidence continues to increase, individuals with diabetes are likely to become an increasingly sizable component of the overall burden of cardiovascular disease (CVD). While the relationship between reduced life expectancy and diabetes is surely multifactorial, diabetic kidney disease (DKD) is particularly pertinent; indeed, compared with nondiabetic individuals, patients with type 1 diabetes have no excess mortality in absence of DKD (2), and the role of chronic kidney disease (CKD) is demonstrably critical in type 2 diabetes (3).

Besides traditional risk factors, such as poor blood glucose or blood pressure control (4), the role of chronic low-grade inflammation is becoming more recognized (5). The tumor necrosis factor (TNF)-$\alpha$ pathway and chronic low-grade inflammation are closely involved in the pathogenesis of diabetes-associated complications (6). In this context, circulating TNF receptors were shown to be associated with diabetic nephropathy in patients with type 1 diabetes (7,8) and T2D (9). However, their relevance for all-cause mortality remains poorly established. Up until now, only one article has evidenced the relationship between TNF receptor 1 (TNFR1) and all-cause mortality in T2D patients, but it did not clearly report the beneficial effect of the TNFR1 determination in addition to established risk factors (10).

To study whether TNF1R predicts mortality, we searched for an association between circulating TNFR1 and all-cause mortality in the context of DKD, using a follow-up study of a single-center cohort of T2D patients (Survie, Diabete de type 2 et Genetique [SURDIAGENE]). Our purpose was to establish the additional predictive value of this biomarker for all-cause mortality in our cohort, on top of established risk factors using the UK Prospective Diabetes Study (UKPDS) outcome equation for mortality.

## RESEARCH DESIGN AND METHODS

### Study Patients

The SURDIAGENE study is based on an inception prospective monocentric cohort and aims to identify the genetic and environmental determinants of microvascular and macrovascular complications in type 2 diabetes (11). Patients with T2D were recruited and followed regularly at the University Hospital of Poitiers, France, between 2002 and 2011. The main exclusion criteria were residence outside the Poitiers region and evidence of nondiabetic renal disease.

At baseline, all patients were examined to collect clinical and biological data. Diabetic retinopathy was staged as nil, background, severe nonproliferative, and proliferative according to retinal photograph and/or classification by a trained ophthalmologist. A history of CVD at baseline was defined as a personal history of myocardial infarction and/or stroke. Our current analysis is restricted to patients with DKD at baseline presenting with proteinuria as defined by a urinary albumin-to-creatinine ratio (uACR) $>$30 mg/mmol and/or an estimated glomerular filtration rate (eGFR) $<$60 mL/min/1.73 $m^2$.

The design was approved by the Poitiers University Hospital Ethic Committee (CPP Ouest 3). All participants in the study gave their written informed consent.

### Outcome Criteria

Living status and cardiovascular and renal end points were determined from patients' hospital records and interviews with their general practitioners every second year since 2007. This analysis takes into account data updated for the fourth quarter of 2011.

The primary end point was time from inclusion to occurrence of all-cause mortality. The cardiovascular cause was specifically reviewed by the adjudication committee. The secondary end point was time to occurrence of to a composite renal outcome in those patients without end-stage renal disease (ESRD) at baseline: development of ESRD requiring renal replacement therapy or sustained (over 1 month) doubling of baseline serum creatinine. Patients moving out of the Poitou-Charentes region were censored at the time of their departure.

We compared the observed risk for all-cause mortality in our cohort with the risk predicted by the latest version of the UKPDS outcome equation (12) using the following variables: age, diabetes duration, sex, ethnicity, current smoking status, systolic blood pressure, $HbA_{1c}$, BMI, eGFR, heart rate, atrial fibrillation, albuminuria, and peripheral vascular disease. Two variables (lipids [HDL cholesterol and LDL cholesterol] and hematology [hemoglobin and white blood cell {WBC} count]) were not entered into the model because they were missing for approximately half of the study population.

### Adjudication Procedure

Each end point was centrally reviewed by two independent physicians and in case of disagreement it was discussed by the entire committee. The hospitalization record or all other relevant supporting documents were used to adjudicate clinical outcomes. CVD was defined according to the International Classification of Diseases Tenth Revision (codes I00–I99; http://apps.who.int/classifications/apps/icd/icd10online/).

### Biobanking: Biological Determinations

Biological resources were processed immediately after collection according to standardized procedures and stored at $-80^{\circ}$C until use. Urinary creatinine was measured using a Hitachi 911 automatic analyzer (Roche Diagnostics, Meylan, France). Serum creatinine and urinary albumin were measured by nephelometry on a Modular System P (Roche Diagnostics GmbH), uACR and renal function with eGFR were determined using the CKD Epidemiology Collaboration formula (13).

Glycated hemoglobin ($HbA_{1c}$) (International Federation of Clinical Chemistry and Laboratory Medicine normal values 4.0–6.0% [20–42 mmol/mol]) was determined using a high-performance liquid chromatography method with an ADAMS A1C HA-8160 analyzer (Menarini, Florence, Italy).

Serum TNFR1 concentrations were measured using human sTNFR1 ELISA (product #BIO94TNFR1; EKF Diagnostics, Dublin, Ireland) according to the manufacturer's instructions. All serum samples were assayed in duplicate, and the mean of the two determinations was considered for statistical analysis. Intra- and interassay coefficients of variation were 1.9% (range 0–11%) and

4.1% (range 2.7–5.3%), respectively. Reference control samples covering the standard curve range were recovered for an average of 99% across all assays ($n$ = 17).

### Statistical Analysis

All statistical analyses were carried out using Statview version 5.0 and SAS version 9.3 software packages (SAS Inc., Cary, NC). Continuous variables are given as mean ± SD or median (interquartile range) if the distribution of the variable was skewed. Categorical variables are given as the number of patients with the respective attribute and the corresponding percentage. Comparisons were conducted using the $t$ test or ANOVA for normally distributed continuous variables or the Mann-Whitney $U$ or Kruskal-Wallis tests for nonnormally distributed variables. The Spearman rank correlation test was used to test the correlation between continuous variables. Comparisons of categorical variables were performed with Pearson $\chi^2$ tests.

To study the relationship between serum TNFR1 concentrations and clinical and biological variables or clinical outcomes, we considered the quartiles of the distribution of the biomarker in our cohort.

We plotted Kaplan-Meier survival curves and compared them with the log-rank test. We used the Cox proportional hazard model for univariate and multivariate analyses. Variables associated with the outcome at $P < 0.05$ on the basis of the univariate models were introduced in the multivariate models. We used eGFR, but not serum creatinine, when considering multivariate models.

We tested for nonlinear associations using cubic splines for TNFR1 and adjusted risk of all-cause mortality, and in a sensitivity analysis we excluded subjects with ESRD at baseline.

To assess the improvement in Cox model performance accomplished by adding TNFR1, we used the integrated discrimination improvement (IDI) index on top of covariates from the UKPDS outcome model for all-cause mortality. A $P$ value $<0.05$ was considered statistically significant.

## RESULTS

### Baseline Characteristics

The SURDIAGENE cohort is made up of 1,470 T2D subjects. A total of 526 subjects fulfilled the selection criteria for the current study, of whom 522 had available serum samples.

The clinical and biological characteristics are summarized in Table 1 according to quartiles of TNFR1 concentrations. Interestingly, those in the fourth quartile,

**Table 1—Baseline clinical and biological characteristics according to quartile distribution of serum TNFR1 concentration**

| Characteristics | All ($n$ = 522) | Quartile 1 ($n$ = 130) | Quartile 2 ($n$ = 130) | Quartile 3 ($n$ = 131) | Quartile 4 ($n$ = 131) | $P$ value |
|---|---|---|---|---|---|---|
| Serum TNFR1 concentration (pg/mL)* | 760–35,177 | 760–1,912 | 1,914–2,508 | 2,524–3,450 | 3,464–35,177 | — |
| Sex: men/women | | | | | | 0.3355 |
| Male | 299 (57) | 66 (51) | 79 (61) | 79 (60) | 75 (57) | |
| Female | 223 (43) | 64 (49) | 51 (39) | 52 (40) | 56 (43) | |
| Age (years) | 70.4 ± 9.4 | 68.4 ± 10.9 | 71.1 ± 8.8 | 71.7 ± 8.6 | 70.4 ± 8.9 | **0.0325** |
| BMI (kg/m$^2$) | 30.5 ± 6.2 | 29.4 ± 5.9 | 30.7 ± 5.3 | 30.8 ± 6.0 | 31.1 ± 7.3 | 0.1083 |
| Active smoking | 38 (7) | 18 (14) | 9 (7) | 7 (5) | 4 (3) | **0.0066** |
| Diabetes duration (years) | 18.3 ± 10.6 | 16.3 ± 10.6 | 18.5 ± 9.8 | 19.6 ± 11.4 | 18.8 ± 10.1 | 0.0642 |
| $HbA_{1c}$, % (mmol/mol) | 7.7 ± 1.5 (61 ± 16.4) | 7.8 ± 1.6 (62 ± 17.5) | 7.8 ± 1.4 (62 ± 15.3) | 7.8 ± 1.6 (62 ± 17.5) | 7.3 ± 1.4 (56 ± 15.3) | **0.0080** |
| Serum creatinine (μmol/L) | 119 (62) | 90 (36) | 109 (40) | 123 (37) | 211 (172) | **<0.0001** |
| eGFR (mL/min/1.73 m$^2$) | 48.9 ± 22.7 | 68.0 ± 20.5 | 54.0 ± 14.9 | 46.8 ± 14.2 | 26.8 ± 18.5 | **<0.0001** |
| uACR (mg/mmol) | 28.5 (111.0) | 19.8 (55.6) | 13.0 (60.1) | 17.4 (97.7) | 163.1 (316.7) | **<0.0001** |
| History of cardiovascular disease† | 142 (27) | 28 (22) | 37 (28) | 41 (31) | 36 (27) | 0.3643 |
| ESRD | 22 (4) | 0 (0) | 0 (0) | 3 (2) | 19 (15) | **<0.0001** |
| Diabetic retinopathy stage‡ | | | | | | **0.0044** |
| Nil | 214 (42) | 67 (54) | 55 (44) | 50 (39) | 42 (34) | |
| Background | 165 (33) | 33 (26) | 43 (34) | 51 (40) | 38 (30) | |
| Severe nonproliferative | 61 (12) | 15 (12) | 9 (7) | 17 (13) | 20 (16) | |
| Proliferative | 65 (13) | 10 (8) | 19 (15) | 11 (9) | 25 (20) | |
| Systolic blood pressure (mmHg) | 137 ± 20 | 139 ± 20 | 135 ± 19 | 137 ± 21 | 139 ± 21 | 0.2603 |
| Diastolic blood pressure (mmHg) | 72 ± 12 | 73 ± 13 | 71 ± 12 | 72 ± 12 | 72 ± 11 | 0.8490 |
| Total cholesterol (mmol/L) | 4.93 ± 1.29 | 5.01 ± 1.21 | 4.88 ± 1.29 | 4.91 ± 1.14 | 4.93 ± 1.51 | 0.8740 |
| Resting heart rate (beats per minute) | 73 ± 13 | 73 ± 14 | 72 ± 11 | 73 ± 14 | 74 ± 14 | 0.5890 |
| ACE-I or ARB use | 383 (73) | 96 (74) | 103 (79) | 101 (77) | 83 (63) | **0.0190** |
| Statin use | 238 (46) | 58 (45) | 56 (43) | 65 (50) | 59 (45) | 0.7401 |

Quantitative variables are described by mean ± SD or median (interquartile range). Otherwise data are $n$ (%). Boldface data indicate $P$ values below the statistical significance threshold. ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker. *Data are minimum–maximum. †Cardiovascular disease defined by a personal history of myocardial infarction and/or stroke. ‡Missing data at baseline for 17 patients.

with the highest TNFR1 concentration, had a significantly lower eGFR value and lower $HbA_{1c}$.

In addition, TNFR1 expressed as a quantitative variable was independently associated with age, BMI, $HbA_{1c}$, eGFR, uACR, and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker use (data not shown). WBC was available for only 226 patients (no clinical and biological difference between those with or without WBC data) and was not correlated with serum TNFR1 concentration (Rho = 0.08; $P$ = 0.206). The mean duration of follow-up was 51 months.

### Serum TNFR1 Concentration and Renal Events

Patients with ESRD at baseline ($n$ = 22) were not taken into account for renal outcomes. During follow-up, a composite renal outcome (ESRD and/or sustained doubling of serum creatinine) occurred in 69 patients (incidence rate 31.2 per 1,000 person-years [95% CI 24.0–38.5]), 39 of which had ESRD (incidence rate 17.7 per 1,000 person-years [95% CI 12.2–23.1]).

When our composite renal outcomes were considered, there was a clear difference between patients in the fourth quartile compared with those in the three others (Table 2). The risk of renal event was obviously greater in those people from the fourth quartile compared with those in the first to third quartiles, even after multiple adjustments (data not shown).

### Serum TNFR1 Concentration and Mortality

During follow-up (corresponding to 2,209 person-years), 196 deaths occurred. The overall mortality rate was 89 per 1,000 person-years (95% CI 76–101), including 129 cardiovascular deaths (58 per 1,000 person-years [95% CI 48–68]) (Table 2); 25 deaths were due to cancer and 42 deaths were from other causes.

The effect of TNFR1 on all-cause mortality is depicted using Kaplan-Meier survival curves according to quartiles of serum TNFR1 concentrations (Fig. 1). Patients in the fourth quartile had an increased risk for death, and approximately half of the patients from the TNFR1 fourth quartile were dead after 4.5 years of follow-up.

In a univariate Cox model analysis, all-cause mortality was significantly associated with TNFR1 concentration, diabetes duration, age, $HbA_{1c}$, and renal function parameters (eGFR and uACR) (Table 3). In a multivariate Cox model analysis, TNFR1 remained significantly associated with the risk of all-cause mortality (for one quartile increase of TNFR: hazard ratio [HR] 1.40 [95% CI 1.11–1.67]) and cardiovascular death (for one quartile increase of TNFR: HR 1.38 [95% CI 1.11–1.73]) independent of other factors. Comparing the fourth quartile to the first three ones, the risk for death was clearly increased (HR 1.62 [95% CI 1.06–2.46]; $P$ = 0.024). As expected, age was the main risk factor for this outcome. Interestingly, the lower the $HbA_{1c}$, the greater the mortality risk (Supplementary Table 1).

A restricted multivariable cubic spline plot for TNFR1 and adjusted risk of overall mortality is presented in Supplementary Fig. 1*A* and *B*. It shows a linear increase of risk for patients with TNRF1 between 500 to 5,000 pg/mL, whereas risk reached a plateau for patients with TNFR1 >5,000 pg/mL (most of them have stage 4 or 5 CKD).

We considered the UKPDS outcome equation for death to evaluate the clinical relevance of TNFR1 concentration in addition to established risk factors. The predicted risk for all-cause mortality according to the UKPDS outcome equation and the observed outcomes are plotted in Supplementary Fig. 2. Considering the IDI strategy, we clearly observed a significant improvement in mortality risk (IDI 0.00363; $P$ = 0.03).

## CONCLUSIONS

Our data showed a clear and graded relationship between concentrations of serum TNFR1 and risk of all-cause mortality. In addition, the risk of renal events (sustained doubling of serum creatinine or ESRD) had significantly and independently increased in our cohort of T2D patients with DKD. The relationship between TNFR1 and mortality was independent of known risk factors such as age, diabetes duration, blood glucose or blood pressure control, and eGFR. Serum concentration of TNFR1 added prognostic information for the risk of death to the UKPDS outcome equation.

Our results are in accordance with recently published data showing a deleterious effect of TNFR1 serum concentrations for renal outcomes in type 1 diabetes and T2D (7,9). Interestingly, the relationship between TNFR1 was positive for ESRD as well as when considering ESRD and/or sustained doubling of serum creatinine. Our findings not only confirm

**Table 2—Incidence rates of clinical outcomes according to quartiles of serum TNFR1 concentrations**

| Variables | All-cause mortality | Cardiovascular mortality | ESRD | ESRD or sustained doubling of creatinine |
|---|---|---|---|---|
| TNFR1 quartile 1 | | | | |
| Events per person-years ($n$) | 31/662.2 | 19/662.2 | 0/662.2 | 7/649.1 |
| Incidence rate (per 1,000 person-years) | 46.8 (30–63) | 28.7 (16–42) | 0.0 (0–0) | 10.8 (3–19) |
| TNFR1 quartile 2 | | | | |
| Events per person-years ($n$) | 43/560.9 | 28/560.9 | 2/554.8 | 7/546.4 |
| Incidence rate (per 1,000 person-years) | 76.7 (54–100) | 49.9 (31–68) | 3.6 (−1 to 9) | 12.8 (3–22) |
| TNFR1 quartile 3 | | | | |
| Events per person-years ($n$) | 49/526 | 31/526 | 5/513.6 | 14/494.2 |
| Incidence rate (per 1,000 person-years) | 93.2 (67–119) | 58.9 (38–80) | 9.7 (1–18) | 28.3 (13–43) |
| TNFR1 quartile 4 | | | | |
| Events per person-years ($n$) | 73/460 | 51/460 | 32/360.1 | 41/347 |
| Incidence rate (per 1,000 person-years) | 158.7 (122–195) | 110.9 (80–141) | 88.8 (58–120) | 118.1 (82–154) |

TNFR1 concentration limits in each quartile are presented in Table 1. Incidence rate of ESRD and ESRD or sustained doubling of serum creatinine was computed on a total of 500 (patients with ESRD at baseline were removed from the calculation).

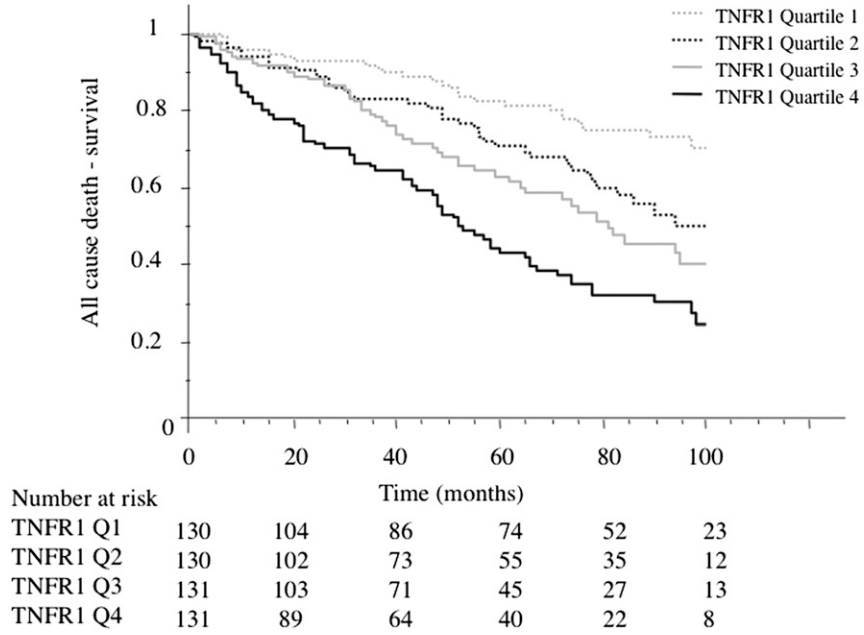


**Figure 1**—Kaplan-Meier survival curves according to serum TNFR1 quartile: first quartile, serum TNFR1 concentration <1,914 pg/mL (gray dotted line); second quartile, 1,914–2,508 pg/mL (black dotted line); third quartile, 2,524–3,450 pg/mL (gray solid line); fourth quartile, ≥3,464 pg/mL (black solid line). Log-rank, 42.796; $P < 0.001$.

the deleterious role of TNFR1 on kidney function but also point to a clear association with all-cause mortality. The effect of TNFR1 concentration on mortality in T2D was recently suggested (10). However, our results are based on much larger numbers of deaths, leading to an adequate statistical power. In addition, we found that in our cohort, risk of death was much greater than risk of developing ESRD, a finding at variance with the data of the Joslin 2 study, in which the two risks were roughly similar (9). Indeed, the particularly elevated risk of death was in good agreement with results from the UKPDS (14), showing that altered renal function in T2D patients was more strongly associated with mortality risk than with ESRD risk. Since TNFR1 was prognostic for both severe renal events and death, and because it remained significantly associated with mortality even after adjusting for renal function, it is unlikely to initially increase in response to decreased renal function and only subsequently lead to death. Interestingly, the number of deaths here was twice those reported in the study from the Joslin Diabetes Center by Lee et al. (10), allowing us to be rather conclusive and to assess the prognostic relevance of this biomarker compared with some existing models.

**Table 3—Association of baseline covariates with all-cause mortality in a univariate Cox model analysis**

| Variables | Hazard ratio | 95% CI | *P* value |
|---|---|---|---|
| Sex (reference: men) | 0.82 | (0.62–1.10) | 0.1816 |
| Age (years) | 1.05 | (1.03–1.06) | **<0.0001** |
| BMI (kg/m$^2$) | 1.00 | (0.98–1.03) | 0.7353 |
| Active smoker | 1.29 | (0.79–2.13) | 0.3142 |
| Diabetes duration (years) | 1.02 | (1.00–1.03) | **0.0104** |
| $HbA_{1c}$* | 0.87 | (0.79–0.96) | **0.0076** |
| $Log_{10}$ serum creatinine (μM/L) | 2.77 | (1.58–4.87) | **0.0004** |
| eGFR (mL/min/1.73 m$^2$) | 0.99 | (0.98–1.00) | **0.0007** |
| $Log_{10}$ uACR | 1.47 | (1.24–1.73) | **<0.0001** |
| History of cardiovascular disease | 1.25 | (0.91–1.70) | 0.1543 |
| ESRD (reference: absence of ESRD) | 1.75 | (0.97–3.13) | 0.0619 |
| Systolic blood pressure (mmHg) | 1.00 | (0.99–1.01) | 0.9557 |
| Diastolic blood pressure (mmHg) | 0.99 | (0.98–1.00) | 0.1592 |
| Total cholesterol (mmol/L) | 1.01 | (0.90–1.13) | 0.9331 |
| Resting heart rate (beats per minute) | 1.01 | (1.00–1.02) | 0.1758 |
| ACE-I or ARB use | 0.97 | (0.71–1.33) | 0.8663 |
| Statin use | 1.00 | (0.75–1.34) | 0.9846 |
| TNFR (per increment of 100 pg/mL) | 1.007 | (1.004–1.01) | **<0.0001** |
| TNFR quartile (reference: Q1) | | | **<0.0001** |
| Q2 | 1.69 | (1.06–2.68) | |
| Q3 | 2.10 | (1.34–3.29) | |
| Q4 | 3.57 | (2.34–5.45) | |

Boldface data indicate *P* values below the statistical significance threshold. *By 1% increase; an increase of $HbA_{1c}$ of 1% corresponds to 10.9 mmol/mol. ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker.

There is also a growing body of evidence of the epidemiological relationship between inflammation and CVD. Epidemiological investigations of the chances of preventing, recognizing early and optimally treating chronic diseases in an elderly population (ESTHER study)

recently showed that low-grade inflammation, represented by proinflammatory cytokines (migration inhibitory factor and adiponectin), was associated with cardiovascular events in diabetic patients with renal dysfunction, whereas no clear effect was evidenced in patients with normal renal function (15). In the Heart Outcomes Prevention Evaluation (HOPE) study, proinflammatory markers were identified as prognostic for cardiovascular outcomes (16). Our current findings add to this literature, showing that the prognostic role of TNFR1 goes beyond established risk factors such as those highlighted in the UKPDS outcome equations. The analysis of specific diabetes-related events such as amputation could help to address the question of a role of TNFR1 in vasculopathy. Such analyses, however, are beyond the scope of this article.

We found that the lower the $HbA_{1c}$, the greater the mortality risk. This point could be considered a counterintuitive finding when considering blood glucose control intervention studies. However, based on the data from Currie et al. (17), who performed an observational study of a large British database, such results also were found, with a U-shaped association between $HbA_{1c}$ and all-cause mortality. Interestingly, in that article, the prevalence of patients with serum creatinine $>130$ μmol/L was higher than in those with $HbA_{1c}$ in the first three deciles compared with the others, in line with our findings of a low $HbA_{1c}$ in patients with CKD being associated with higher mortality risk.

Some caveats must be made regarding our findings. Indeed, the population considered here is rather old (average age 70.4 years), making it mandatory to replicate our findings before any generalization of them. It is also important to note, however, that this biomarker was relevant in such a population, among whom not only age drives the prognosis.

One key question is the mechanistic relationship between TNFR1 and renal and mortality outcomes. It has yet to be clearly established whether high concentrations of soluble TNFR1 act to protect cells from TNF-α-driven inflammation or whether they reflect a deleterious mechanism per se. In addition, more precise knowledge of soluble TNFR1 could help to predict more accurately the result of targeting this system to improve prognosis in T2D. Although some speculations on the interrelated effects of TNFR1 on kidney structure and renal outcome have already been put forward (18), our present data suggest a more generalized mode of action that leads to death.

The biology of the soluble form of TNFR1 is largely unknown. Some data seem to suggest that the majority of soluble TNFR1 is excreted in exosomes rather than as the product of a cleavage of the extracellular portion of the receptor (19). In addition, the exosomic form is not capable of intrinsic cell signaling, thereby making it a possible decoy for TNF-α (19). A previous in vitro functional study suggested that TNFR1 at high concentrations could reduce the effect of TNF-α (20). Whether one form or the other of TNFR1 is associated with renal or mortality outcome is beyond the scope of this article, but the development of a biological assay that is able to distinguish between these forms could help address this issue.

Some limitations to our study should be acknowledged. The question of recruitment is crucial in our population. We recruited an inception hospital-based population, possibly leading to the recruitment of patients with a higher risk for mortality. We strongly believe that we have effectively limited recruitment bias, since the risk factors associated in our study with all-cause mortality, such as age, renal function, or systolic blood pressure, are well established. Moreover, our data have been shown to be in good agreement with the UKPDS outcome equation.

Although serum concentrations of TNFR1 were determined using the same kit, they were much higher in the subgroup of SURDIAGENE patients with DKD than in patients recruited to the Joslin 2 study (9). Since we did not perform any direct comparison between patients included in the two studies, the sizable difference can be explained by the following factors: age (SURDIAGENE patients were obviously older), geographical/ethnic origin, therapeutic (SURDIAGENE patients were more often treated with renin angiotensin system blockers), or severity of DKD (more severe in SURDIAGENE patients). However, even when focusing on patients without stage 5 DKD at baseline, we still found high TNFR1 concentrations.

Finally, we could not include WBC count or LDL-cholesterol/HDL-cholesterol in the UKPDS outcome equation because this information was missing for a substantial proportion of our study population. The lack may be critical insofar as WBC count could constitute a surrogate marker for inflammation. Of note, TNFR1 and WBC count were not correlated. In addition, in our sensitivity analysis, the addition of WBC count to TNFR1 in a bivariate analysis did not substantially modify the HR between TNFR1 and all-cause mortality.

Altogether, our results suggest that TNFR1 could be a major risk factor for all-cause mortality. Nonetheless, its clinical utility remains to be established. It can be considered in different ways: either as a sole prognostic factor leading to improved risk recognition and that has proven interesting as a supplement to the covariates used in the UKPDS outcome equation or as an important factor associated with personalized medicine and leading to specific interventions, some of which may target this pathway. Only future trials will allow this point to be conclusively established.

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**Author Contributions.** P.-J.S. researched data, wrote the manuscript, and contributed

to the discussion. E.G., S.R., and G.V. performed statistical analysis, edited the manuscript, and contributed to the discussion. G.D., J.-M.H., C.H.-D., P.L., D.M., V.R., R.R., and P.Z. edited the manuscript and contributed to the discussion. P.S. researched data, edited the manuscript, and contributed to the discussion. S.H. designed the study, researched data, wrote the manuscript, and contributed to the discussion. S.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## Appendix

**Center Organization.** Recruiting physicians: Samy Hadjadj (Coordinator), Frédérique Duengler, Louis Labbé, Aurélie Miot, Xavier Piguel, Florence Torremocha, Pierre-Jean Saulnier, and Richard Maréchaud.

**Baseline Data Case Review.** All patients' records were reviewed to ascertain the following points: type 2 diabetes, diabetic kidney disease, diabetic retinopathy, and cardiovascular disease. The clinicians involved in this process are warmly thanked: Daniel Herpin and Philippe Sosner (Cardiology), Frank Bridoux (Nephrology), Helene Manic (Ophthalmology), and Samy Hadjadj (Diabetology).

**Adjudication Procedure.** Case Inquiry: Samy Hadjadj (Coordinator), Sonia Brishoual, Céline Divoy, Cécile Demer, Aurélie Miot, Xavier Piguel, Florence Torremocha, Nathalie Fauvergue, Séverin Carasson, Pierre-Jean Saulnier, and Philippe Sosner. Local Coordination: Stéphanie Ragot (coordinator & biostatistician) and Fabrice Lebel (data manager). Adjudication Committee: Jean-Michel Halimi (Chairman, Tours), Grégory Ducrocq (Paris Bichat), Charlotte Hulin-Delmotte (Poitiers), Pierre Llatty (Poitiers), David Montaigne (Lille), Vincent Rigalleau (Bordeaux), Ronan Roussel (Paris Bichat), and Philippe Zaoui (Grenoble). Quality Control (INSERM CIC 1402): Pierre-Jean Saulnier, Astrid de Hautecloque, Frederike Limousi, Nathalie Fauvergue, Sofia Hermann, and Sonia Brishoual.

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