Association Between Urinary Markers of Nucleic Acid Oxidation and Mortality in Type 2 Diabetes

A population-based cohort study

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OBJECTIVE—We recently showed that RNA oxidation, estimated by urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo), independently predicted mortality in a cohort of 1,381 treatment-naive patients with newly diagnosed type 2 diabetes. In the present investigation, we analyzed urine collected 6 years after the diagnosis to assess the association between urinary markers of nucleic acid oxidation and mortality in patients with established and treated diabetes.

RESEARCH DESIGN AND METHODS—We used data from the 970 patients who attended the screening for diabetes complications 6 years after the diagnosis. Cox proportional hazards regression was used to examine the relationship between urinary markers of DNA oxidation (8-oxo-7,8-dihydro-2′-deoxyguanosine [8-oxodG] [$n = 938$]) and RNA oxidation (8-oxoGuo [$n = 936$]) and mortality.

RESULTS—During a median of 9.8 years of follow-up, 654 patients died. Urinary 8-oxoGuo assessed 6 years after the diagnosis was significantly associated with mortality. The multivariate-adjusted hazard ratios for all-cause and diabetes-related mortality of patients with 8-oxoGuo levels in the highest quartile compared with those in the lowest quartile were 1.86 (95% CI 1.34–2.58) and 1.72 (1.11–2.66), respectively. Conversely, 8-oxodG was not associated with mortality. In addition, we found an association between changes in 8-oxoGuo from diagnosis to 6-year follow-up and mortality, with increased risk in patients with an increase and decreased risk in patients with a decrease in 8-oxoGuo.

CONCLUSIONS—The RNA oxidation marker 8-oxoGuo is an independent predictor of mortality in patients with established and treated type 2 diabetes, and changes in 8-oxoGuo during the first 6 years after diagnosis are associated with mortality.

The increasing prevalence of diabetes together with the associated morbidity and mortality calls for additional preventive and therapeutic strategies. New biomarkers that can be used in therapy control and risk stratification as an alternative to current methods are needed and can facilitate a more individualized and sufficient treatment of diabetes. Evidence derived from both epidemiological and mechanistic studies suggests that oxidative stress has an important role in mediating the pathologies of diabetes complications (1–6). Although studies have shown associations between markers of oxidative stress and diabetes complications, the predictive value of these markers has not been validated in large-scale prospective studies (7,8).

A number of studies indicate that urinary markers of nucleic acid oxidation could be useful biomarkers in diabetes. Associations between urinary excretion of the DNA oxidation marker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and diabetes or diabetes-related quantitative traits have been shown (8), but no similar studies have been performed for the ribonucleoside counterpart 8-oxo-7,8-dihydroguanosine (8-oxoGuo). The current knowledge of the prognostic importance of urinary markers of nucleic acid oxidation is limited to one study (9).

We previously investigated the association between markers of nucleic acid oxidation and mortality in the Diabetes Care in General Practice cohort—a population-based cohort of 1,381 patients newly diagnosed with diabetes—and showed that urinary excretion of the RNA oxidation marker 8-oxoGuo measured shortly after diagnosis of type 2 diabetes predicted mortality independently of conventional risk factors (9). It is, however, not known whether urinary 8-oxoGuo also predicts mortality in the established and treated diabetic state or whether changes in urinary 8-oxoGuo are associated with changes in mortality.

To investigate the association between the urinary markers of DNA (8-oxodG) and RNA (8-oxoGuo) and oxidation and mortality in established and treated type 2 diabetes, we used data from the Diabetes Care in General Practice cohort obtained 6 years after diagnosis. In addition, we combined these data with data from the time of diagnosis to assess the association between changes in these markers and mortality.

RESEARCH DESIGN AND METHODS

—In the Diabetes Care in General Practice study (10), 474 general practitioners agreed to include all subjects on their practice list who fulfilled the following criteria: newly diagnosed diabetes based on hyperglycemia symptoms and/or raised blood glucose values, diagnosised between 1 March 1989 and 28 February 1992 and aged ≥40 years. The diagnosis was subsequently confirmed with a single fasting whole blood/plasma glucose value of ≥7.0/8.0 mmol/L, measured in a major laboratory. The protocol-based exclusion criteria were life-threatening somatic disease, severe mental illness, or unwillingness to participate. After exclusion of 162 patients, the original study population consisted of 1,381 newly diagnosed diabetic patients. Based on the onset of insulin treatment within 180 days of diagnosis, ~97.5% were considered to have type 2 diabetes (10). Freshly voided morning urine samples were collected from all patients at the time of diagnosis and again at the sixth annual screening for diabetes complications (6-year follow-up). In the present investigation, we used data from the 970 patients who attended the screening. The protocol was approved by the ethics committee of Copenhagen and Frederiksberg, and informed consent was obtained from all patients.

Assessments

The urine samples were assayed between 2009 and 2010 for the oxidatively modified guanine nucleosides 8-oxodG and 8-oxoGuo using a validated method of ultraperformance liquid chromatography and tandem mass spectrometry (11). For 32 (3.3%) and 34 (3.5%) patients, respectively, no valid assessments of 8-oxodG and 8-oxoGuo were obtained. 8-oxodG and 8-oxoGuo were normalized against urinary creatinine concentration. The assessment of the remaining patient characteristics at baseline and at 6-year follow-up has been described previously (10). Cohabitation status, education level, and height (used in BMI calculation) were only assessed at diagnosis.

The vital status of all the patients was certified on 1 January 2009 through the Danish Civil Registration System (12). The vital status of one patient could not be assessed because this person had emigrated in 1992. Diabetes-related death was defined as at least one of the following entries in the Danish National Register of Causes of Death (13): sudden death or death from myocardial infarction, stroke, renal disease, hyperglycemia, hypoglycemia, or

peripheral vascular disease. The cause of death was not recorded in the register in three cases.

Statistical analysis

The participants were grouped according to the quartiles of their urinary 8-oxodG and 8-oxoGuo levels in order to examine the associations between patient characteristics at 6-year follow-up and the corresponding levels of oxidative stress. The medians and interquartile ranges (for continuous characteristics) or percentages (for categorical characteristics) were reported for each quartile, and associations were assessed by Kruskal-Wallis or χ^2 tests, respectively.

The associations between oxidative stress and all-cause mortality and diabetes-related mortality, respectively, were analyzed in Cox proportional hazards regression models based on time from 6-year follow-up to death or censoring. Oxidative stress was represented by the natural logarithm of 8-oxodG and 8-oxoGuo and by a four-class ordinal variable corresponding to the quartiles of the distribution. Two models were estimated for each of the oxidative stress variables and each of the outcomes: a univariate (unadjusted) model and a multivariate model. Covariates included in the latter model were age, sex, diabetes duration, smoking status, cohabitation status, physical activity, education, BMI, presence or absence of hypertension and of microalbuminuria, A1C, total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, history of acute myocardial infarction and stroke, and antidiabetes treatment.

For investigation of the influence of changes in oxidative stress from diagnosis to the 6-year follow-up on subsequent mortality, two 16-class variables were created where the classes corresponded to the combination of the quartiles at diagnosis and at 6-year follow-up of 8-oxodG and 8-oxoGuo, respectively. These variables were then used in Cox proportional hazards models as described above to assess the significance of upward and downward movements between quartiles. Only patients with a valid measurement both at diagnosis and at assessment 6 years after diagnosis were included in this analysis.

The proportional hazards assumption was tested by adding the interactions between each of the independent variables and the logarithm of time since diagnosis to the model; a joint test of these interactions tests the assumption.

The assumption was not violated in any of the models.

In addition, to determine the possible additional prognostic value of adding urinary 8-oxoGuo to established biomarkers and risk factors, we generated receiver operating characteristic (ROC) curves for all-cause mortality, and the areas under the curves (AUCs) were calculated.

Reported *P* values are two sided. Analyses were performed with SAS version 9.2.

RESULTS

—Tables 1 and 2 show characteristics of the study participants at 6-year follow-up according to quartiles of 8-oxodG and 8-oxoGuo. The median age at 6-year follow-up was 69.2 (interquartile range 59.9–77.3), with an equal distribution of men and women (51.4 and 48.6%, respectively).

For both 8-oxodG and 8-oxoGuo, participants in the highest quartiles were older, were more often women, and had lower levels of serum creatinine. In addition, participants in the highest quartiles of 8-oxodG had lower total cholesterol, and those in the highest quartile of 8-oxodG had lower BMI than participants in the lower quartiles. The level of A1C differed between the quartiles of 8-oxodG, but no trend across quartiles was observed. Participants in the highest quartiles of 8-oxoGuo were more often nonsmokers, less physically active, and more often living alone at the time of diagnosis. Differences in antidiabetes and antihypertension treatment between the quartiles of 8-oxoGuo were observed but with no trend across quartiles, except for the use of diuretics, where those in the highest quartiles more frequently used diuretics (Table 2).

Nucleic acid oxidation and mortality

The study observation time ranged from 26 September 1995 to 1 January 2009, and the median duration of follow-up was 9.8 years (interquartile range 4.2–13.3). During this period, 654 (61.2%) patients had died; 407 deaths were regarded as diabetes related.

Patients who died between 6-year follow-up and 1 January 2009 had significantly higher urinary 8-oxoGuo excretion at 6-year follow-up than survivors (median 3.89 nmol/mmol creatinine [interquartile range 3.09–4.91] vs. 3.43 nmol/mmol creatinine [2.75–4.16]; *P* < 0.0001). In contrast, no significant difference was found for 8-oxodG (2.08 nmol/mmol creatinine [1.60–2.73] vs. 2.02 nmol/mmol creatinine [1.57–2.63]; *P* = 0.25).

Table 1—*Characteristics at 6-year follow-up according to quartiles of 8-oxodG*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	$P*$
N	234	235	234	235	
8-oxodG level (nmol/mmol creatinine)	<1.59	1.59–2.05	2.06–2.69	>2.69	
Male sex	62.0	48.5	49.6	45.1	0.002
Age (years)	66.1 (58.1–74.4)	69.4 (61.1–76.9)	67.7 (58.8–76.9)	71.1 (61.3–78.9)	0.003
Duration of diabetes (years)	5.4 (4.9–6.0)	5.5 (4.9–6.1)	5.5 (4.9–6.0)	5.4 (4.9–6.0)	0.75
Smoking status					0.34
Never	31.8	27.5	37.5	35.9	
Previous	38.3	38.7	33.0	32.7	
Current	30.0	33.8	29.5	31.4	
Physical activity					0.32
Low	30.7	26.7	25.1	34.7	
Moderate	61.5	63.8	66.4	59.4	
High	7.8	9.5	8.5	5.9	
Cohabitation status (living alone)	25.5	29.9	27.0	29.7	0.67
Education (basic)	75.2	80.9	78.2	80.1	0.47
A1C	8.5 (7.7–9.7)	8.7 (7.9–9.8)	8.9 (7.9–9.9)	8.5 (7.4–9.9)	0.046
Total cholesterol (mmol/L)	6.1 (5.3–6.8)	6.2 (5.4–7.0)	5.8 (5.2–6.7)	6.0 (5.1–6.8)	0.017
Fasting triglycerides (mmol/L)	1.92 (1.26–2.66)	1.85 (1.27–2.62)	1.80 (1.17–2.68)	1.69 (1.23–2.43)	0.53
Serum creatinine (μmol/L)	93.0 (83.0–110.0)	91.0 (82.0–103.0)	89.0 (79.0–100.0)	85.5 (77.0–99.0)	<0.001
Urinary albumin (mg/L)	11.9 (5.6–29.1)	10.9 (5.7–25.9)	12.5 (4.9–30.5)	11.8 (5.2–25.2)	0.90
Hypertension†	74.8	74.9	74.4	68.5	0.33
BMI (kg/m^2)	28.5 (25.8–32.1)	28.9 (26.0–32.3)	28.7 (25.7–32.7)	27.0 (25.0–29.7)	<0.001
Retinopathy	16.1	17.0	14.8	11.6	0.43
Peripheral neuropathy	25.3	24.1	23.8	29.0	0.56
History of AMI	12.1	10.2	9.4	6.8	0.28
History of stroke	8.6	8.5	6.8	9.8	0.72
Antidiabetes treatment					0.50
Insulin	15.8	11.1	13.3	9.8	
Oral antidiabetes treatment	54.3	57.5	58.1	61.3	
Diet only	29.9	31.5	28.6	28.9	
Antihypertension treatment					
Calcium channel blockers	16.3	16.6	13.3	11.5	0.32
ACE inhibitors	19.3	23.4	19.7	17.9	0.49
Diuretics	42.1	38.7	34.6	35.3	0.32
β-Blockers	8.2	6.8	11.1	6.0	0.18
Other antihypertension treatment	2.2	1.3	1.3	1.7	0.86
Lipid-lowering drugs	6.9	3.8	3.4	3.8	0.24

Data are percent or median (interquartile range) unless otherwise indicated. AMI, acute myocardial infarction. *P values are from χ^2 tests (for categorical data) or from ANOVA (for continuous data). †Hypertension was defined as systolic/diastolic blood pressure $\geq$160/90 mmHg or the use of antihypertension drugs.

Kaplan-Meier analysis estimates of survival according to quartiles of urinary 8-oxodG and 8-oxoGuo at 6-year follow-up are shown in Supplementary Fig. 1. The results of the unadjusted and adjusted Cox regression analyses are shown in Table 3.

At 6-year follow-up, only 8-oxoGuo was significantly associated with mortality. The unadjusted hazard ratios for all-cause and diabetes-related mortality of patients with 8-oxoGuo levels in the highest quartile compared with those in the lowest quartile were 2.09 (95% CI 1.62–2.67; $P < 0.001$) and 1.94 (1.40–2.69; $P < 0.001$), respectively. The multivariate-adjusted hazard ratios for all-cause and diabetes-related mortality of patients with 8-oxoGuo levels in the highest quartile compared with those in the lowest quartile were 1.86 (1.34–2.58; $P < 0.001$) and 1.72 (1.11–2.66; $P = 0.02$), respectively.

When log8-oxodG and log8-oxoGuo were considered as continuous covariates, the results were similar to those described above (Table 3). The multivariate-adjusted hazard ratios for all-cause and diabetes-related mortality per unit increase in log8-oxoGuo were 1.82 (95% CI 1.36–2.42; $P < 0.001$) and 1.64 (1.13–2.37; $P = 0.009$), respectively.

Other analyses
We analyzed the significance of changes in the nucleic acid oxidation markers from diagnosis to 6-year follow-up. For both 8-oxodG and 8-oxoGuo, ~30% of the patients in the second and third quartiles remained in the same quartile as at diagnosis, whereas this figure was ~45% for patients in the highest and lowest quartiles (Table 4). The unadjusted and multivariate-adjusted hazard ratios for all-cause mortality of patients who changed quartile from diagnosis to 6-year follow-up compared with those who remained in the same quartile are shown in Table 4.

Table 2—*Characteristics at 6-year follow-up according to quartiles of 8-oxoGuo*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P^*
N	234	234	234	234	
8-oxoGuo level					
(nmol/mmol creatinine)	<2.94	2.94–3.64	3.65–4.66	>4.66	
Male sex	69.2	54.7	44.0	37.6	<0.001
Age (years)	63.6 (55.3–71.3)	68.2 (59.2–75.9)	70.0 (61.9–77.7)	72.7 (65.8–79.5)	<0.001
Duration of diabetes (years)	5.4 (4.9–5.9)	5.5 (4.8–6.1)	5.4 (4.9–5.9)	5.5 (4.9–6.0)	0.91
Smoking status					0.005
Never	24.1	31.9	34.7	42.1	
Previous	38.2	35.4	37.9	31.0	
Current	37.7	32.7	27.4	26.9	
Physical activity					<0.001
Low	19.7	25.1	32.2	40.0	
Moderate	70.9	66.5	59.8	54.0	
High	9.4	8.4	7.9	6.1	
Cohabitation status (living alone)	15.4	29.4	30.8	36.4	<0.001
Education (basic)	75.3	78.0	78.3	82.7	0.29
A1C	8.6 (7.8–9.7)	8.5 (7.7–9.6)	8.8 (7.8–10.1)	8.6 (7.7–9.9)	0.47
Total cholesterol (mmol/L)	5.9 (5.2–6.6)	6.1 (5.2–6.9)	6.1 (5.4–6.8)	6.0 (5.2–7.0)	0.64
Fasting triglycerides (mmol/L)	1.67 (1.20–2.45)	1.88 (1.23–2.66)	1.86 (1.24–2.66)	1.80 (1.27–2.65)	0.45
Serum creatinine (μmol/L)	92.0 (85.0–105.0)	89.0 (80.0–103.0)	88.0 (79.0–101.0)	88.0 (78.0–104.5)	0.001
Urinary albumin (mg/L)	11.9 (5.1–26.6)	11.0 (5.1–27.0)	10.2 (5.4–27.5)	13.0 (6.1–30.5)	0.49
Hypertension†	71.4	69.7	76.1	75.2	0.34
BMI (kg/m^2)	28.7 (25.8–32.0)	28.1 (25.3–31.9)	28.5 (25.9–32.4)	27.9 (25.1–31.3)	0.30
Retinopathy	14.2	14.5	13.5	17.6	0.66
Peripheral neuropathy	19.9	27.0	28.0	26.9	0.17
History of AMI	10.8	9.0	7.3	11.2	0.45
History of stroke	7.3	7.3	9.4	9.8	0.65
Antidiabetes treatment					0.009
Insulin	17.5	8.6	15.0	8.6	
Oral antidiabetes treatment	50.9	62.4	54.7	63.7	
Diet only	31.6	29.1	30.3	27.8	
Antihypertension treatment					
Calcium channel blockers	14.5	11.5	15.5	15.8	0.54
ACE inhibitors	21.8	18.4	26.6	13.7	0.005
Diuretics	34.2	31.2	41.2	43.6	0.018
β-Blockers	9.0	7.3	8.6	7.3	0.86
Other antihypertension treatment	2.1	0.9	1.3	2.1	0.61
Lipid-lowering drugs	6.0	3.0	5.2	3.9	0.41

Data are percent or median (interquartile range) unless otherwise indicated. AMI, acute myocardial infarction. *P values are from χ^2 tests (for categorical data) or from ANOVA (for continuous data). †Hypertension was defined as systolic/diastolic blood pressure ≥160/90 mmHg or the use of antihypertension drugs.

For 8-oxoGuo, there was a clear and significant trend for increased risk in patients who changed to higher quartiles and decreased risk in patients who changed to lower quartiles. This trend was seen for all quartiles of 8-oxoGuo at diagnosis but was most pronounced for patients with 8-oxoGuo in the highest quartile: the unadjusted and multivariate-adjusted hazard ratios of patients who changed from the highest to the lowest quartile of 8-oxoGuo compared with those who remained in the highest quartile were 0.38 (95% CI 0.16–0.89) and 0.31 (0.14–0.68), respectively. For 8-oxodG, this trend was less obvious and was only significant for patients with 8-oxodG in the lowest and highest quartiles at diagnosis.

The ability of 8-oxoGuo and other biomarkers and risk factors to predict mortality was evaluated by ROC curves. The AUC was 0.63 (95% CI 0.59–0.67) for a model including the traditional biomarkers in diabetes: A1C, fasting triglycerides, total cholesterol, urinary albumin, systolic blood pressure, and BMI. Adding 8-oxoGuo to this model increased the AUC to 0.67 (0.64–0.72) (Supplementary Fig. 2). AUCs for a multivariate model—including all the covariates in our multivariate Cox proportional hazards regression model with and without 8-oxoGuo—were 0.88 (0.86–0.91) and 0.87 (0.86–0.91), respectively.

CONCLUSIONS—In this study, we found that urinary excretion of the RNA oxidation marker 8-oxoGuo in a freshly voided morning urine sample 6 years after diagnosis of diabetes independently predicted all-cause and diabetes-related mortality, whereas the DNA oxidation marker 8-oxodG did not. The prognostic information of 8-oxoGuo was independent of other characteristics of patients with type

Table 3—Relationship of 8-oxodG and 8-oxoGuo with all-cause and diabetes-related mortality

	Unadjusted hazard ratio (95% CI)	P	Multivariate-adjusted hazard ratio (95% CI)*	P
All-cause mortality				
8-oxodG				
Quartile 1	1.0		1.0	
Quartile 2	1.01 (0.79–1.30)	0.92	1.02 (0.75–1.38)	0.92
Quartile 3	1.03 (0.81–1.32)	0.80	1.14 (0.84–1.54)	0.40
Quartile 4	1.18 (0.93–1.51)	0.18	1.26 (0.94–1.71)	0.14
Log 8-oxodG	1.20 (0.97–1.48)	0.09	1.27 (0.98–1.64)	0.07
8-oxoGuo				
Quartile 1	1.0		1.0	
Quartile 2	1.26 (0.97–1.63)	0.09	1.12 (0.82–1.54)	0.48
Quartile 3	1.51 (1.16–1.95)	0.002	1.55 (1.13–2.13)	0.007
Quartile 4	2.09 (1.62–2.67)	<0.001	1.86 (1.34–2.58)	<0.001
Log 8-oxoGuo	2.01 (1.63–2.49)	<0.001	1.82 (1.36–2.42)	<0.001
Diabetes-related mortality				
8-oxodG				
Quartile 1	1.0		1.0	
Quartile 2	0.97 (0.71–1.32)	0.85	1.03 (0.71–1.51)	0.86
Quartile 3	0.90 (0.66–1.23)	0.51	1.07 (0.72–1.58)	0.75
Quartile 4	1.13 (0.84–1.54)	0.42	1.18 (0.79–1.74)	0.42
Log 8-oxodG	1.12 (0.86–1.46)	0.41	1.20 (0.86–1.66)	0.29
8-oxoGuo				
Quartile 1	1.0		1.0	
Quartile 2	1.40 (1.00–1.94)	0.05	1.31 (0.87–1.96)	0.20
Quartile 3	1.68 (1.21–2.32)	0.002	1.71 (1.13–2.58)	0.01
Quartile 4	1.94 (1.40–2.69)	<0.001	1.72 (1.11–2.66)	0.02
Log 8-oxoGuo	2.00 (1.53–2.61)	<0.001	1.64 (1.13–2.37)	0.009

*Covariates included in the multivariate model were age, sex, diabetes duration, smoking status, cohabitation status, physical activity, education, BMI, A1C, presence or absence of hypertension and of microalbuminuria (urinary albumin ≥15 or >15 mg/L), total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, history of acute myocardial infarction and stroke, and antidiabetes treatment.

2 diabetes that have been linked to mortality: most importantly, age, sex, A1C, lipids, urinary albumin excretion, blood pressure, smoking, and preexisting cardiovascular disease (14–19).

In addition, we found that changes in 8-oxoGuo from the time of diagnosis to 6-year follow-up were associated with mortality, with increased risk of death in patients with an increase, and decreased risk of death in patients with a decrease in 8-oxoGuo. This finding supports the notion that RNA oxidation is not only a predictor of risk in diabetes but also a risk factor, i.e., that modification of RNA oxidation also modifies risk.

To our knowledge, our population-based study of diabetic patients is the only study of the association between urinary excretion of markers of oxidative stress and mortality in diabetic patients. As in the current study, urinary 8-oxoGuo assessed at the time of diagnosis was shown to be an independent predictor of mortality (9). The association between 8-oxoGuo and mortality seemed stronger at 6-year follow-up than at diagnosis. At diagnosis, the risk of death was ~50% higher among those in the highest quartile than among those in the lowest quartile, whereas this figure was ~90% at 6-year follow-up.

ROC curve analyses showed that 8-oxoGuo added prognostic information to other established biomarkers in diabetes. However, in the multivariate model including all the covariates in our multivariate Cox proportional hazards regression model, 8-oxoGuo did not significantly improve the predictive accuracy of the model.

RNA oxidation could be an epiphenomenon rather than a contributing pathophysiological mechanism, but it would still function as a marker of generalized intracellular oxidative stress. However, the fact that only 8-oxoGuo was shown to be an independent predictor of mortality suggests that RNA oxidation plays a more important role in diabetic patients than DNA oxidation and generalized intracellular oxidative stress. The exact pathophysiological mechanisms behind diabetes complications are still elusive. It has been suggested that mitochondrial dysfunction, with failure of complete oxidation leading to overproduction of superoxide by the mitochondrial electron-transport chain, plays a key role in the biochemical abnormalities leading to vascular complications in diabetes (20). Superoxide is readily converted into hydrogen peroxide through the action of superoxide dismutase, and in the presence of iron, hydrogen peroxide is reduced to hydroxyl radicals by a Fenton reaction. The highly reactive hydroxyl radicals then oxidize DNA and RNA among many other macromolecules (20). Compared with DNA, RNA is more prone to oxidative damage because of its widespread distribution in close proximity to sites of reactive oxygen species generation, its single-stranded nature, and the lesser association with protecting proteins. Surprisingly, the role of RNA oxidation in human disease has only recently been investigated, predominantly in degenerative brain diseases (21–30). On the basis of the limited evidence available, we suggest a possible mechanism by which RNA oxidation could be involved in the development of diabetes complications. RNA oxidation is observed in both mRNA and noncoding RNA. Oxidized bases on mRNA may cause ribosome stalling on the mRNA with production of truncated proteins or slow the translational process, leading to a reduction in protein expression (31). Since the noncoding RNAs, which include rRNA, tRNA, and mRNAs, play critical regulatory roles in mRNA translation, oxidation of these could further negatively affect the translational process, leading to smaller quantities of protein overall and/or defective proteins, which may have detrimental effects on a variety of distinct cellular processes that lead to changes in proliferation and apoptosis (31–33). Apoptosis plays an important role in the development of the atherosclerotic plaque (34), and through this pathway increased RNA oxidation may be involved in the development of the vascular complications in diabetes. It should be emphasized that the described possible mechanism is a suggestion based on available evidence and that no

Table 4—Relationship between changes in 8-oxodG and 8-oxoGuo from diagnosis to 6-year follow-up and all-cause mortality

Quartile at diagnosis	N (%)	Unadjusted hazard ratio (95% CI)	Multivariate-adjusted hazard ratio (95% CI)*	P_{trend}
8-oxodG				
Quartile 1 at diagnosis				
Follow-up quartile				
1	105 (46.7)	1.00	1.00	0.02
2	66 (29.3)	1.41 (0.93–2.14)	1.57 (0.92–2.68)	
3	35 (15.6)	1.33 (0.79–2.24)	1.80 (0.92–3.52)	
4	19 (8.4)	1.19 (0.60–2.34)	2.29 (1.01–5.20)	
Quartile 2 at diagnosis				
Follow-up quartile				
1	64 (27.6)	1.16 (0.66–2.04)	1.42 (0.84–2.42)	0.71
2	66 (28.5)	1.00	1.00	
3	62 (26.7)	0.96 (0.53–1.74)	1.02 (0.57–1.82)	
4	40 (17.2)	1.11 (0.58–2.13)	1.43 (0.73–2.78)	
Quartile 3 at diagnosis				
Follow-up quartile				
1	34 (14.5)	1.25 (0.65–2.40)	1.24 (0.73–2.18)	0.70
2	63 (26.8)	1.06 (0.60–1.89)	0.85 (0.49–1.46)	
3	74 (31.5)	1.00	1.00	
4	64 (27.2)	1.31 (0.76–2.27)	1.26 (0.73–2.18)	
Quartile 4 at diagnosis				
Follow-up quartile				
1	23 (10.0)	0.97 (0.50–1.86)	0.33 (0.14–0.77)	0.04
2	38 (16.5)	1.15 (0.69–1.93)	0.98 (0.55–1.75)	
3	60 (26.0)	0.76 (0.46–1.26)	1.30 (0.80–2.10)	
4	110 (47.6)	1.00	1.00	
8-oxoGuo				
Quartile 1 at diagnosis				
Follow-up quartile				
1	104 (44.6)	1.00	1.00	0.002
2	56 (24.0)	1.40 (0.80–2.47)	0.89 (0.50–1.59)	
3	47 (20.2)	1.34 (0.73–2.45)	1.34 (0.75–2.41)	
4	26 (11.2)	2.37 (1.20–4.66)	1.59 (0.77–3.30)	
Quartile 2 at diagnosis				
Follow-up quartile				
1	63 (27.6)	0.72 (0.36–1.41)	0.64 (0.35–1.14)	0.02
2	73 (32.0)	1.00	1.00	
3	56 (24.6)	1.57 (0.87–2.83)	1.30 (0.71–2.37)	
4	36 (15.8)	1.58 (0.79–3.15)	1.11 (0.54–2.25)	
Quartile 3 at diagnosis				
Follow-up quartile				
1	40 (16.9)	1.05 (0.57–1.93	0.84 (0.44–1.59)	0.0002
2	58 (24.5)	0.83 (0.47–1.48)	0.71 (0.40–1.24)	
3	72 (30.4)	1.00	1.00	
4	67 (28.3)	1.15 (0.67–1.96)	1.17 (0.69–1.96)	
Quartile 4 at diagnosis				
Follow-up quartile				
1	21 (9.5)	0.38 (0.16–0.89)	0.31 (0.14–0.68)	<0.0001
2	44 (19.8)	1.03 (0.62–1.71)	0.55 (0.32–0.92)	
3	56 (25.2)	1.19 (0.76–1.87)	0.71 (0.43–1.15)	
4	101 (45.5)	1.00	1.00	

*Covariates included in the multivariate model were age, sex, diabetes duration, smoking status, cohabitation status, physical activity, education, BMI, A1C, presence or absence of hypertension and of microalbuminuria (urinary albumin ≥15 or <15 mg/L), total cholesterol, triglycerides, serum creatinine, presence or absence of retinopathy and of peripheral neuropathy, history of acute myocardial infarction and stroke, and antidiabetes treatment.

conclusion regarding a causal relationship between RNA oxidation, vascular complications, and mortality can be drawn from the present association.

Important limitations of this study include the reliance on a single measurement of the nucleic acid oxidation markers in a morning spot urine sample and the need to elaborate the potential mechanisms underlying our findings. On the other hand, the fact that a significant association between 8-oxoGuo and mortality was shown both at diagnosis (9) and at follow-up and the relation between changes in RNA oxidation and mortality support the assumption that the association is not a chance finding. Another limitation is that only BMI was used to evaluate obesity. However, it has been shown that waist circumference and waist-to-hip ratio may be better predictors of mortality than BMI (35). Unfortunately, these anthropometric measures were not assessed. In addition, glucose variability, which has been shown to exhibit a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia (36,37), was not assessed in the study. Finally, the lack of information on diet and consumption of antioxidants, which potentially could affect the level of oxidative stress, should be mentioned. The main strengths of our study include the prospective design, the large sample size, the long follow-up period, and the low attrition rate.

The findings of this study may provide new insight into the pathophysiological mechanisms responsible for the development of complications in diabetes but also indicate that measurement of 8-oxoGuo could have clinical applications. In the prevention of complications in diabetic patients, multivariate risk factor approaches are used to assess risk (38). Biomarkers used in these approaches include A1C, lipids, and urinary albumin. Our data show that urinary 8-oxoGuo predicts mortality in type 2 diabetic patients independent of other conventional risk factors including these currently used biomarkers. Measurement of urinary 8-oxoGuo could therefore provide additional information about risk that may be useful for identifying patients who would benefit the most from intensified treatment or specific treatment strategies. A multivariate risk factor approach with the combined use of 8-oxoGuo and other known risk factors could therefore be useful for improving risk stratification of diabetic patients. It should be emphasized,

though, that the clinical significance of using this marker is unknown, since the clinical utility of 8-oxoGuo remains to be determined.

Although the clinical trials conducted to date have failed to provide adequate support for the use of antioxidants in diabetes, recent studies of bardoxolone methyl (39), showing beneficial effect on glomerular filtration rate in type 2 diabetic patients, suggest that targeting the Nrf2 (nuclear factor erythroid 2–related factor 2) pathway may be a useful strategy to counteract oxidative stress in diabetes. Investigating the effect of activators of Nrf2 on RNA oxidation may provide interesting information that could shed more light on the role of RNA oxidation in diabetes. In addition, intervention studies should be initiated to investigate the effect of different treatment modalities of diabetes (e.g., glycemic control, exercise, diet, and weight control) on RNA oxidation.

Further experimental work is needed to establish the exact mechanisms responsible for the association between 8-oxoGuo and mortality in diabetes patients, and studies should be initiated to evaluate the potential clinical applications of 8-oxoGuo as a biomarker in diabetes that could be used for risk stratification, selection of appropriate therapeutic intervention, or monitoring response to therapy.

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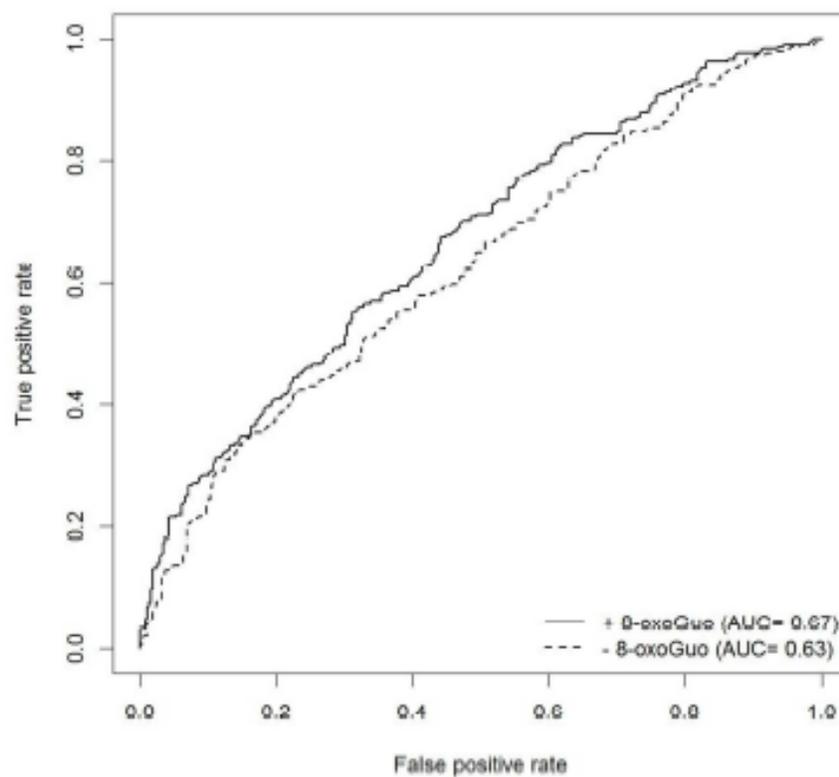
SUPPLEMENTARY DATA

Supplementary Figure 1. Unadjusted Kaplan-Meier curves showing overall survival according to quartiles of 8-oxodG and 8-oxoGuo, respectively.
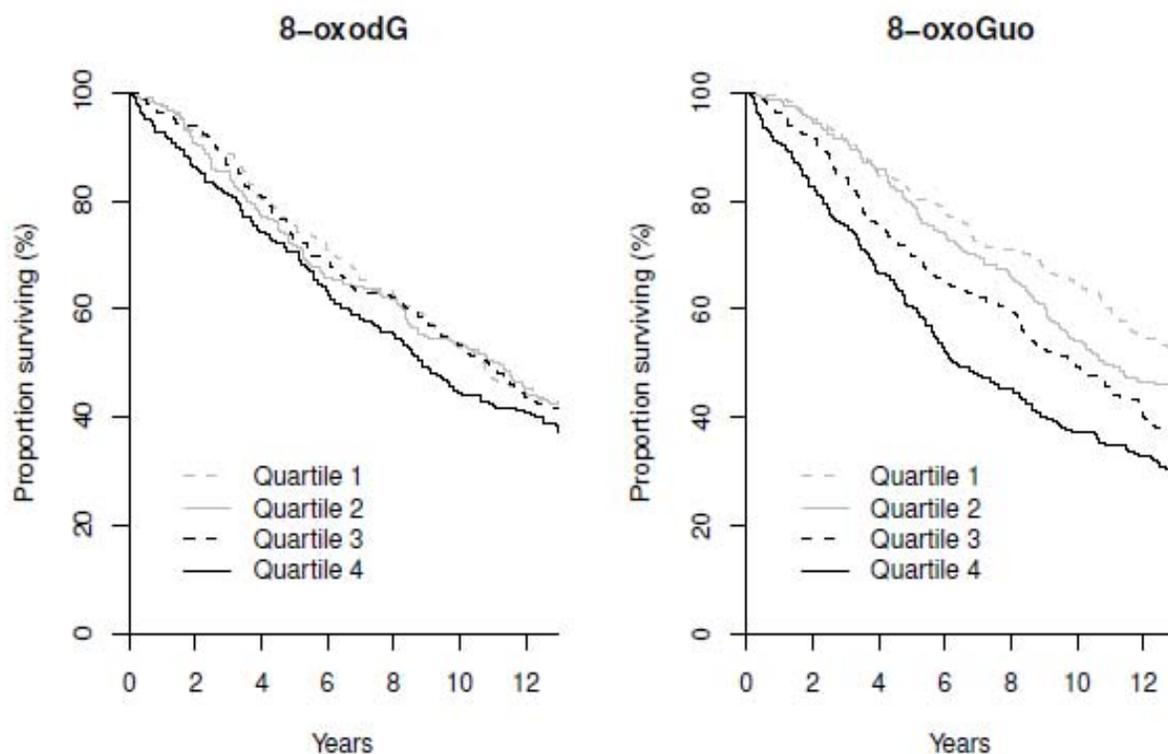
SUPPLEMENTARY DATA

Supplementary Figure 2. ROC curves demonstrating the ability of 8-oxoGuo and other biomarkers to predict mortality.
The AUC for a model including traditional biomarkers in diabetes (without 8-oxoGuo) was 0.63 (95% CI, 0.59–0.67) and for a model including the traditional biomarkers in diabetes and 8-oxoGuo it was 0.67 (95% CI, 0.64–0.72).

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