Use of Vascular Assessments and Novel Biomarkers to Predict Cardiovascular Events in Type 2 Diabetes: The SUMMIT VIP Study

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OBJECTIVE

Cardiovascular disease (CVD) risk prediction represents an increasing clinical challenge in the treatment of diabetes. We used a panel of vascular imaging, functional assessments, and biomarkers reflecting different disease mechanisms to identify clinically useful markers of risk for cardiovascular (CV) events in subjects with type 2 diabetes (T2D) with or without manifest CVD.

RESEARCH DESIGN AND METHODS

The study cohort consisted of 936 subjects with T2D recruited at four European centers. Carotid intima-media thickness and plaque area, ankle-brachial pressure index, arterial stiffness, endothelial function, and circulating biomarkers were analyzed at baseline, and CV events were monitored during a 3-year follow-up period.

RESULTS

The CV event rate in subjects with T2D was higher in those with (n = 440) than in those without (n = 496) manifest CVD at baseline (5.53 vs. 2.15/100 life-years, P < 0.0001). New CV events in subjects with T2D with manifest CVD were associated with higher baseline levels of inflammatory biomarkers (interleukin 6, chemokine ligand 3, pentraxin 3, and hs-CRP) and endothelial mitogens (hepatocyte growth factor and vascular endothelial growth factor A), whereas CV events in subjects with T2D without manifest CVD were associated with more severe baseline atherosclerosis (median carotid plaque area 30.4 mm^2 [16.1–92.2] vs. 19.5 mm^2 [9.5–40.5], P = 0.01). Conventional risk factors, as well as measurements of arterial stiffness and endothelial reactivity, were not associated with CV events.

CONCLUSIONS

Our observations demonstrate that markers of inflammation and endothelial stress reflect CV risk in subjects with T2D with manifest CVD, whereas the risk for CV events in subjects with T2D without manifest CVD is primarily related to the severity of atherosclerosis.

Diabetes is an important risk factor for cardiovascular disease (CVD) and is associated with a twofold excess risk of acute myocardial infarction and stroke (1). A recent large Swedish registry study showed that although the incidence of cardiovascular (CV) events has declined substantially in subjects with diabetes between 1998 and 2014, it still remains significantly higher than in subjects without diabetes (2). With the

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worldwide adult prevalence of diabetes rising from 4.7% in 1980 to 8.5% in 2014, the CV complications of diabetes represent a major public health challenge (3). The increased CV risk associated with diabetes remains essentially the same when adjusting for conventional risk factors (1). Accordingly, traditional risk score calculators are less useful in diabetes (4,5). This has not been a major clinical concern because most guidelines have considered all subjects with diabetes as having high risk based on studies demonstrating that the CV risk is equivalent to subjects without diabetes with a previous coronary event (6). However, studies that are more recent have shown that the CV risk in type 2 diabetes (T2D) is highly heterogeneous and that many subjects with T2D have much lower risk of CVD than subjects with established CVD and no diabetes (7–10). Hence, there is an urgent need to improve CVD risk prediction in T2D.

The Innovative Medicine Initiative project SUMMIT (Surrogate Markers for Micro- and Macrovascular Hard Endpoints for Innovative Diabetes Tools) was initiated to identify novel markers for prediction of CV complications in diabetes. Given the poor risk prediction in individuals with diabetes based on traditional CV risk factors alone and the still elusive causes behind the increased CV risk in individuals with diabetes, we wanted to assess the ability of a panel of noninvasive vascular imaging, functional vascular tests, and emerging biomarkers to predict CV risk in subjects with T2D. To meet this end, we performed the SUMMIT Vascular Imaging Prediction (SUMMIT VIP) study. As there is a growing population of patients with T2D with clinically manifest CVD that is at a very high risk for new events (11), we included both subjects with and without prevalent CVD.

RESEARCH DESIGN AND METHODS

Study Population

The baseline study cohort consisted of 458 subjects with T2D and clinically manifest CVD (T2D/CVD) and 527 subjects with T2D but without clinical signs of CVD recruited from existing population cohorts and hospital registers at the university hospitals in Malmö (Sweden), Pisa (Italy), Dundee (U.K.), and Exeter (U.K.) between November 2010 and June 2013. Diabetes was defined based on contemporary or historical evidence of hyperglycemia (according to World Health Organization 1998 criteria; fasting plasma glucose >7.0 mmol/L or 2-h plasma glucose >11.1 mmol/L, or both) or by current medication with insulin, sulphonylureas, metformin, or other antidiabetic drugs. Subjects diagnosed with T2D <35 years of age or treated with insulin within 12 months of diagnosis were not included in the study. Classification of CVD included nonfatal acute myocardial infarction, hospitalized unstable angina, resuscitated cardiac arrest, any coronary revascularization procedure, nonfatal stroke, transient ischemic attack confirmed by a specialist, lower extremity artery disease defined as ankle-brachial pressure index (ABPI) <0.9 with intermittent claudication, or prior corrective surgery, angioplasty, or above-ankle amputation. T2D with and without CVD were matched at each center for sex, age (±5 years), and duration of diabetes (±5 years). Exclusion criteria included renal replacement therapy, malignancy requiring active treatment, end-stage renal disease, any chronic inflammatory disease on therapy, previous bilateral carotid artery invasive interventions, or atrial fibrillation. Subjects with T2D with CVD were excluded if the CVD event occurred >5 years prior to the diagnosis of T2D. Demographics and clinical characteristics, including medication and physical and laboratory examinations, were obtained according to a predefined study protocol at all four participating centers. Study subjects were invited to a follow-up visit after 36 months, and information of incident CVD events (same criteria as used for inclusion) was recorded. A total of 760 study subjects (81.2%) attended the follow-up visit. For those that did not attend the follow-up visit, information regarding clinical events was obtained through medical records or telephone interviews. Forty-nine subjects (5.0%) were lost to follow-up. The study was approved by the local ethical review boards and performed in accordance with the principles of the Declaration of Helsinki. All study subjects provided written informed consent.

Vascular Assessments

Intima-media thickness (IMT) in the right and left common carotid artery (CCA) and the carotid bulbs, as well as total carotid plaque area, was determined by ultrasound. Plaques were defined as focal thickenings (≥0.8 mm) of the artery wall. The length and height of each individual plaque were measured to calculate plaque area. The interobserver variability of plaque area measurements was 8.9 ± 4.6%. The total plaque area represents the sum of the area of all plaques identified in the left and right carotid arteries. In average, we identified 2.4 plaques per study subject. The median height of the plaques was 1.9 mm (interquartile range [IQR] 1.5–2.5) and the median length 11.2 mm (IQR 8.0–15.9). Segments with plaques were included in the IMT measurements. Endothelial function was measured using an EndoPat device (Itamar Medical, Caesarea Industrial Park, Israel) to estimate the endothelium-dependent postischemic hyperemia in response to 5 min of arterial occlusion. Arterial stiffness was assessed by calculating carotid-femoral pulse wave velocity (PWV) using a Sphygmocor device (Atcor Medical, West Ryde, New South Wales, Australia). Left and right ABPI were calculated. The ABPI was calculated as the ratio between the highest systolic blood pressure value from the foot and the highest blood pressure from the arm on the same side of the body. Detailed information about the methods used for vascular assessments, as well as data regarding intra- and interobserver variability and calibration between centers, has been published previously (12).

Biomarker Analysis

Plasma levels of biomarkers reflecting inflammation (interleukin 6 [IL-6], chemokine ligand 3 [CCL3], and pentraxin 3), endothelial growth activation (hepatocyte growth factor, placental growth factor, and vascular endothelial growth factor A [VEGF A]), extracellular matrix proteolysis (matrix metalloproteinase 3, 7, and 12 [MMP-3, -7, and -12]), apoptosis (Fas, TNF receptor 1, and TRAIL receptor 2), as well as other emerging CV risk markers (N-terminal prohormone of brain natriuretic peptide [NT-proBNP], growth differentiation factor 15 [GDF-15], and fatty acid binding protein 4 [FABP-4]) were analyzed by the proximity extension assay technique using the Proseek Multiplex CVD$^{96\times96}$ reagents kit (Olink Bioscience, Uppsala, Sweden) at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden, as previously described (13). All samples

were analyzed in the same run. Data analysis was performed by a preprocessing normalization procedure using Olink Wizard for GenEx (Multid Analyses, Gothenburg, Sweden). Values are presented as arbitrary units. Data regarding intra- and interassay variations as well as general calibrator curves to calculate the approximate concentrations are available on the OLINK home page (http://www.olink.com).

Statistics

Values are presented as mean and SD for continuous variables with normal distribution and as median and IQR for skewed variables. Biomarker values were log transformed when used in statistical analyses. Differences in clinical characteristics between the groups with or without new CV events were investigated using χ^2, Student *t*, or Mann-Whitney *U* tests, as appropriate. Logistic regression was used to test for associations between baseline clinical characteristics and incident CV events (fatal or nonfatal) in subjects with T2D and prevalent CVD at baseline. The additional value of biomarkers to a reference model to predict CV events during follow-up was assessed by the integrated discrimination improvement (IDI) and by comparing areas under the receiver operating characteristic (AUROC) curves. Analyses were done using SPSS statistics version 22 and in R version 3.3.0 (using the PredictABEL package to calculate IDI and the pROC package to compare AUROCs). All statistical analyses were done in accordance with the original protocol of the study.

RESULTS

The baseline investigation included 458 subjects with T2D and CVD (myocardial infarction, stroke, or lower extremity arterial disease) and 527 subjects with T2D but without clinically manifest CVD. The clinical characteristics of the study cohort have been previously published (12). Fatal and nonfatal CV events were registered during a 3-year follow-up period. Forty-nine subjects (5.0%) were excluded from the study due to lack of information on clinical events during follow-up. Of the remaining 936 subjects, 105 suffered a CV event during follow-up (3.6 CV events/100 life-years). A breakdown of the components of the composite incident CV events in the two groups is shown in Supplementary Table 1. There were also 12 deaths from noncardiovascular causes and 8 deaths from unknown cause. Subjects with T2D and manifest CVD at baseline had a more than twofold higher CV event rate than those free of CVD at baseline (5.5 vs. 2.2/100 life-years, $P < 0.0001$).

Markers for CV Events at Follow-up in Subjects With T2D and Manifest CVD

There were no differences in major CV risk factors between subjects with or without CV events during follow-up in the two study groups (Table 1). Occurrence of a new CV event in the T2D/CVD group was associated with higher baseline HbA_{1c} (Table 2). Table 2 also shows CV and antidiabetic medications at the baseline and follow-up visits. Insulin treatment was more common among those with a new event. However, when including both HbA_{1c} and insulin treatment in a binary logistic regression model together with age, sex, duration of diabetes, smoking, BMI, triglycerides, LDL, HDL, and estimated glomerular filtration rate, only HbA_{1c} remained significantly associated with a new CV event (hazard ratio 1.03 [95% CI 1.01–1.03]). There was no major change in the type of antidiabetic treatment during the study period. Subjects with a CV event during follow-up were more often on statin therapy at the follow-up visit (Table 2).

With the exception of an increased IMT in the left carotid bulb, there were no significant differences in carotid IMT, total carotid plaque area, PWV, endothelial reactivity, or ABPI between those with and without a new CV event (Table 3). However, baseline plasma levels of endothelial mitogens and biomarkers reflecting inflammation, such as IL-6, CCL3, pentraxin 3, and hs-CRP, as well as MMP-12, NT-proBNP, and FABP-4, were higher in subjects with a new event (Table 3). In subjects with T2D and manifest CVD, the discrimination slope of a binary logistic regression model with IL-6 and risk factors (age, sex, duration of diabetes, current smokers, total cholesterol, HDL, HbA_{1c}, systolic blood pressure, and ethnicity) was significantly

Table 1—Baseline clinical characteristics for subjects with diabetes with or without a CV event during follow-up

	CVD at baseline (*n* = 440)			No CVD at baseline (*n* = 496)		
	No CV event (*n* = 367)	CV event (*n* = 73)	*P*	No CV event (*n* = 464)	CV event (*n* = 32)	*P*
Age (years)	69.4 ± 8.5	69.3 ± 8.7	NS	66.5 ± 8.7	68.2 ± 6.1	NS
Sex (% males)	73.4	65.6	NS	62.5	62.5	NS
Current smokers (%)	9.5	16.4	NS	9.1	15.6	NS
Duration of diabetes (years)	12.1 ± 8.6	13.5 ± 8.8	NS	9.1 ± 7.0	11.5 ± 6.3	NS
BMI (kg/m^2)	29.9 ± 4.7	30.7 ± 5.6	NS	30.6 ± 5.4	30.4 ± 4.8	NS
Lipids						
LDL (mmol/L)	2.06 ± 0.77	2.08 ± 0.75	NS	2.41 ± 0.93	2.24 ± 0.76	NS
HDL (mmol/L)	1.20 ± 0.36	1.19 ± 0.33	NS	1.32 ± 0.38	1.30 ± 0.41	NS
Triglycerides (mmol/L)	1.42 (1.02–2.08)	1.45 (1.05–1.84)	NS	1.35 (1.00–1.97)	1.40 (0.90–2.43)	NS
Blood pressure						
Systolic (mmHg)	138 ± 20	140 ± 17	NS	136 ± 18	137 ± 17	NS
Diastolic (mmHg)	76 ± 10	74 ± 9	NS	78 ± 10	77 ± 9	NS
Renal function						
eGFR (mL/min/1.73 m^2)	74.8 ± 26.9	78.0 ± 27.6	NS	85.1 ± 20.7	81.0 ± 20.0	NS

Variables with normal distribution are shown as mean ± SD and skewed variables as median (IQR). Statistical comparisons between subjects with and without events during follow-up were done using Student *t* test for variables with normal distribution and with Mann-Whitney *U* test for skewed variables. χ^2 test was used for categorical variables. eGFR, estimated glomerular filtration rate.

Table 2—Antidiabetic medication and HbA_{1c} at the baseline and 3-year follow-up investigation

	CVD at baseline (n = 440)			No CVD at baseline (n = 496)		
	No CV event (n = 367)	CV event (n = 73)	P	No CV event (n = 464)	CV event (n = 32)	P
Baseline						
Statin (%)	88.9	80.6	(0.05)	61.3	75.0	NS
ACE inhibitors (%)	54.1	44.4	NS	38.5	34.4	NS
β-Blockers (%)	57.4	56.9	NS	17.8	9.4	NS
Antiplatelet (%)	71.9	75.0	NS	24.6	31.2	NS
Glitazones (%)	6.3	3.0	NS	7.2	15.6	NS
Metformin (%)	65.0	61.4	NS	71.4	81.3	NS
Insulin (%)	29.3	45.7	0.007	15.8	25.0	NS
Sulfonylurea (%)	29.9	20.0	NS	29.7	21.8	NS
DPP-4 inhibitors (%)	11.3	4.3	NS	11.3	6.3	NS
Incretin analogs (%)	5.5	2.9	NS	5.2	3.1	NS
HbA_{1c} (mmol/mol)	57.7 ± 12.9	62.8 ± 18.7	0.036	56.1 ± 13.6	59.3 ± 12.9	NS
HbA_{1c} (%)	7.43 ± 1.18	7.90 ± 1.71	0.036	7.28 ± 1.24	7.56 ± 1.18	NS
	No CV event (n = 276)	CV event (n = 51)	P	No CV event (n = 397)	CV event (n = 24)	P
Follow-up						
Statin (%)	79.7	94.6	0.03	63.1	75.0	NS
ACE inhibitors (%)	47.0	43.2	NS	36.3	55.0	NS
β-Blockers (%)	59.9	60.5	NS	19.6	15.0	NS
Antiplatelet (%)	79.1	72.7	NS	26.5	55.0	0.008
Glitazones (%)	4.7	0	NS	5.5	5.0	NS
Metformin (%)	63.5	65.8	NS	68.9	85.0	NS
Insulin (%)	30.0	44.7	NS	21.6	35.0	NS
Sulfonylurea (%)	27.3	21.6	NS	25.4	30.0	NS
DPP-4 inhibitors (%)	13.4	8.1	NS	13.4	15	NS
Incretin analogs (%)	6.5	2.7	NS	5.7	0	NS
HbA_{1c} (mmol/mol)	46.8 ± 23.8	39.3 ± 23.6	NS	43.1 ± 23.3	50.1 ± 32.2	NS
HbA_{1c} (%)	6.43 ± 2.18	5.75 ± 2.16	NS	6.10 ± 2.13	6.79 ± 2.94	NS

Data are percentage of subjects treated with each medication, except HbA_{1c} values are shown as mean ± SD. Between-group comparisons were done using Student t test. χ^2 test was used for categorical variables. DPP-4, dipeptidyl peptidase 4.

improved (by 2.7% points) compared with a model without IL-6 (IDI 0.027 [95% CI 0.0064–0.048], P = 0.010). Similarly, the discrimination slope of a binary logistic regression model with hs-CRP and risk factors was significantly improved (by 1.6% points) compared with a model without hs-CRP (IDI 0.016 [95% CI 0.0025–0.031], P = 0.021). The AUROC curve was significantly increased with the addition of IL-6 (P = 0.02) or hs-CRP (P = 0.02) to the risk factor model (AUROC of IL-6 and risk factor model 0.68 [95% CI 0.60–0.75], AUROC of hs-CRP and risk factor model 0.68 [95% CI 0.61–0.75], AUROC of risk factor model 0.60 [95% CI 0.51–0.69]; P = 0.02). Addition of hs-CRP to the IL-6 model did not significantly increase the AUROC further. Risk reclassification with the addition of IL-6 or hs-CRP to the model was mainly downward (Fig. 1*A–D*).

Markers for CV Events at Follow-up in Subjects With T2D Without Manifest CVD

There were no significant differences in conventional CV risk factors or medication at baseline between those with and without a CV event during follow-up in

Table 3—Baseline vascular measurements in subjects with diabetes with or without a CV event during follow-up

	CVD at baseline (n = 440)			No CVD at baseline (n = 496)		
	No CV event (n = 367)	CV event (n = 73)	P	No CV event (n = 464)	CV event (n = 32)	P
CCA IMT, right (mm)	0.97 ± 0.25	0.92 ± 0.20	NS	0.89 ± 0.20	1.00 ± 0.23	0.002
Carotid bulb IMT, right (mm)	1.14 (0.96–1.62)	1.38 (1.01–1.84)	NS	1.03 (0.87–1.24)	1.28 (0.85–1.55)	(0.07)
CCA IMT, left (mm)	0.97 ± 0.25	0.87 ± 0.25	NS	0.92 ± 0.24	1.07 ± 0.49	0.001
Carotid bulb IMT, left (mm)	1.13 (0.95–1.47)	1.27 (1.03–1.67)	0.045	1.05 (0.88–1.27)	1.20 (0.95–1.78)	0.04
Total plaque area (mm^2)	30.4 (15.3–61.4)	36.0 (17.6–68.6)	NS	19.5 (9.5–40.5)	30.4 (16.1–92.2)	0.01
PWV (m/s)	11.8 ± 3.2	11.3 ± 2.3	NS	10.9 ± 2.6	11.6 ± 2.5	NS
Reactive hyperemia index	2.10 ± 0.56	2.16 ± 0.55	NS	2.20 ± 0.65	2.04 ± 0.79	NS
ABPI, right	1.11 ± 0.22	1.05 ± 0.28	(0.07)	1.20 ± 0.15	1.20 ± 0.32	NS
ABPI, left	1.11 ± 0.23	1.10 ± 0.28	NS	1.18 ± 0.28	1.18 ± 0.29	NS

Variables with normal distribution are shown as mean ± SD and skewed variables as median (IQR). Statistical comparisons between subjects with and without events during follow-up were done using Student t test for variables with normal distribution and with Mann-Whitney U test for skewed variables.

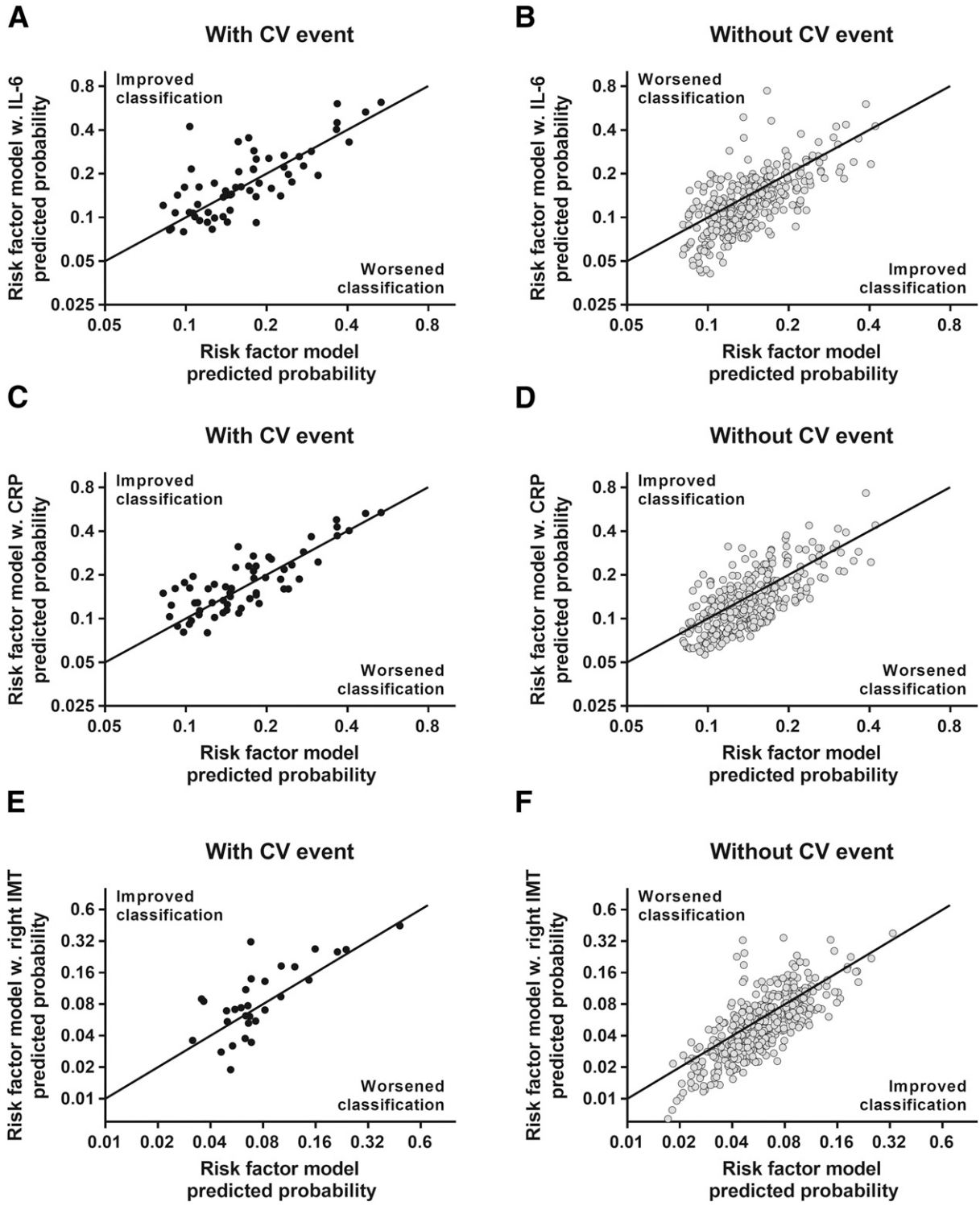

Figure 1—Scatter plots of predicted probabilities of risk factor models with and without biomarkers. Predicted probabilities for models with IL-6 in addition to risk factors (age, sex, duration of diabetes, current smokers, total cholesterol, HDL, HbA_{1c}, systolic blood pressure, and ethnicity) in subjects with T2D with manifest CVD and with CV event (*A*) or without CV event (*B*) during follow-up. Predicted probabilities for models with hs-CRP in addition to risk factors in subjects with T2D with manifest CVD and with CV event (*C*) or without CV event (*D*) during follow-up. Predicted probabilities for models with right CCA IMT in addition to risk factors in subjects with T2D without manifest CVD and with CV event (*E*) or without CV event (*F*) during follow-up. The 45° line designates equal predicted probabilities of the models.

the T2D/non-CVD group (Tables 1 and 2). Subjects with a CV event during follow-up were more often on antiplatelet therapy at the follow-up visit (Table 2).

Those with a CV event had increased IMT in both the left and right bulb and the right CCA, as well as an increased total carotid plaque area (Table 3). PWV, endothelial reactivity, and ABPI were not associated with the occurrence of CV events. Subjects with CV events also had higher baseline plasma levels of the

apoptosis marker TRAIL receptor 2 and of GDF-15, but did not demonstrate the same elevation in endothelial mitogens and inflammatory biomarkers as subjects with T2D with manifest CVD that suffered a new event (Table 4). In the T2D/non-CVD group, the discrimination slope of a binary logistic regression model with right CCA IMT and risk factors (age, sex, duration of diabetes, current smokers, total cholesterol, HDL, HbA_{1c}, systolic blood pressure, and ethnicity) was significantly improved (by 2.4% points) compared with a model without IMT (IDI right CCA IMT 0.024 [95% CI 0.0035–0.045], $P = 0.022$) (Fig. 1*E* and *F*). There was no significant difference in AUROC with the addition of right CCA IMT to the risk factor model ($P = 0.10$).

CONCLUSIONS

Using a panel of conventional risk factors, vascular assessments, and emerging biomarkers, we demonstrate in the current study that different markers predict risk for CV events in patients with T2D with and without manifest CVD. Subjects with T2D with manifest CVD that developed a new event had higher baseline plasma levels of hs-CRP, proinflammatory cytokines, endothelial mitogens, MMP-12, FABP-4, and the cardiac stress marker NT-proBNP but were not characterized by more severe atherosclerosis as assessed by carotid IMT (except from a marginally thicker IMT in left carotid bulb) or ABPI. The biological process that results in elevated levels of endothelial mitogens remains to be fully characterized, but it is likely to involve endothelial stress. Except for a higher HbA_{1c}, there were no differences in conventional risk factors between those with and without a new CV event. NT-proBNP is an established marker of CV risk. Notably, NT-proBNP only predicted CV events in subjects with established CVD in the current study. Other studies have identified elevated NT-proBNP as a CV risk factor in subjects with T2D (14), but to our knowledge, it has previously not been shown that this primarily is the case for subjects with T2D with prevalent CVD. Increased arterial stiffness and endothelial dysfunction as assessed by reduced vasodilatation after transient ischemia are well-established vascular complications in diabetes and have been associated with increased CV risk (15–18). In accordance, subjects with T2D were found to have increased PWV and a lower reactive hyperemia index at the SUMMIT VIP baseline investigation (12). In spite of this, neither of these measures predicted the occurrence of a new event in subjects with established CVD in the current study.

Development of a CV event in subjects with T2D without manifest CVD at baseline was associated with increased carotid atherosclerosis as assessed by the CCA and carotid bulb IMT, as well as by increased total carotid plaque area at the baseline investigation. However, biomarkers were less good predictors, with only GDF-15 and the apoptosis marker TRAIL receptor 2 being higher in those with a CV event. Moreover, there were no differences in conventional risk factors between those with and without a CV event.

Our observations are in accordance with previous observations that conventional risk factors are poor predictors of CV events in subjects with T2D; however, they suggest some important alternatives. We found that biomarkers reflecting inflammation, as well as endothelial and cardiac stress, are predictors of CV events in subjects with diabetes and manifest CVD, whereas carotid IMT is a better predictor of risk in subjects with diabetes without manifest CVD. Increased carotid IMT is a well-established CV risk factor in the general population (19). In accordance, subjects with T2D with manifest CVD at the baseline

Table 4—Baseline biomarkers in subjects with diabetes with or without a CV event during follow-up

	CVD at baseline (n = 440)			No CVD at baseline (n = 496)		
	No CV event (n = 367)	CV event (n = 73)	P	No CV event (n = 464)	CV event (n = 32)	P
Inflammation						
IL-6	42.8 (29.8–68.1)	58.5 (42.1–93.5)	0.00005	34.1 (23.8–52.7)	39.5 (24.2–58.0)	NS
CCL3 (MIP-1α)	4.8 (3.9–5.9)	5.1 (4.2–6.7)	0.008	4.6 (3.9–5.9)	4.7 (3.9–5.4)	NS
Pentraxin 3	2.1 (1.7–2.6)	2.3 (2.0–2.7)	0.043	2.1 (1.7–2.6)	2.1 (1.8–2.6)	NS
hs-CRP (mg/L)	1.46 (0.69–3.30)	2.74 (1.30–4.68)	0.00005	1.48 (0.66–2.95)	2.20 (0.70–4.38)	NS
Endothelial mitogens						
Hepatocyte growth factor	122 (95–148)	134 (107–169)	0.029	110 (88–135)	112 (89–146)	NS
Placental growth factors	189 (153–253)	207 (156–250)	NS	167 (138–204)	184 (143–223)	0.08
VEGF A	1,520 (1,199–1,934)	1,624 (1,246–2,131)	0.045	1,409 (1,136–1,783)	1,558 (1,199–1,824)	NS
Matrix proteolysis						
MMP-3	2.6 (2.1–3–5)	2.6 (2.2–3.3)	NS	2.4 (1.9–2.9)	2.2 (2.0–2.6)	NS
MMP-7	517 (333–780)	545 (342–750)	NS	410 (282–580)	539 (347–691)	NS
MMP-12	172 (11–249)	204 (147–289)	0.025	125 (92–180)	130 (102–234)	0.09
Apoptosis						
TNF receptor 1	7,231 (5,743–9,153)	7,033 (5,873–9,793)	NS	6,295 (5,220–7,591)	6,451 (5,433–7,899)	NS
TRAIL receptor 2	3.9 (2.7–5.3)	4.2 (2.8–5.4)	NS	3.3 (2.5–4.1)	4.0 (3.1–4.4)	0.039
Fas	231 (186–274)	218 (179–276)	NS	210 (175–247)	212 (169–254)	NS
Other						
NT-proBNP	26.2 (14.3–43.6)	38.6 (20.5–58.9)	0.001	14.3 (9.8–26.0)	16.2 (10.3–22.7)	NS
GDF-15	1,458 (1,044–2,154)	1,541 (1,143–2,073)	NS	1,121 (830–1,632)	1,483 (1,180–1,898)	0.005
FABP-4	10.7 (7.8–14.9)	13.7 (8.5–19.8)	0.01	9.6 (7.3–12.6)	10.6 (7.6–17.2)	NS

All data are median (IQR). hsCRP values are mg/L while all others are given as arbitrary units. Statistical comparisons between subjects with and without events during follow-up were done on log_2-transformed values using Student t test. MIP-1α, macrophage inflammatory protein 1α.

investigation had significantly greater carotid IMT than those without manifest CVD (12). Hence, there seems to be a clear association between atherosclerosis severity and CV risk in subjects with T2D, but this association diminishes in subjects with manifest CVD. One possible explanation for this could be that a more intense medical intervention in subjects with manifest CVD allows other risk factor mechanisms than those traditionally associated with atherosclerosis progression to become more important as causes of CV events (20). Hence, biomarkers that associate with CV events in this group could provide information regarding such alternative mechanisms. In the present studies, we found that subjects with new events had higher baseline levels of proinflammatory biomarkers and endothelial mitogens, suggesting the presence of an inflammatory state involving endothelial stress that persists in the presence of statin treatment. In this context, it is interesting to note that the recently published Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) showed that IL-1β antibody treatment lowered the rate of recurrent events in patients with a history of myocardial infarction and elevated hs-CRP in spite of statin treatment (21). The mechanisms that maintain vascular inflammation in statin-treated patients remain to be fully characterized but may involve factors such as altered shear stress over stenotic plaques, intraplaque accumulation of cholesterol crystals, autoimmune responses against modified plaque antigens, and chronic infections (22). It is also possible that the difference in factors predicting CV events in subjects with T2D with and without clinically manifest CVD is due to a more advanced stage of vascular disease in the former group.

Our study has both strengths and limitations. The strengths include the comprehensive vascular assessments in combination with a number of established and emerging biomarkers reflecting possible mechanisms responsible for development of CV complications in subjects with T2D. The study is also unique in that it compares risk assessments in subjects with or without established CVD. The lack of assessments of the coronary arteries and the relatively limited number of CV events during follow-up, particularly in the group without CVD at baseline, represent important limitations. As we used treatment with antidiabetic medication to define the presence of T2D, we cannot exclude that some subjects with prediabetes were included in the study. However, it is unlikely that this should have any major influence on the results of the study. Finally, we used a lower threshold for defining the presence of carotid plaques (focal IMT thickenings ≥0.8 mm) than that used in many other studies.

In conclusion, our observations demonstrate that markers of inflammation and endothelial stress are elevated in subjects with T2D with manifest CVD that develop a new event, suggesting that these patients may benefit from novel anti-inflammatory CV therapy. The risk for CV events in subjects with T2D without manifest CVD is primarily related to the severity of atherosclerosis.

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