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Circulating Inflammatory Markers and the Risk of Vascular Complications and Mortality in People With Type 2 Diabetes and Cardiovascular Disease or Risk Factors: The ADVANCE Study

C-reactive protein (CRP), fibrinogen, and interleukin-6 (IL-6) are associated with cardiovascular disease (CVD) and death in general populations. However, studies of these factors in type 2 diabetes are limited. We studied their associations with the risk of major macrovascular events, microvascular complications, and mortality in patients with type 2 diabetes who participated in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) Study. Plasma CRP, fibrinogen, and IL-6 levels were determined in a case-cohort study (*n* = 3,865) nested within the 11,140 men and women with type 2 diabetes and baseline CVD or risk factors in the ADVANCE Study. All three biomarkers of inflammation were associated with an increased risk of macrovascular events and death in analyses adjusted for age, sex, and treatment groups. After further adjustment, only IL-6 was an independent predictor of macrovascular events (hazard ratio per SD increase 1.37 [95% CI 1.24–1.51]) and death (1.35 [1.23–1.49]). IL-6 significantly improved the prediction of macrovascular events and death. After adjustment, none of the markers predicted microvascular complications. We conclude that IL-6 levels, but not CRP or fibrinogen levels, add significantly to the prediction of macrovascular events and mortality in individuals with type 2 diabetes who have baseline CVD or risk factors.

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Type 2 diabetes mellitus (T2DM) is a major and growing health problem worldwide, increasing the risk of both macrovascular and microvascular disease, as well as nonvascular mortality (1). Although control of blood pressure, lipid, and blood glucose levels are proven strategies in reducing the risk of cardiovascular complications (2,3), other less classical risk factors contribute to the

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cardiovascular risk associated with T2DM (1). Inflammation plays a role in atherothrombosis and its clinical complications (4), and prospective studies of generally healthy persons (and meta-analyses of such studies) have established that circulating levels of inflammatory markers including C-reactive protein (CRP), fibrinogen, leukocytes, and albumin are associated with risks of macrovascular disease and total mortality (5–7). Persons with diabetes have higher levels of CRP (7) and fibrinogen (6) compared with those without diabetes, but limited numbers of individuals with diabetes have been included in published prospective studies of general populations (6,7); there are only three previous studies of cohorts with T2DM and CRP (8–10) or fibrinogen (11) levels. These previous reports studied only mortality (9–11) or cardiovascular events only in men (8). Hence, there is a need for studies to evaluate these associations in large cohorts of people with diabetes.

While they have several potential pathogenic roles, the causality of increased CRP and fibrinogen levels in individuals with cardiovascular disease (CVD) has not yet been established (6,7). They may be downstream markers of the expression of proinflammatory cytokines (12) such as the key "messenger" cytokine interleukin-6 (IL-6). A meta-analysis (13) of prospective studies of IL-6 in generally healthy persons has reported a stronger association of long-term IL-6 levels with risk of coronary heart disease (CHD) compared with CRP or fibrinogen levels; and (in contrast to CRP or fibrinogen) a recent meta-analysis (14) of genetic studies has suggested a causal role for IL-6 in the condition. Proinflammatory cytokines, including IL-6, may play a role in the pathogenesis of obesity and insulin resistance (15), and in the cardiovascular complications of obesity and T2DM (16,17). However, there is only one previous prospective study of IL-6 and complications in T2DM: the ESTHER Study (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung) (18,19), in which 161 subjects experienced a primary cardiovascular event, and IL-6 was associated only with the risk of cardiovascular events in those with renal dysfunction (19).

We therefore performed a nested case-cohort study of the associations of baseline circulating levels of CRP, fibrinogen, and IL-6 and risk of major macrovascular and microvascular complications and death from any cause in men and women with T2DM who had either baseline CVD or risk factors and who participated in the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) Study (clinical trial reg. no. NCT00145925, clinicaltrials.gov) (2,3). Our aims were *1*) to assess their potential clinical utility as predictors of vascular events and mortality; and *2*) to discuss their potential causal significance, which is currently being evaluated in other studies of functional genotypes and specific drug antagonists (6,7,14).

RESEARCH DESIGN AND METHODS

The design and results of the ADVANCE randomized clinical trial have been previously published (2,3,20). Participants were men and women in 20 countries from Asia, Australasia, Europe, and North America, aged ≥55 years, who had received a diagnosis of type 2 diabetes after the age of 30 years. In addition, they were required to have a history of CVD (stroke, myocardial infarction, transient ischemic attack, unstable angina, coronary or peripheral revascularization, amputation, macroalbuminuria, proliferative retinopathy or photocoagulation, macular edema, or blindess in one eye) or one or more additional cardiovascular risk factors (T2DM duration >10 years, age ≥65 years, current cigarette smoking, total cholesterol >6.0 mmol/L, HDL cholesterol <1.0 mmol/L, or microalbuminuria) (20). The study made two randomized comparisons: a double-blind assessment of the efficacy of fixed combination therapy with perindopril-indapamide (2 mg/0.625 mg for 3 months increasing, if tolerated, to 4 mg/1.25 mg) versus placebo, and an open-label evaluation of an intensive glucose-lowering regimen using modified release gliclazide, with a target HbA_{1c} level of ≤6.5%, versus standard guideline-based glycemic control. A total of 11,140 participants were randomized, and the median duration of follow-up was 5 years.

Nonfasting blood samples were taken at baseline, anticoagulated with EDTA, and stored centrally at −80°C for a median of 7.8 years prior to transportation to the University of Glasgow coagulation laboratory. Samples were available from all countries involved in the ADVANCE Study, except China and India, giving a total population of 7,376 trial participants who contributed samples. Using a nested case-cohort study design (21), a random subcohort of 3,500 samples was selected plus samples from all additional individuals who had experienced a macrovascular event or a microvascular complication, or had died during the follow-up period (n = 697). High-sensitivity CRP and fibrinogen levels were assayed by immunonephelometry (ProSpec; Dade Behring, Milton Keynes, U.K.) and high-sensitivity IL-6 levels by ELISA (R&D Systems, Oxford, U.K.). Intra-assay and interassay coefficients of variation were 4.7 and 8.3%, 2.6 and 5.3%, and 7.5 and 8.7%, respectively.

Major macrovascular events were cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. Major microvascular events were new or worsening nephropathy (i.e., development of macroalbuminuria, doubling of serum creatinine level to at least 200 μmol/L, need for renal replacement therapy, or death due to renal disease) or retinopathy (i.e., development of proliferative retinopathy, macular edema, diabetes-related blindness, or retinal photocoagulation therapy).

Hazard ratios for linear effects of each of the three inflammatory biomarkers on each of the three studied outcomes (major macrovascular events, worsening nephropathy, and worsening retinopathy) were obtained from weighted Cox regression models using the STSELPRE procedure for case-cohort analyses in the Stata package. CRP and IL-6 were log-transformed to remove the effects of their skewness. Linearity of relationships among log CRP, log IL-6, and fibrinogen was verified using restricted linear splines, and by dividing each variable into ordinal groups according to fifths and testing for linearity and nonlinearity (22). To enable direct comparison among the three biomarkers, results were produced for a 1 SD increment (based on the entire sample). Three models, with different sets of potential confounding variables, were fitted for each of the nine inflammatory biomarker/outcome combinations: model 1 with age, sex, and randomized treatment; model 2 with, in addition to the variables in model 1, duration of diabetes, current smoking, systolic blood pressure, BMI, albumin/creatinine ratio (AC ratio), estimated glomerular filtration rate (eGFR), glycated hemoglobin (HbA_{1c}), plasma glucose, total and HDL cholesterol, and triglycerides; and model 3 with, in addition to the variables in model 2, the other two biomarkers. For the variables found to be independent predictors in model 2, the ability to discriminate risk (23,24) and reclassify risk—using the integrated discrimination index (IDI) (25) and the net reclassification improvement (NRI) (25)—were assessed using methods suitable for survival data and applied to the subcohort.

RESULTS

Table 1 shows the baseline demographic, clinical, and laboratory characteristics of the case-cohort data set, classified by outcome status. Of the 4,197 participants, 332 (7%) had missing or unusable blood samples. Clinical details and the mean or median values of the inflammatory biomarkers relating to the remaining 3,865 individuals are shown in Table 1. The mean age of the study cohort was 66.9 years (SD 6.6), and 61% of the cohort were male. During 5 years of follow-up, 709 patients experienced a major macrovascular event, 439 patients experienced a microvascular complication, and 706 patients died (Table 1). (Note that patients could appear in more than one of the three columns.)

Table 1—Baseline demographic, clinical, and laboratory characteristics classified by outcome status

Characteristics	Macrovascular events (*n* = 709)	Microvascular events (*n* = 439)	Death (*n* = 706)	None of the three outcomes (*n* = 2,502)	Overall (*n* = 3,865)
Male subjects, *n* (%)	491 (69.3)	295 (67.2)	492 (69.7)	1,427 (57.0)	2,358 (61.0)
Current smokers, *n* (%)	105 (14.8)	61 (13.9)	119 (16.9)	363 (14.5)	583 (15.1)
History of CVD, *n* (%)	347 (48.9)	156 (35.5)	312 (44.2)	787 (31.5)	1,345 (34.8)
Age (years)					
Mean (SD)	69.01 (6.55)	66.50 (6.52)	69.96 (6.65)	66.08 (6.45)	66.88 (6.61)
Median (Q1, Q3)	69 (65, 74)	66 (61, 71)	70 (66, 75)	66 (61, 71)	67 (62, 71)
Duration of diabetes (years)	9.00 (6.99)	9.77 (6.88)	9.04 (7.47)	7.31 (6.08)	7.86 (6.41)
BMI (kg/m^2)	29.65 (4.99)	30.18 (5.39)	29.57 (5.12)	30.17 (5.31)	30.06 (5.26)
Blood pressure (mmHg)					
Systolic	150.94 (22.99)	150.54 (21.95)	149.84 (23.63)	146.31 (21.09)	147.55 (21.66)
Diastolic	81.61 (11.43)	81.14 (11.32)	80.77 (11.81)	81.91 (10.55)	81.62 (10.88)
Cholesterol (mmol/L)					
Total	5.10 (1.17)	5.12 (1.10)	5.05 (1.14)	5.17 (1.19)	5.13 (1.17)
HDL	1.17 (0.31)	1.18 (0.33)	1.18 (0.31)	1.25 (0.33)	1.22 (0.33)
Triglycerides (mmol/L)	1.61 (1.20, 2.30)	1.79 (1.22, 2.56)	1.60 (1.20, 2.30)	1.70 (1.20, 2.34)	1.70 (1.20, 2.35)
HbA_{1c} (%)	7.60 (1.58)	7.79 (1.57)	7.60 (1.56)	7.29 (1.33)	7.42 (1.43)
AC ratio (μg/mg)	22.22 (8.84, 70.72)	48.35 (14.14, 132.60)	21.22 (8.57, 65.42)	11.76 (5.57, 28.29)	15.03 (6.54, 41.42)
Glucose (mmol/L)	8.59 (2.83)	9.08 (3.44)	8.56 (2.93)	8.34 (2.53)	8.47 (2.72)
eGFR	67.45 (17.83)	67.06 (19.45)	66.43 (17.79)	73.31 (15.97)	71.48 (16.86)
Fibrinogen (g/L)	4.10 (1.03)	4.05 (1.06)	4.17 (1.16)	3.93 (0.92)	3.99 (0.98)
CRP (mg/L)	2.01 (0.93, 4.35)	1.72 (0.91, 3.52)	2.05 (1.01, 4.67)	1.74 (0.84, 3.91)	1.81 (0.88, 4.06)
IL-6 (pg/mL)	2.70 (1.94, 4.05)	2.37 (1.74, 3.45)	2.82 (1.98, 4.28)	2.13 (1.53, 3.11)	2.31 (1.62, 3.40)

Data are based on nonfasting blood samples. Values are given as mean (SD) or median (Q1, Q3), unless otherwise stated.

Supplementary Table 1 shows these results for subjects without a history of microvascular or macrovascular disease at baseline (n = 2,270).

Supplementary Table 2 shows the associations among CRP, fibrinogen, and IL-6 levels, and baseline levels of macrovascular CVD and risk factors (as well as associations among CRP, fibrinogen, and IL-6 levels), after adjustment for age, sex, and randomized treatment allocations. Male patients had lower levels of fibrinogen and CRP than female patients, but higher levels of IL-6. Current smokers had higher levels of all three biomarkers. IL-6, but not CRP or fibrinogen, was associated with baseline macrovascular CVD. All three biomarkers were modestly associated with BMI (Spearman correlations [r]: fibrinogen r = 0.133; CRP r = 0.242; IL-6 r = 0.207) and moderately with each other (fibrinogen and CRP r = 0.414; fibrinogen and IL-6 r = 0.301; CRP and IL-6 r = 0.500). Associations with other continuous variables were weaker.

After adjustment for age, sex, and randomized treatment (model 1), fibrinogen and IL-6 levels were associated with all three study outcomes, whereas CRP level was associated with macrovascular events and death, but not with microvascular complications (Table 2). Further adjustment for key clinical risk factors (model 2) removed the effects of fibrinogen and IL-6 levels on microvascular complications and fibrinogen on macrovascular events; the remaining associations were attenuated, but still significant (Table 2). The risk of a macrovascular event was increased by 11% for every extra SD of log CRP, and by 37% for every extra SD of log IL-6; corresponding results for death were similar, at 15 and 35%. After additional adjustments for the other two inflammatory markers (model 3), only the associations of IL-6 with macrovascular events and death remained significant (Table 2). Results were similar in participants with and without baseline CVD (Fig. 1), except that the positive association between fibrinogen and death was only significant in the subgroup without overt disease at baseline. Results were also similar in participants with (n = 1,047) and without (n = 2,818) renal dysfunction at baseline (Supplementary Fig. 1). The numbers of individual macrovascular and microvascular outcomes in the whole study population are shown in Supplementary Table 3. Supplementary Table 4 shows that IL-6 level was significantly ($P < 0.0001$) associated with risks of myocardial infarction, stroke, cardiovascular death, and noncardiovascular death but not with risks of new or worsening nephropathy, retinopathy, or neuropathy.

Table 3 shows the discrimination and reclassification statistics for all three inflammatory markers and outcomes. Although the IDI and (with one exception) the continuous version of the NRI were significant in each case, the change in C-statistic and categorical NRI were only significant in the case of IL-6, which added significantly to both the discrimination and reclassification of both macrovascular events and death. Discrimination increased by 0.012 for macrovascular events and by 0.016 for death. The categorical NRI values were 0.03 and 0.06 for macrovascular events and death, respectively.

Table 2—Risks of macrovascular events, microvascular events, and death for a 1 SD increment in circulating inflammatory biomarkers

Variables	Macrovascular events	Microvascular events	Death
CRP[a]			
Model 1	1.19 (1.09–1.29)	1.00 (0.91–1.11)	1.25 (1.15–1.36)
P value	0.001	0.95	0.001
Model 2	1.09 (0.99–1.19)	0.92 (0.83–1.02)	1.14 (1.04–1.26)
P value	0.07	0.10	0.005
Model 3	0.95 (0.85–1.05)	0.88 (0.77–1.00)	0.98 (0.88–1.09)
P value	0.30	0.05	0.69
Fibrinogen[b]			
Model 1	1.16 (1.07–1.26)	1.14 (1.02–1.27)	1.24 (1.14–1.36)
P value	0.005	0.02	0.001
Model 2	1.05 (0.95–1.15)	1.03 (0.92–1.15)	1.11 (1.01–1.23)
P value	0.35	0.63	0.03
Model 3	0.99 (0.90–1.09)	1.08 (0.94–1.23)	1.04 (0.93–1.15)
P value	0.84	0.28	0.51
IL-6[c]			
Model 1	1.41 (1.31–1.52)	1.15 (1.04–1.26)	1.46 (1.35–1.57)
P value	0.001	0.005	0.001
Model 2	1.31 (1.19–1.43)	1.00 (0.89–1.12)	1.36 (1.24–1.49)
P value	0.001	0.98	0.001
Model 3	1.34 (1.21–1.49)	1.04 (0.91–1.19)	1.36 (1.23–1.50)
P value	0.001	0.59	0.001

Data are as hazard ratios (95% CIs), unless otherwise stated. [a]Log scale; 1 SD = 1.1. [b]Log scale; 1 SD = 0.98. [c]Log scale; 1 SD = 0.6.

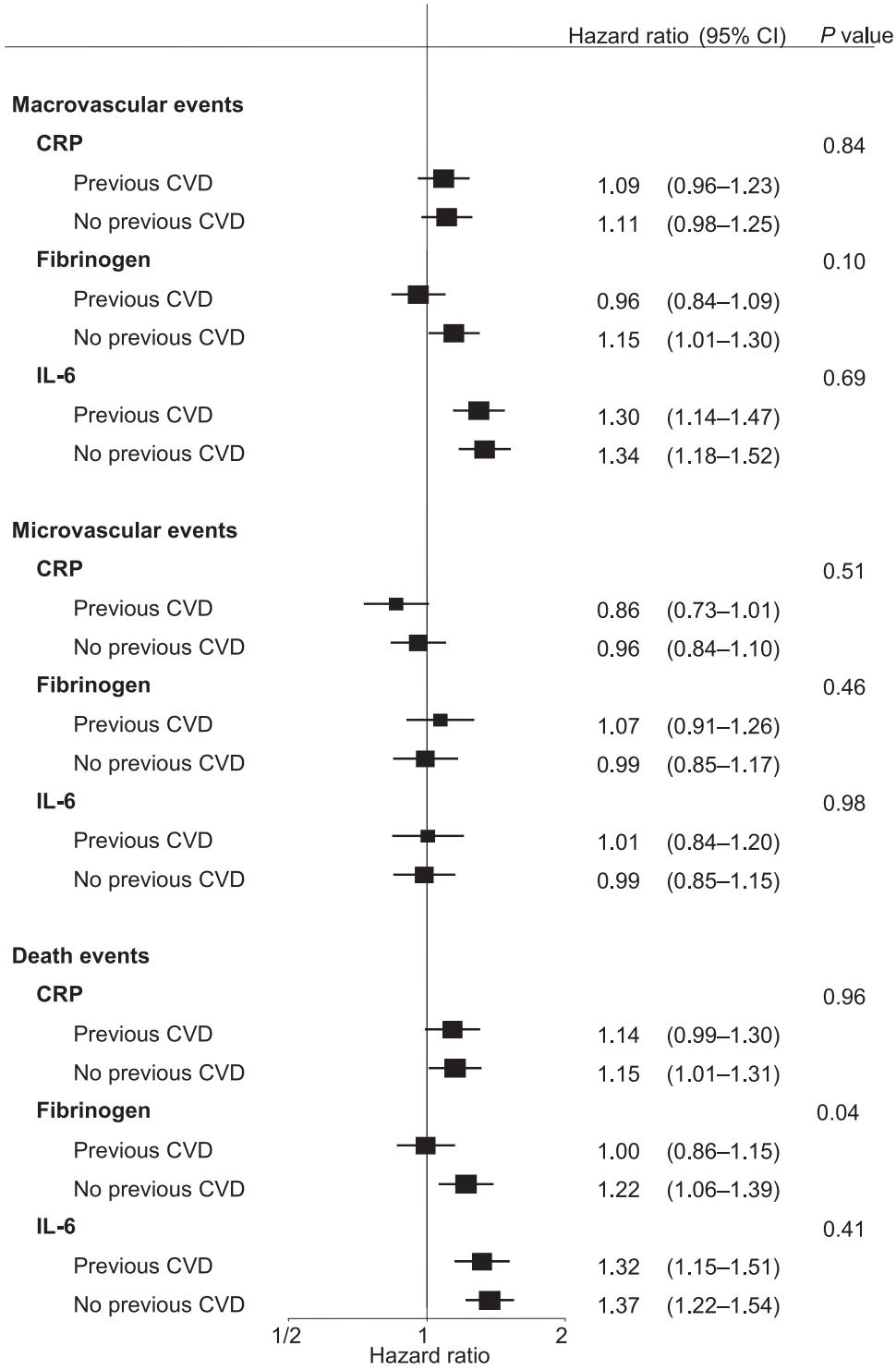

Figure 1—Comparison of associations of circulating inflammatory biomarkers with study outcomes in participants with and without previous CVD. The *P* values refer to tests of interaction by history of previous CVD.

DISCUSSION

This is the largest prospective study to date assessing the relationship among levels of circulating inflammatory markers, risk of vascular events (both macrovascular and microvascular), and death in persons with T2DM. It shows that, after adjustment for potential confounding factors, higher levels of CRP were associated with an increased risk of macrovascular events and mortality, whereas higher levels of fibrinogen were associated weakly with mortality. However, these associations were weak, and were abolished by further adjustment for IL-6. In contrast, IL-6 showed stronger associations with the

Table 3—Circulating inflammatory biomarkers after adjustment for age, sex, randomized treatment allocations, duration of diabetes, current smoking, history of CVD, systolic blood pressure, BMI, AC ratio, eGFR, HbA_{1c}, glucose, total and HDL cholesterol, and triglyceride levels

Variables	Macrovascular events	Microvascular events	Death
Base* C-statistic	0.682 (0.661–0.703)	0.730 (0.705–0.756)	0.686 (0.665–0.707)
CRP			
C-statistic	0.683 (0.662–0.705)	0.731 (0.705–0.756)	0.692 (0.671–0.712)
P value	0.33	0.44	0.09
IDI	0.0004 (−0.0017–0.0026)	0.016 (0.011–0.021)	0.007 (0.003–0.011)
P value	0.636	<0.001	<0.001
Relative IDI (%)	0.55 (−2.2–3.4)	23.6 (17.8–29.2)	10.5 (4.8–16.5)
NRI			
Continuous	0.008 (−0.124–0.131)	0.316 (0.175–0.443)	0.130 (0.004–0.253)
P value	0.17	<0.001	0.04
Categorical	0.002 (−0.019–0.024)	0.003 (−0.038–0.042)	−0.019 (−0.061–0.021)
P value	0.34	0.16	0.33
Fibrinogen			
C-statistic	0.684 (0.663–0.706)	0.731 (0.705–0.756)	0.688 (0.667–0.709)
P value	0.24	0.78	0.33
IDI	0.000 (−0.002–0.002)	0.013 (0.010–0.016)	0.009 (0.004–0.014)
P value	0.98	<0.001	<0.001
Relative IDI (%)	0.0 (−2.7–2.7)	19.2 (15.9–22.4)	13.0 (6.3–20.7)
NRI			
Continuous	0.057 (−0.070–0.184)	0.283 (0.145–0.413)	0.110 (−0.013–0.240)
P value	0.35	<0.001	0.08
Categorical	0.009 (−0.012–0.031)	−0.006 (−0.048–0.036)	0.005 (−0.029–0.042)
P value	0.41	0.76	0.51
IL-6			
C-statistic	0.692 (0.671–0.713)	0.731 (0.705–0.755)	0.702 (0.681–0.722)
P value	0.012	0.37	<0.001
IDI	0.011 (0.007–0.015)	0.013 (0.010–0.016)	0.024 (0.018–0.031)
P value	<0.001	<0.001	<0.001
Relative IDI (%)	13.7 (8.8–19.1)	19.4 (15.8–22.8)	36.9 (27.8–47.6)
NRI			
Continuous	0.194 (0.073–0.320)	0.206 (0.072–0.340)	0.295 (0.168–0.415)
P value	0.006	0.002	<0.001
Categorical	0.034 (−0.006–0.077)	0.008 (−0.031–0.045)	0.053 (0.002–0.103)
P value	0.09	0.50	0.04

Data are reclassification and discrimination statistics (95% CI), unless otherwise stated. NRI categories were 5 and 10% 5-year risk. Biomarkers were all analyzed in continuous form, after log transformations for CRP and IL-6. The *P* value for the C-statistic relates to the increase when adding the biomarker to the base model. *Using model 2, described in Table 2.

risk of macrovascular events and mortality and remained an independent predictor of these outcomes after adjustment for other risk factors and for levels of CRP and fibrinogen. In addition, the incorporation of IL-6 levels significantly, albeit modestly, improved risk prediction that was based on clinical factors alone. After adjustment for clinical risk factors, none of the three inflammatory markers were associated with future microvascular complications.

Relationships Between Inflammatory Biomarkers and Clinical Risk Factors

As expected (6,7,13), all three inflammatory markers were associated with BMI and current smoking. However, only IL-6 level was associated with pre-existing CVD. In addition, the levels of all three markers were modestly correlated.

CRP and Fibrinogen

Large individual patient meta-analyses of general population studies have suggested that the association between CRP and fibrinogen levels and CHD are weaker in patients with diabetes than those without the condition (6,7). In the most recent meta-analysis (26), the addition of CRP or fibrinogen to a clinical risk score in general populations increased the 10-year NRI by 1.52 and 0.83%, respectively. Previous reports of CRP or fibrinogen in cohorts with T2DM have reported associations with cardiovascular and total mortalities (9–11) or with cardiovascular events only in men (8). The current, and largest, study of all vascular complications and mortality in men and women with diabetes confirms previous reports that CRP (9,10) and fibrinogen (11) levels are associated with cardiovascular and total mortality,

confirms two previous reports (10,19) that CRP level is associated with cardiovascular events, and confirms that CRP level is not clinically useful for prediction of cardiovascular events (19) or mortality (9). Our study is also consistent with a recent report (27) from nine community-based prospective cohort studies, which observed that the associations of fibrinogen level with CVD and mortality (which were similar in people with and without diabetes) did not improve the predictive accuracy of established risk factors. In addition to such confirmation, our findings are novel in showing that neither CRP nor fibrinogen level is clinically useful for the prediction of macrovascular events, microvascular events, or total mortality.

IL-6

High IL-6 levels are associated with obesity and insulin resistance (15), and, together with other proinflammatory cytokines, may play a role in the pathogenesis of CVD (12–17). In two large population-based cohorts of middle-aged individuals without known CVD, and in an associated meta-analysis, higher IL-6 levels were associated with an increased incidence of CHD during long-term follow-up (13), an association that became more apparent after levels were corrected for intraindividual variation in IL-6 levels (13). However, there is only one previous prospective study associating levels of IL-6 with the risk of vascular complications or mortality in T2DM: the ESTHER Study (18,19), in which 161 subjects experienced a primary cardiovascular event and IL-6 was associated only with risk of cardiovascular events in those with renal dysfunction (19).

The current study is novel in that it provides the first evidence, in patients with diabetes, that IL-6 levels show significant associations (which are stronger than CRP or fibrinogen level) with macrovascular complications (n = 709) and mortality (n = 706) in patients with T2DM, and it shows that IL-6 level adds significantly to their prediction from conventional risk factors. The C-statistic for macrovascular events increased by 0.012 when IL-6 was added to the prognostic model; this should be interpreted as increasing the chance of correctly discriminating between a pair of subjects with diabetes, only one of whom will go on to experience a macrovascular event within 5 years, by 0.012 (or 1.2%). For death, the corresponding increase is estimated to be 0.016 (or 1.6%). In the area of CVD prognosis, it is notoriously difficult to achieve a substantial increase in the C-statistic, largely because the classical risk factors (especially age) discriminate so well already, and because the scale of C-statistics is narrow (0.5–1). Although 0.012 seems small in absolute terms, this level of increment is not atypical for novel cardiovascular biomarkers that have been suggested for clinical use, acknowledging the reality that the remaining unexplained risk is likely to be explained in many small accumulating steps. At least in part because of the difficulty in interpreting the C-statistic, Pencina et al. (25) introduced the IDI and NRI, used here in the form suitable for survival data. Of these, the one we consider to be most clinically relevant is the categorical NRI, which measures the net improvement in prediction across clinical risk thresholds (of 5 and 10% 5-year risk). The IDI and continuous (threshold-free) NRI are less clinically useful because they may be influenced by outliers. For macrovascular events, adding IL-6 to the base prognostic set improved the categorical NRI by 0.03, sometimes roughly interpreted as an improvement of 3%. For death, the same statistic was 0.06, roughly interpreted as a 6% improvement. Taking all the metrics together, we conclude that IL-6 gives a moderate improvement in predicting who will have a macrovascular event within the next 5 years and a more substantial, albeit not major, improvement in predicting who will die within 5 years among high-risk subjects with diabetes. We thus suggest that IL-6 levels be considered for inclusion in future clinical and biomarker prediction scores for T2DM patients.

With regard to translational potential, the strong association of IL-6 levels with macrovascular events in patients with type 2 diabetes was not attributable to confounding by conventional risk factors, and it highlights the potential causal importance of proinflammatory cytokine levels in the macrovascular complications of diabetes. A potential causal role for IL-6 level is suggested by a recent meta-analysis of general population studies (14) in which not only IL-6 levels but also an associated functional mutation in the IL-6 receptor gene (rs 8192284) was associated with CHD risk (a positive Mendelian randomization study). The IL-6 receptor is also a susceptibility location with genome-wide significance for coronary artery disease (28). In a recent report, an inflammatory risk score comprising five inflammatory gene polymorphisms, including a single nucleotide polymorphism in the IL-6 gene (rs 1800795), was associated with risk of ischemic stroke in a prospective cohort of subjects with T2DM (29). We suggest that further studies of IL-6 and IL-6 receptor gene polymorphisms are required to establish whether or not genetic determinants of IL-6 levels are associated with the macrovascular complications of T2DM. If so, the translational importance of our findings could be further investigated by studies of IL-6 antagonists (14). The current study suggests that a 1 SD reduction in log(IL-6) level by IL-6 antagonists might reduce the risk of myocardial infarction, stroke, or death by about a quarter.

In contrast to macrovascular events, none of the three inflammatory biomarkers was independently associated with the risk of microvascular events. These findings suggest that upregulated inflammation may be less important in pathogenesis of microvascular compared with macrovascular complications of type 2 diabetes. The absence of an association between inflammation and microvascular disease contrasts with cross-sectional data.

For example, in a nested case-control study of 543 patients with type 1 diabetes who participated in the Epidemiology and Prevention of Diabetes (EURODIAB) Prospective Complications Study, higher levels of CRP, IL-6, and tumor necrosis factor-α were associated with increased urinary albumin excretion and more severe retinopathy, even after adjustment for confounding factors such as age, sex, duration of diabetes, systolic blood pressure, and HbA_{1c} level (30). Similarly, in patients with T2DM, higher white cell counts, though still within the normal range, were associated with an increased prevalence of retinopathy and albuminuria (31).

Strengths and Limitations

The strengths of the current study include its size, international recruitment, rigorous definition of outcomes, completeness of follow-up, and adjustment for major risk factors. The case-cohort study is an ideal design for biomarker research when, as here, there are several outcomes of interest (21). We show a comprehensive set of reclassification statistics, using contemporary methods. Of these, we believe that the categorical NRI, with clinically relevant thresholds, is the most appropriate measure for clinical decision making since it estimates the independent effect of a biomarker without being affected by changes in risk estimation at relatively unimportant extreme values.

One limitation of our study is the selection of persons with T2DM who had either baseline CVD or risk factors. While our findings cannot be generalized to persons with T2DM who have neither CVD nor risk factors, the baseline characteristics of the ADVANCE Study cohort are comparable to several other observational studies at the community level (32), and hence it seems reasonable to conclude that its results are broadly generalizable, particularly for relative risks. Nevertheless, the selection criteria in the ADVANCE Study may have affected the IDI and NRI, which will not be constant across all subgroups; for example, each may differ between nonsmokers in the ADVANCE Study (who, by the selection criteria, had to have another risk factor if they were free of CVD) and nonsmokers in general diabetes populations aged ≥55 years. Another limitation is that only single measures of variables of interest were used in estimating the incremental effect of the biomarkers. However, single measures are the only viable option in risk scoring, the basic component of prognostic modeling, which was the primary focus of this research.

Conclusions

We conclude that IL-6 levels, but not CRP or fibrinogen levels, independently improve the clinical prediction of macrovascular events and mortality in persons with type 2 diabetes who have baseline CVD or risk factors. We also conclude that further studies exploring a potential causal role for IL-6 in the cardiovascular complications associated with type 2 diabetes are warranted, including studies of functional genotypes and, possibly, of IL-6 antagonists.

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Author Contributions. G.L. wrote the initial drafts of the manuscript, which were revised for scientific content by the other authors, and performed the laboratory analyses. M.W. wrote the initial drafts of the manuscript, which were revised for scientific content by the other authors, performed the statistical analyses, and designed the biomarker substudy. G.H. designed the biomarker substudy. A.R. performed the laboratory analyses. Q.L. performed the statistical analyses. S.H., M.M., P.H., A.P., N.P., and J.C. collected the data. M.W. 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.

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