Cardiovascular and All-Cause Mortality Risk Associated With Urinary Excretion of 8-oxoGuo, a Biomarker for RNA Oxidation, in Patients With Type 2 Diabetes: A Prospective Cohort Study

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OBJECTIVE

Cardiovascular mortality risk remains high among patients with type 2 diabetes. Oxidative stress indicated by high urinary excretion of the biomarker for RNA oxidation, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), is associated with an increased risk of death in newly diagnosed and treated patients. We assessed whether 8-oxoGuo is associated with specific cardiovascular and all-cause mortality risk.

RESEARCH DESIGN AND METHODS

Urinary biomarkers for nucleic acid oxidation were measured in a cohort of patients with type 2 diabetes aged ≥60 years (*n* = 1,863), along with biochemical measurements, questionnaire findings, and Central Person Registry information to estimate the hazard ratios (HRs) for log2-transformed RNA oxidation using Cox regression.

RESULTS

During the 5-year follow-up, 173 of 1,863 patients had died (9.3%), including 73 patients who died of cardiovascular disease (42.2%). Doubling of RNA oxidation was associated with an HR of all-cause mortality of 2.10 (95% CI 1.63–2.71; $P < 0.001$) and an HR of cardiovascular death of 1.82 (95% CI 1.20–2.77; $P = 0.005$) after multiple adjustments. The 5-year absolute risks (ARs) of all-cause mortality (AR 13.9 [95% CI 10.8–17.0] vs. AR 6.10 [95% CI 4.00–8.30]) and cardiovascular mortality (AR 5.49 [95% CI 3.44–7.55] vs. AR 3.16 [95% CI 1.59–4.73]) were approximately two times higher in the highest quartile of RNA oxidation than in the lowest quartile.

CONCLUSIONS

We conclude that high RNA oxidation is associated with all-cause and cardiovascular mortality risk in patients with type 2 diabetes. Targeting oxidative stress via interventions with long-term follow-up may reveal the predictive potential of the biomarker 8-oxoGuo.

Several recent large clinical intervention trials have challenged the isolated effect of optimizing glycemia on cardiovascular complications and mortality in type 2 diabetes (1,2). Meta-analysis of trials for intensive glucose control did not demonstrate a reduction in cardiovascular deaths, although events were reduced (3), and trials for

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newer glucose-lowering agents also failed to meet the primary cardiovascular outcome targets (4). Despite several decades of specific type 2 diabetes treatment, only two recent clinical trials, LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) (5) and EMPA-REG (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) (6), demonstrated the effects of liraglutide and empagliflozin on macrovascular morbidity and mortality, whereas the SUSTAIN-6 (Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes) (7) trial reported the effects of semaglutide on composite outcomes. Nevertheless, effective pharmacological prevention of cardiovascular events remains limited to conventional and nondiabetes-specific treatments, including the control of hypertension and hyperlipidemia, which have no effect on glucose control (8).

Oxidative stress is an alternative potential explanatory mechanism underlying glucose toxicity for both micro- and macrovascular diabetic complications (9–15). Oxidative stress may be defined as an increase in pro-oxidants, such as reactive oxygen species (ROS), in excess of the antioxidant capacity within the cell, which subsequently leads to oxidative modifications of cellular components, such as RNA and DNA (10). Although compelling evidence targeting oxidative stress, either directly or indirectly, may yield clinical benefits, further studies are needed (16,17).

Recently, in a cohort of 1,381 newly diagnosed, treatment-naive patients with type 2 diabetes, we showed that high urinary excretion of 8-oxo-7,8-dihydroguanosine (8-oxoGuo), a marker of RNA oxidation, was associated with increased mortality (18). The association was confirmed in the same cohort 6 years after diagnosis (19), and alterations in RNA oxidation were associated with similar changes in the risk of all-cause mortality during the same time period (19).

We hypothesized that 8-oxoGuo predicts all-cause mortality and cause-specific cardiovascular death in patients with long-term type 2 diabetes receiving state-of-the-art nonpharmacological and pharmacological treatment. Therefore, we examined mortality risk in a large cohort of patients with type 2 diabetes of variable duration, treated according to best practice clinical guidelines.

RESEARCH DESIGN AND METHODS

Study Design and Cohort

The Vejle Diabetes Biobank cohort recruited patients aged between 25 and 75 years with type 2 diabetes from 31 December 2006 onwards (20). Patients with diabetes were identified using the Danish Personal Identification Number and the Danish Civil Registration System based on the presence of at least one of the following criteria: high glycated hemoglobin (HbA_{1c}) value (one value of $HbA_{1c} \geq 6.6\%$ [48.6 mmol/mol] in the laboratory database from 1996 to 2006), minimum three HbA_{1c} measurements in the laboratory database from 2002 to 2006, antidiabetic medication prescriptions in the Danish National Prescription Registry, and/or diabetes diagnosis registered in the Danish National Patient Registry (20). Furthermore, based on the results of the questionnaire administered on the day of the health examination, 57 patients self-reported type 2 diabetes (2%). Individuals diagnosed with type 1 diabetes and those who did not acknowledge having diabetes were excluded. Data were derived from a questionnaire (covering smoking status, exercise habit, genetic disposition of diabetes, and antidiabetic medications and other medications), a health examination (including weight and height measurements to calculate BMI, and systolic and diastolic blood pressure measured with Omron M5 Professional [Osaka, Japan] in sitting position after a 5-min rest), biochemical measurements (HbA_{1c}, total cholesterol, HDL and LDL, C-reactive protein [CRP], albumin, and creatinine levels), and measurements of urinary 8-oxoGuo and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). In the Vejle Diabetes Biobank, 56% of the patients with type 2 diabetes received oral antidiabetics exclusively, 11% insulin alone, and 11% insulin combined with oral antidiabetics; 22% did not receive antidiabetic medication but did receive dietary and related lifestyle change advice (20). Additional information on the cohort and the full study protocol are available online (20). The biochemical measurements were analyzed using standard methods as previously described in the Vejle Diabetes Biobank (20).

We stratified the patients according to age (<50, 50–60, and ≥60 years) and analyzed the number of total events. Among the 827 patients aged <60 years, only 28 died during follow-up. Therefore, we only included patients aged ≥60 years to generate a reliable survival model. We further chose 5 years as the cutoff for the follow-up period since most patients could be evaluated by this time due to the limited follow-up duration (median follow-up 6.3 years) and because the occurrence of cardiovascular death was known.

The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from each patient. Study approval was granted on 3 April 2013 by the local ethics committee of the Region of Southern Denmark (S-20080097, amendment protocol 37831) and reported to the Danish Data Protection Agency.

Measurements of Urinary Nucleic Acid Biomarkers

We used validated and highly specific ultraperformance liquid chromatography tandem mass spectrometry to detect 8-oxoGuo levels, which were then compared with the levels of 8-oxodG (the biomarker for DNA modification by oxidation) in spot urine samples corrected for urinary creatinine (21). The validation procedures were in accordance with the U.S. Food and Drug Administration guidelines, and the lower limit of quantification was 1.0 nmol/L for both 8-oxoGuo and 8-oxodG (21). According to recently published data, the accuracy of this method was 98.7% for 8-oxoGuo and 95.7% for 8-oxodG (21). The average within-day precision was 2.9% for 8-oxoGuo and 3.7% for 8-oxodG; average between-day precision was 1.5% for 8-oxoGuo and 3.4% for 8-oxodG (21). Specificity was achieved by measuring two characteristic fragmentation ions (quantifier and qualifier ions) and applying the relevant acceptance criteria for the response ratio between the two ions (21).

In the study, 2,727 spot urine measurements of 8-oxoGuo and 8-oxodG were available. Urine samples (one per patient) were collected between March 2007 and May 2010 and stored at −80°C. The samples were stored for up to 5 years post-study. The samples were analyzed between August 2012 and December 2013 and stored at −20°C during this time period, which is considered stable for the nucleic acid oxidation markers for up to 15 years (22).

Outcomes

The predefined primary outcome was all-cause mortality. The secondary outcome was cardiovascular mortality, which was defined as at least one cardiovascular diagnosis on the death certificate.

The vital status was recorded on 31 December 2013 by the Danish Civil Registration System via Statistics Denmark, where all deaths are recorded within 2 weeks (23). However, registration of the causes of death is delayed by 1 year, and therefore, the cardiovascular deaths were recorded on 31 December 2012 based on the "death due to cardiovascular disease" entries in the Danish National Registry of Causes of Death (23).

Statistical Analyses

Differences between sexes in the baseline characteristics were analyzed using the Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables. All-cause mortality was analyzed using the Kaplan-Meier method, and cardiovascular death was determined using the Aalen-Johansen method (24). The rationale for this distinction is that the Kaplan-Meier method cannot deal with competing risks (25). Reported were the absolute 5-year risks (ARs) across the 8-oxoGuo quartiles. Multiple Cox regression was used to analyze the associations between 8-oxoGuo and the cause-specific hazard of the cardiovascular end point. The model was adjusted for age, systolic blood pressure, smoking, albuminuria, BMI, LDL levels, and CRP levels and was further stratified according to sex. The models were considered stable based on fitted models with 1,000 bootstrap samples. Covariates included in the model were based on evidence from the literature. Complete case analyses were performed for 1,863 patients (37 missing values were

Table 1—Baseline characteristics of 1,900 patients with type 2 diabetes, all of whom were stratified at baseline by age (≥60 years) and sex

Baseline characteristics	Missing values	All	Women	Men	*P* value (women vs. men)
No. of patients		1,900	698	1,202	
8-oxoGuo (nmol/mmol creatinine)	0	2.83 (2.32–3.46)	3.10 (2.58–3.79)	2.65 (2.24–3.26)	<0.001
8-oxodG (nmol/mmol creatinine)	0	1.72 (1.31–2.26)	1.88 (1.44–2.44)	1.64 (1.24–2.10)	<0.001
Age (years)	0	67.0 (63.0–71.0)	67.0 (63.0–71.0)	67.0 (63.0–71.0)	0.914
Genetic disposition	0	893 (47.0)	387 (55.4)	506 (42.1)	<0.001
Smoker	4				<0.001
Never		596 (31.4)	334 (47.9)	262 (21.8)	
Previous		937 (49.3)	247 (35.4)	690 (57.4)	
Daily		291 (15.3)	90 (12.9)	201 (16.7)	
Occasionally		72 (3.8)	25 (3.6)	47 (3.9)	
Exercise	6	1,423 (75.1)	542 (78.1)	881 (73.4)	0.027
BMI	6				0.019
<25 kg/m^2		234 (12.4)	101 (14.6)	133 (11.1)	
25–30 kg/m^2		768 (40.5)	257 (37.1)	511 (42.5)	
>30 kg/m^2		892 (47.1)	335 (48.3)	557 (46.4)	
HbA_{1c} (% DCCT)	9	6.8 (6.3–7.5)	6.7 (6.3–7.4)	6.8 (6.3–7.5)	0.314
HbA_{1c} (mmol/mol)	9	51 (45–58)	50 (45–57)	51 (45–58)	0.314
Cholesterol (mmol/L)	7	4.20 (3.70–4.70)	4.35 (3.90–4.90)	4.00 (3.50–4.60)	<0.001
Triglycerides (mmol/L)	7	1.50 (1.09–2.10)	1.54 (1.14–2.10)	1.46 (1.06–2.09)	0.063
HDL (mmol/L)	7	1.25 (1.04–1.51)	1.40 (1.18–1.64)	1.18 (0.98–1.40)	<0.001
LDL (mmol/L)	7	2.16 (1.74–2.68)	2.21 (1.81–2.72)	2.13 (1.69–2.66)	0.002
CRP (mg/L)	7	1.80 (0.90–3.90)	2.20 (1.10–4.57)	1.70 (0.80–3.60)	<0.001
Plasma albumin (g/L)	7	45.0 (43.0–47.0)	45.0 (43.0–46.0)	45.0 (43.0–47.0)	0.972
Plasma creatinine (μmol/L)	25	81.5 (70.2–94.5)	70.7 (61.8–81.1)	87.0 (77.7–100.1)	<0.001
Urinary albumin (mg/L)	0	6.80 (4.27–12.6)	5.65 (3.70–8.90)	7.80 (4.80–17.40)	<0.001
Urinary creatinine (μmol/L)	0	7.20 (4.97–10.2)	5.60 (4.10–8.10)	8.00 (5.90–11.10)	<0.001
Systolic blood pressure (mmHg)	22	151 (138–166)	149 (136–163)	153 (140–167)	<0.001
Diastolic blood pressure (mmHg)	23	84 (77–92)	82 (75–90)	85 (79–92)	<0.001
Current use of insulin	0				0.556
No		1,493 (78.6)	548 (78.5)	945 (78.6)	
One type		308 (16.2)	109 (15.6)	199 (16.6)	
Two or more types		99 (5.2)	41 (5.9)	58 (4.8)	
Metformin	0	964 (50.7)	356 (51.0)	608 (50.6)	0.897
ACE inhibitors	0	739 (38.9)	230 (33.0)	509 (42.3)	<0.001
AT2 antagonists	0	365 (19.2)	147 (21.1)	218 (18.1)	0.134
Lipid-lowering drugs	0	1,442 (75.9)	545 (78.1)	897 (74.6)	0.101

Data are presented as median with the upper and lower quartile, *n*, or *n* (%) unless otherwise noted; the number of missing values is also included. The Mann-Whitney *U* test was performed for continuous variables, and the χ^2 test was used for categorical variables. AT2, angiotensin II receptor type 1; DCCT, Diabetes Control and Complications Trial.

excluded). Sensitivity analyses for the cardiovascular end point for males in the cohort were carried out due to concerns about the differences in risk factors in the baseline characteristics. Subanalyses of hypertensive patients were performed to address organ damage.

In addition, correlations between the baseline variables were assessed by the Kendall τ rank correlation coefficient. For comparison, all analyses were also repeated for 8-oxodG (DNA oxidation). The level of significance was set at 5%. Statistical analyses were performed with R version 3.3.2 (26).

RESULTS

The baseline characteristics of the 1,900 patients with type 2 diabetes aged ≥60 years are shown in Table 1. Their median age was 67 years, and 63.3% were male. In the cohort, 47.0% of patients had a family history of type 2 diabetes, 47.1% of patients had a BMI >30 kg/m^2, and 49.3% had previously smoked. The median HbA_{1c} level was 6.8% (51 mmol/mol). The median blood pressure was 151/84 mmHg, and the median levels of 8-oxoGuo and 8-oxodG were 2.83 nmol/mmol of creatinine (interquartile range [IQR] 2.32–3.46) and 1.72 nmol/mmol of creatinine (IQR 1.31–2.26), respectively. The additional biochemical measurements were within the normal range. Self-reported pharmacological treatments in the group were as follows: 38.9% of the patients were treated with ACE inhibitors, 19.2% were treated with angiotensin II receptor antagonists, 50.7% were treated with metformin, 75.9% were treated with lipid-lowering drugs, and 21.4% were treated with insulin. Women had higher baseline levels of both 8-oxoGuo and 8-oxodG than men (Table 1).

After 5 years of follow-up, 173 of the 1,863 patients with type 2 diabetes had died; of these, 73 died of cardiovascular disease. The age- and sex-adjusted hazard ratio (HR) for all-cause mortality was 2.19 (95% CI 1.71–2.80; $P < 0.001$), whereas that in the fully adjusted model was 2.10 (95% CI 1.63–2.71; $P < 0.001$) for a doubling of 8-oxoGuo levels; however, no significant difference was noted when 8-oxodG levels were considered. The cause-specific Cox regression model showed an age- and sex-adjusted HR for death due to cardiovascular disease of 1.92 (95% CI 1.26–2.91; $P = 0.002$), whereas that in the fully adjusted model was 1.82 (95% CI 1.20–2.77; $P = 0.005$) for a doubling of 8-oxoGuo levels; however, no significant difference was noted when 8-oxodG levels were considered (Fig. 1). Subanalyses for males in the cohort showed an age-adjusted HR for death of cardiovascular disease of 1.84 (95% CI 1.16–2.92; $P = 0.009$) and the fully adjusted model yielded an HR of 1.71 (95% CI 1.08–2.73; $P = 0.023$) for a doubling of 8-oxoGuo levels. Subanalyses for hypertensive patients showed an HR for all-cause mortality as 2.14 (95% CI 1.46–3.15; $P < 0.001$) in the age- and sex-adjusted model, and the fully adjusted model showed an HR of 2.13 (95% CI 1.44–3.14; $P < 0.001$) for a doubling in 8-oxoGuo levels. The HRs for cardiovascular death were unstable with wide CIs and nonsignificant P values.

The all-cause mortality across the quartiles of 8-oxoGuo and 8-oxodG is shown in Fig. 2. The 5-year AR of all-cause mortality was more than two times higher (AR 13.9 [95% CI 10.8–17.0] vs. AR 6.1 [95% CI 4.0–8.3]) for the highest quartile of 8-oxoGuo than that for the lowest. The Aalen-Johansen estimate showed that the 5-year AR of cardiovascular death was 1.7 times higher (AR 5.49 [95% CI 3.44–7.55] vs. AR 3.16 [95% CI 1.59–4.73]) for the highest quartile of 8-oxoGuo than that for the lowest (Fig. 3).

The Kendall τ rank correlation coefficient for HbA_{1c} and 8-oxoGuo was −0.01 ($P = 0.395$), whereas that for albuminuria and 8-oxoGuo was −0.03 ($P = 0.098$). Moreover, the coefficient for HbA_{1c} and 8-oxodG was −0.08 ($P < 0.001$), and that for albuminuria and 8-oxodG was −0.05 ($P < 0.001$).

CONCLUSIONS

In the current study, we found that high urinary excretion of 8-oxoGuo, a biomarker of RNA oxidation, predicts cardiovascular death in patients with type 2 diabetes. We conclude that high RNA oxidation, measured using the biomarker 8-oxoGuo, can be used to predict the macrovascular complications of type 2 diabetes, independent of the currently available biomarkers.

Nobel Prize winner J.D. Watson previously proposed type 2 diabetes as a redox disease (27). Although the concept of an imbalance between reductive and oxidative reactions is controversial, both the micro- and macrovascular complications of type 2 diabetes have been associated with ROS overproduction and mitochondrial dysfunction (13,14). However, whereas microvascular complications are reportedly related to hyperglycemia, macrovascular complications may be associated with insulin resistance and free fatty acid oxidation to a greater extent (9,11,12,15).

The relevance of oxidative stress or intracellular RNA oxidation in type 2

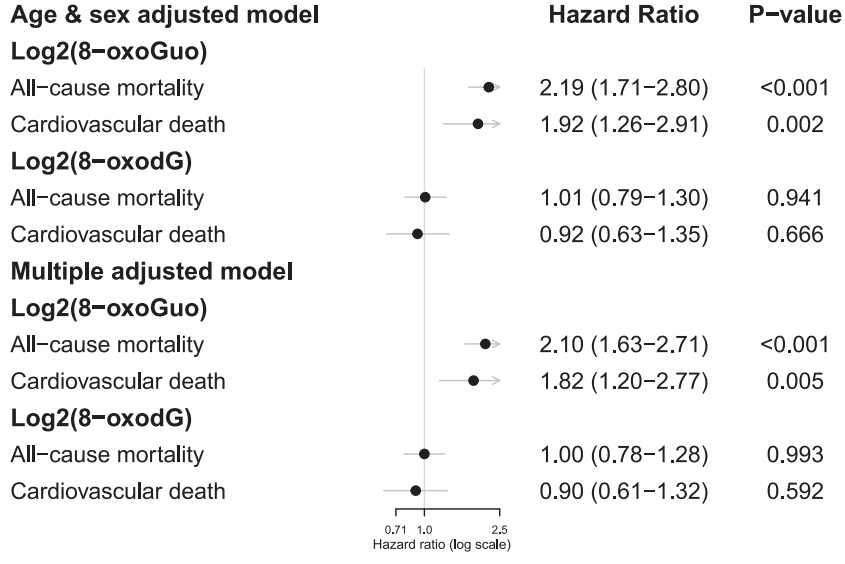
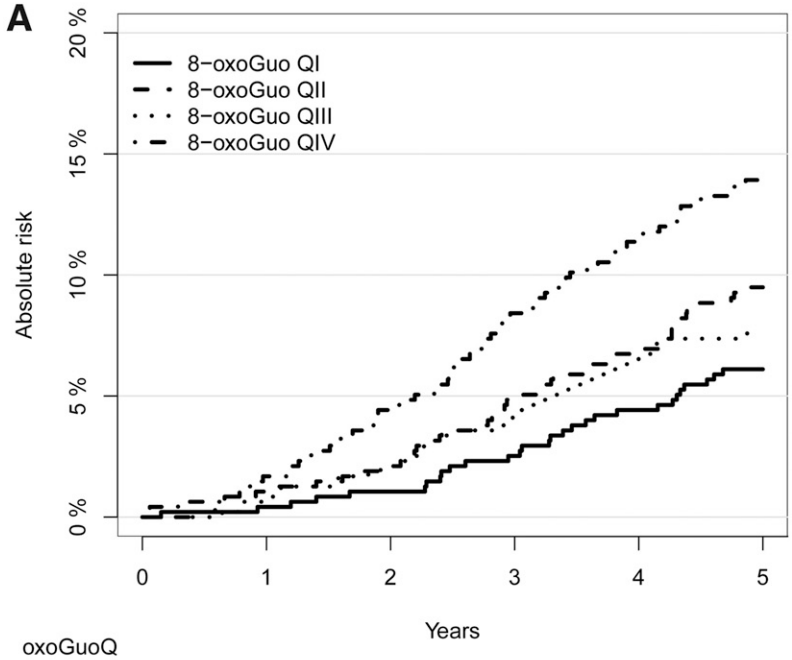
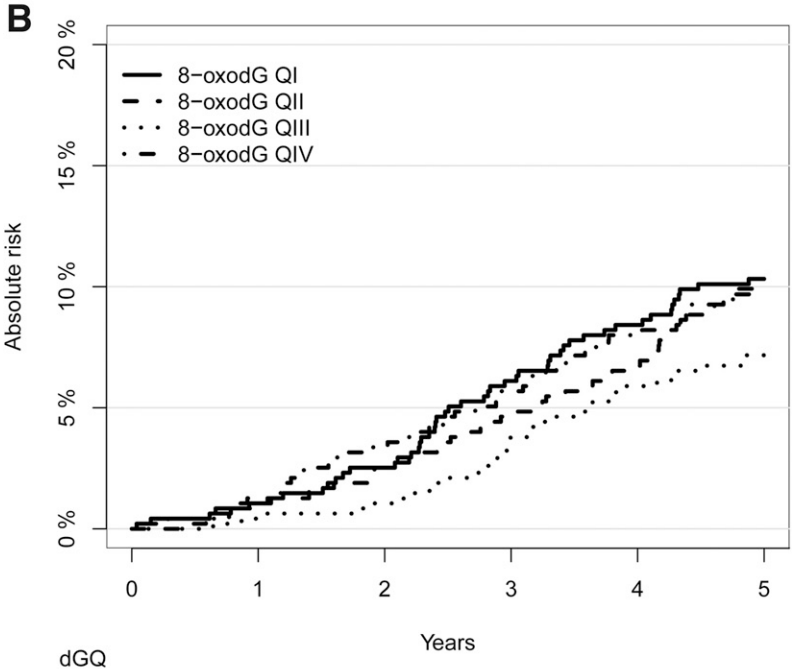


Figure 1—Multiple adjusted Cox regression analyses, which included mutual adjustments for age, sex, systolic blood pressure, smoking, albuminuria, BMI, LDL levels, and CRP levels. The overall number of patients with type 2 diabetes was 1,863 due to a number of missing values among the adjusted variables in the multiple Cox regression. Sex-stratified Cox models did not indicate any change in the results.



oxoGuoQ											
8−oxoGuo QI:	475	474	473	471	470	465	463	457	454	449	416
8−oxoGuo QII:	475	474	470	468	466	458	452	447	443	433	397
8−oxoGuo QIII:	475	475	471	469	465	459	454	449	444	440	414
8−oxoGuo QIV:	475	472	467	462	454	447	435	427	421	413	370



dGQ											
8−oxodG QI:	475	473	470	468	463	452	446	438	435	427	396
8−oxodG QII:	475	474	469	467	463	458	453	448	443	433	400
8−oxodG QIII:	475	475	472	472	470	465	457	453	447	444	411
8−oxodG QIV:	475	473	470	463	459	454	448	441	437	431	390

Figure 2—*A* and *B*: Kaplan-Meier estimates of AR (cumulative incidence) of all-cause mortality across the quartiles (QI to QIV) of 8-oxoGuo and 8-oxodG, with numbers at risk in each quartile shown below the plots.

diabetes is supported by evidence from animal models, and 8-oxoGuo has been found to be a more sensitive nucleic acid marker of diabetes morbidity and mortality than 8-oxodG (28). Moreover, a cross-sectional study indicated notably higher 8-oxoGuo levels in patients with type 2 diabetes with diabetic macrovascular complications, as well as higher 8-oxodG levels (although to a lesser extent) (29).

In this study, we did not observe any association between HbA_{1c} or plasma glucose and 8-oxoGuo, consistent with the view that 8-oxoGuo excretion provides additional information about deleterious intracellular oxidative imbalance that is not provided by blood glucose biomarkers.

A wide range of biomarkers are available for the diagnostic stratification, staging, and monitoring of treatment responses in type 2 diabetes. From a mechanistic point of view, the urinary marker of RNA modification by oxidation reflects intracellular events that are not indicated by HbA_{1c} or microalbuminuria. Oxidative modifications of nucleic acids have been extensively studied, although more evidence is available for the modification of DNA than of RNA (10). RNA repair mechanisms remain largely unresolved but are believed to involve degradation and elimination pathways (10). Nevertheless, evidence of RNA oxidation has emerged over the last decade. It has been postulated that up to 40% of RNA oxidation results from direct strand scission due to hydroxyl radicals mainly produced in the mitochondria (in the Fenton/Haber-Weiss reaction) during cell metabolism (30,31). In a previous review, we presented a number of studies showing that RNA is an important biomarker of disease (10). In fact, RNA is more prone to oxidation than nuclear DNA because of its cytosolic location closer to the mitochondria, single-stranded structure, and lack of protective proteins (10). Consequently, compared with nuclear DNA, RNA is a primary target for ROS-induced oxidative damage. Moreover, a previous study on Alzheimer disease examining neurons from human autopsies found that the level of oxidized RNA was higher than that of both oxidized nuclear and mitochondrial DNA (32).

Furthermore, RNA oxidation has been proposed as a mechanism of disease development by causing mutation or misfolding of proteins that have impaired functions and result in cellular stress upon accumulation (10,33). RNA oxidation has also been implicated in ribosomal stalling that leads to decreased protein expression and has also been associated with both coding and translational errors (31). The observation that the ROS-induced

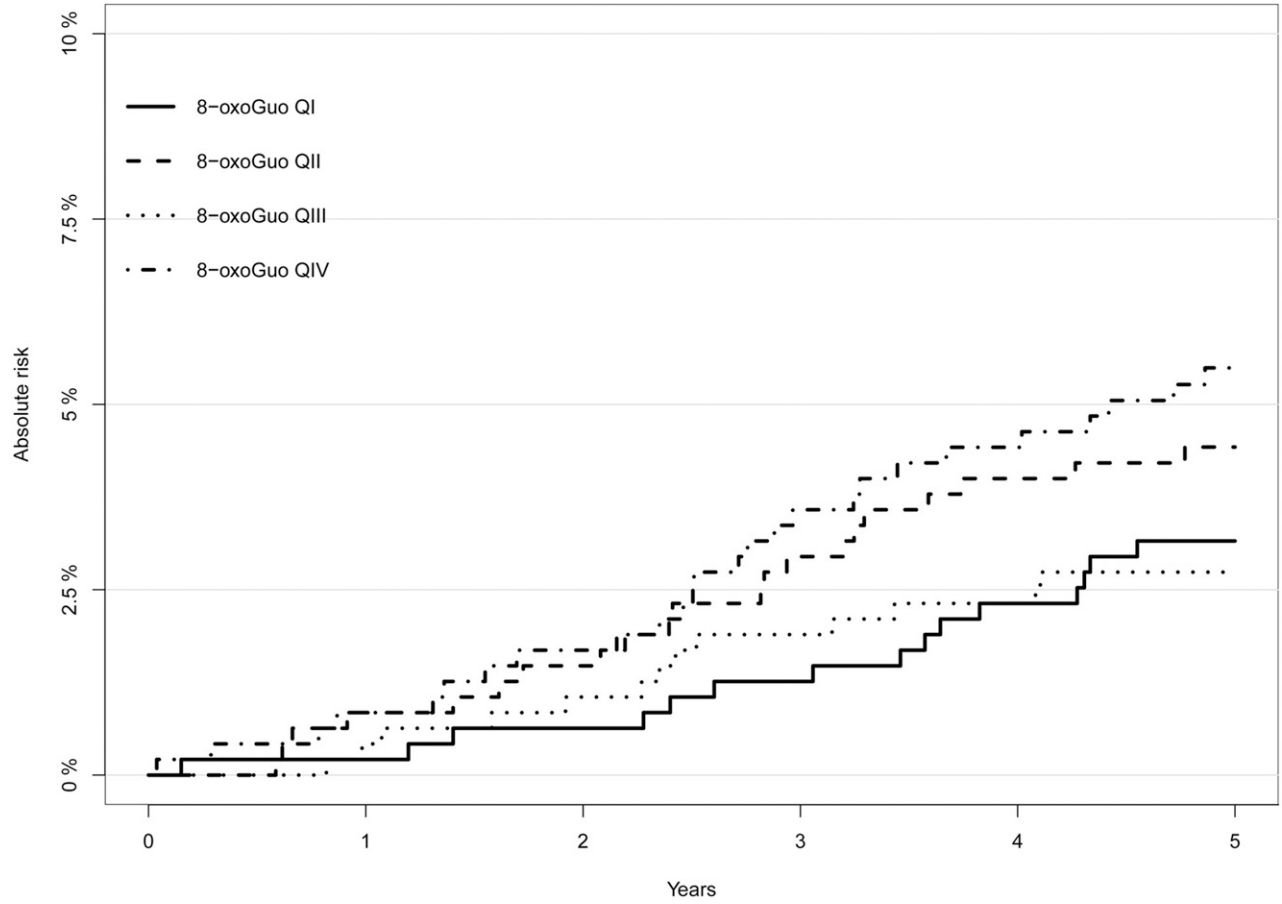


Figure 3—Aalen-Johansen estimates of AR (cumulative incidence) of cardiovascular death across the quartiles (QI to QIV) of 8-oxoGuo.

oxidative damage of certain microRNAs is linked to increased apoptosis in animal models of heart ischemia/reperfusion (34) also supports the hypothesis of oxidized RNA as a possible pathogenic mechanism.

The DNA nucleoside 8-oxodG was not associated with survival in this study. It is postulated that 8-oxodG reflects the events in the cell nucleus (35). Mitochondrial DNA constitutes 1% of total DNA, and oxidative damage to mitochondrial DNA is three- to ninefold greater than that of nuclear DNA in mammalian tissue (36); therefore we estimated that the contribution of mitochondrial DNA to oxidative damage is only 3–8%. The 8-oxodG derived from the cell nucleus is located at a considerable distance from the mitochondria, compared with the cytoplasmic location of RNA, and DNA is also protected by its double-stranded structure and histone proteins. Moreover, DNA has numerous DNA repair systems, such as the nucleotide and base excision repair mechanisms (10). Therefore, cell compartmentation and DNA repair mechanisms may be responsible for the difference in DNA and RNA oxidation levels.

To our knowledge, pharmacological targeting of the biomarkers of RNA or DNA oxidation has not yet revealed treatment options for patients with type 2 diabetes. In our previous studies, we did not find any alteration in RNA oxidation (measured by 8-oxoGuo levels) after short-term statin treatment in healthy volunteers (21), and we did not observe any effect of treatment with angiotensin II antagonists in patients with type 2 diabetes (37). However, the targeting of RNA oxidation via specific antidiabetic treatment may yield interesting results. The pivotal role of oxidative stress in diabetic complications, despite a proposed "unifying mechanism" for numerous pathways within the cells, complicates the progress of clinical beneficial antioxidants (11,15,16).

Evidence from diabetes and other fields (aging and other diseases with robust evidence of RNA oxidative damage) advocates a multilevel approach for antioxidant therapy in targeting ROS metabolism, compared with the simplistic interventions that have failed thus far (31). Yet, a growing body of evidence suggests that antioxidant substances in currently available antidiabetic therapy may help (16,17).

The strength of this study is the large size of the cohort, which was treated according to the best practice guidelines. There is evidence that high RNA oxidation is associated with an increased mortality risk in two independent cohorts with type 2 diabetes (18,19). A further strength of this study is the method used for quantifying RNA oxidation. On oxidation, RNA is degraded and the oxidized nucleoside is excreted into urine, which can be assessed and interpreted to reflect intracellular disturbances in metabolism (28). The measurement of the oxidative modified nucleoside ensures that it does not originate from intestinal absorption, thus minimizing the contribution of diet and bacterial breakdown (38,39).

The limitations of this study include the epidemiological design, the relatively

short follow-up duration, and the recording of late diabetes complications from hospital discharge diagnoses. We tested the prognostic aspects of the biomarker 8-oxoGuo (using the area under the curve and the Brier score) via bootstrapping cross-validation (data not shown). However, due to the low event rate, the prediction of mortality risk with 8-oxoGuo was not significantly better than survival models without 8-oxoGuo. The relatively low percentage of death due to cardiovascular disease could be explained by the short follow-up duration but may also depend on how clinicians report the causes of death in the death certificates. In the current study, we found that hypertensive patients had an increased risk of all-cause but not cardiovascular mortality. Although this observation may be attributed low power, we have recently observed that obesity in men, but not hypertension, is associated with high 8-oxoGuo levels (40).

Although not commercially available thus far, our method for analyzing 8-oxoGuo can be easily taught by skilled technicians at most hospital laboratories. Unlike the commercially available ELISA method, the ultraperformance liquid chromatography tandem mass spectrometry can distinguish between RNA and DNA modifications, as well as other epitopes. Thus, in the future, our analyses could be part of standard biomarkers used by the physicians in a clinical setting. Furthermore, using this method can unravel antioxidant features of currently available treatments in the clinic.

In summary, we found that 8-oxoGuo is associated with both all-cause and specific cardiovascular mortality risk in patients with type 2 diabetes, irrespective of the disease duration and HbA_{1c} levels. In addition, with this larger and up-to-date study, now two independent cohorts provide a rational foundation for the continued examination of the role of oxidative stress in type 2 diabetes and for the identification of novel drug targets and pharmacological therapies.

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