Prognostic Importance of C-Reactive Protein in High Cardiovascular Risk Patients With Type 2 Diabetes Mellitus: The Rio de Janeiro Type 2 Diabetes Cohort Study

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Background—The prognostic value of C-reactive protein (CRP) is controversial in type 2 diabetes mellitus. We aimed to assess it in a cohort of high cardiovascular risk diabetic patients.

Methods and Results—CRP was measured at baseline and during the second year of follow-up in 616 patients. The primary end points were a composite of total fatal and nonfatal cardiovascular events (CVEs), major CVEs, and all-cause and cardiovascular mortalities. Association between baseline and second-year CRP with end points were evaluated by multivariable Cox survival analyses. Baseline median CRP was 2.8 mg/L (interquartile range: 1.2–6.0 mg/L), and 47.8% of the patients either increased or persisted with high CRP levels during the first 2 years of follow-up. After a median follow-up of 8.4 years, 131 total CVEs occurred (89 major CVEs), and 129 patients died (53 of cardiovascular causes). Baseline and second-year CRP, analyzed as a continuous variable and dichotomized at >3.0 mg/L, were significantly associated with total and major CVEs occurrence (with adjusted hazard ratios between 1.22 and 1.34 for increments of 1-SD log of continuous CRP, and between 1.47 and 1.89 for dichotomized CRP), but not with mortality. Additionally, increasing CRP levels or persisting with high levels were associated with a 1.84 (95% CI: 1.10–3.06) excess risk of major CVEs, independent of baseline CRP values.

Conclusions—Baseline and serial changes in CRP levels provide cardiovascular risk prediction independent of standard risk factors and glycemic control, and may be useful to refine cardiovascular risk stratification in high-risk patients with type 2 diabetes mellitus. (***J Am Heart Assoc***. 2016;5:e004554 doi: 10.1161/JAHA.116.004554)

Key Words: cardiovascular events • C-reactive protein • predictors • type 2 diabetes mellitus

Systemic inflammation has great importance in the development and progression of atherosclerosis.[1] C-reactive protein (CRP), a pentameric acute-phase reactant molecule primarily produced by hepatocytes in response to stimulation from interleukin-6 and tumor necrosis factor, hence a distal biomarker of inflammation,[2] is a potential marker of cardiovascular risk in diverse clinical scenarios, such as in patients with hypertension,[3,4] and with stable and unstable coronary heart disease,[5,6] as well as in apparently healthy populations.[7,8] Values of serum CRP above 3.0 mg/L are generally regarded as related to a worse cardiovascular prognosis in clinical practice, although the importance of CRP to improving cardiovascular risk prediction besides conventional risk factors still remains disputable; and it is generally recommended to be useful in patients at intermediate risk (5–20% cardiovascular risk at 10 years).[9,10]

Patients with type 2 diabetes mellitus are usually considered per se as at intermediate-to-high cardiovascular risk. Nevertheless, the prognostic importance of CRP in patients with type 2 diabetes mellitus is still controversial, with some studies showing that it was associated with occurrence of cardiovascular events (CVEs),[11–17] whereas other studies have rejected this observation.[18–22] The factors underlying these disparate findings are not clear, but may involve several factors, such as different diabetic populations with different baseline cardiovascular risks and CRP values, and different assessed cardiovascular end points over variable follow-up periods. Otherwise, few studies evaluated serial changes in CRP levels during follow-up as a potential additional cardiovascular risk marker.[15,21,22] The Rio de Janeiro Type 2 Diabetes (RIO-T2D) cohort study is a prospective observational cohort of high-risk type 2 diabetic individuals, most with already-established micro- or macrovascular complications,

with up to 11 years of follow-up under standardized treatment and detailed serial collection of several clinical and laboratory variables.[23–27] Here, we aimed to assess the value of baseline and serial changes in CRP levels during the first 2 years of follow-up as a factor associated with the future occurrence of CVEs and mortality.

Methods

Patients and Baseline Procedures

This was a prospective study with the first 616 patients from the RIO-T2D cohort study enrolled between August 2004 and December 2010 in the type 2 diabetes mellitus outpatient clinic of our tertiary-care University Hospital and followed up until December 2015. All participants gave a written informed consent and the local Ethics Committee had previously approved the study protocol. The enrollment criteria, baseline protocol, and diagnostic definitions have been detailed previously.[23–27] In brief, inclusion criteria were all adult type 2 diabetic individuals up to 80 years old with either any microvascular or macrovascular complication, or with at least 2 other modifiable cardiovascular risk factors. Exclusion criteria were morbid obesity (body mass index ≥40 kg/m^2), advanced renal failure (serum creatinine >180 μmol/L or estimated glomerular filtration rate <30 mL/min per 1.73 m^2) or the presence of any serious concomitant disease limiting life expectancy. For this study, patients with any chronic inflammatory disease, such as bronchial asthma, rheumatoid arthritis, systemic lupus erythematosus, chronic virus hepatitis and inflammatory bowel disease, or with acute infections at the time of blood sampling were excluded. All were submitted to a standard protocol that included a complete clinical examination, laboratory evaluation, and 24-hour ambulatory blood pressure (BP) monitoring. Diagnostic criteria for diabetic chronic complications were detailed previously.[23–27] In brief, coronary heart disease was diagnosed by clinical, electrocardiographic criteria, or by positive ischemic stress tests. Cerebrovascular disease was diagnosed by history and physical examination and peripheral arterial disease by ankle–brachial index <0.9. Diabetic retinopathy was evaluated by an ophthalmologist. The diagnosis of nephropathy required at least 2 albuminurias ≥30 mg/24 h or confirmed reduction of glomerular filtration rate (<60 mL/min per 1.73 m^2, estimated by the Chronic Kidney Disease Epidemiology Collaboration equation; or serum creatinine >130 μmol/L). Peripheral neuropathy was ascertained by clinical examination (knee and ankle reflex activities, and feet sensations with the Semmes-Weinstein monofilament, vibration with a 128-Hz tuning fork, pinprick, and temperature.

Clinic BP was measured 3 times using a digital oscillometric BP monitor (HEM-907XL; Omron Healthcare, Kyoto, Japan)

with a suitable sized cuff on 2 occasions 2 weeks apart at study entry. The first measure of each visit was discarded and BP considered was the mean between the last 2 readings of each visit. Arterial hypertension was diagnosed if mean systolic BP was ≥140 mm Hg or diastolic BP was ≥90 mm Hg or if antihypertensive drugs had been prescribed. Ambulatory BP monitoring was recorded in the following month using Mobil O Graph (version 12) equipment. Parameters evaluated were mean 24-hour systolic blood pressure and diastolic blood pressure. Laboratory evaluation included fasting glycemia, glycated hemoglobin, serum creatinine, and lipids. Albuminuria was evaluated in 2 nonconsecutive sterile 24-hour urine collections. CRP levels were measured at baseline and during the second year of follow-up by high-sensitivity immunonephelometry (lower detection limit: 0.1 mg/L; intra-assay and interassay variation coefficients: 3.1–4.0% and 2.5–3.8%, respectively). Any CRP value above 10 mg/L was repeated, and the lowest value was considered. For the second-year CRP analyses, 25 patients were excluded because of having a CVE during the first 2 years of follow-up and 78 patients because of not collecting the second-year CRP sample, totaling 513 patients in these analyses.

Follow-Up and End Points

The patients were followed up regularly at least 3 times a year, or more frequently as needed, until December 2015. The observation period for each patient was the number of months from the date of the first clinical examination to the date of the last clinical visit in 2015 or the date of the first end point. Except for the deceased ones, no patient was lost from follow-up. There were 4 primary end points: a composite of all fatal or nonfatal CVEs, major CVEs, all-cause mortality, and cardiovascular mortality. Total CVEs were the following: fatal or nonfatal acute myocardial infarctions, sudden cardiac deaths, new-onset heart failure, death from progressive heart failure, any myocardial revascularization procedure (either surgical or not), fatal or nonfatal strokes, any aortic or lower limb revascularization procedure (surgical or not), any amputation above the ankle, and deaths from aortic or peripheral arterial disease. Major CVEs were nonfatal acute myocardial infarctions and strokes plus all cardiovascular deaths. End points were ascertained from medical records, death certificates, and interviews with attending physicians and patient families, by a standard questionnaire reviewed by 2 independent observers.

Statistical Analysis

Continuous variables were described as means and SD or medians and interquartile range). Survival analyses were performed by Kaplan–Meier estimation of event-free survival

curves, compared by log-rank tests, and by multivariate Cox proportional hazards regression. For patients with multiple events, analysis was restricted to the first event under study. Baseline and second-year CRP were analyzed both as a continuous variable ($\log_e$-transformed due to its skewed distribution), and dichotomized at 2 cut-off values: the classic one (3.0 mg/L), and the upper tertile subgroup (in comparison to the lower tertile group). To assess CRP changes during the first 2 years of follow-up, patients were divided into 2 subgroups based on having either increased or persisted with high CRP, or on having either reduced or persisted with low CRP on the second-year measurement in relation to the baseline measurement. To define these subgroups, we categorize CRP levels at baseline into quartiles and CRP levels were considered to be unchanged if CRP at the second-year examination fell within the same quartile. If this occurred on the first and second quartiles, then patients were considered to have persisted with low CRP values; and if this occurred on the third and fourth quartiles, then patients were considered as persisting with high CRP values. CRP was considered to have decreased if the second-year CRP changed to a lower quartile (for example, from the fourth quartile at baseline to the third quartile on the second year, and so on); and it was considered to have increased if it changed to a higher quartile (eg, from the first quartile at baseline to the second quartile on the second year, and so on). This means that a patient in the second quartile at the second-year examination, for example, could be in the subgroup of patients who persisted with low CRP values (if he/she was also in the second quartile of CRP at baseline), in the subgroup who increased CRP (if he/she was in the first quartile at baseline), or in the subgroup who decreased CRP (if he/she was in the third or fourth quartiles at baseline). For statistical analyses, the subgroups that persisted with low values or decreased CRP, and the subgroups that persisted with high values or increased CRP were joined into single groups (with the subgroup who persisted with low or decreased CRP as the reference subgroup). First, CRP parameters were adjusted for age and sex and then fully adjusted for all potential risk factors: age, sex, body mass index, diabetes mellitus duration, smoking status, physical inactivity, arterial hypertension, number of antihypertensive drugs in use, 24-hour systolic blood pressure, presence of macrovascular and microvascular complications at baseline, mean glycated hemoglobin, high-density lipoprotein and low-density lipoprotein cholesterol during the first year of follow-up, and insulin, statins, and aspirin use. CRP changes analysis was further adjusted for their respective baseline values. Results were presented as hazards ratios with their 95% CIs. The proportional hazards assumption was tested by inspection of log-minus-log curves and no violation was observed. To assess whether CRP improved risk prediction, we compared the predictive performance of Cox models with and without CRP by calculating the Akaike information criterion (AIC), which carries a penalty for the number of variables used in the model and therefore can be compared directly across models with differing numbers of variables.[28] A lower AIC value indicates a better prediction. In interaction analysis, interaction terms were tested between dichotomized CRP ($\geq$3.0 mg/L) and age (<60 or $\geq$60 years), sex, obesity (body mass index <30 or $\geq$30 kg/m^2), presence of macrovascular and microvascular complications, and glycemic control (mean HbA$_{1c}$ <7.5% or $\geq$7.5%). In alternative analyses, patients with events during the first 2 years of follow-up were excluded from examination for possible reverse causality between CRP levels and outcomes, and patients with CRP values >10.0 mg/L (34 patients, 5.5%) were excluded because of other potential inflammatory conditions for CRP elevation. Statistics were performed with SPSS version 19.0 (SPSS Inc, Chicago, IL), and a 2-tailed P<0.05 was considered significant.

Results

Baseline Characteristics and Follow-Up End Points

Baseline median CRP was 2.8 mg/L (interquartile range: 1.2–6.0 mg/L), and median second-year CRP was 2.8 mg/L (interquartile range: 1.5–6.1 mg/L). Overall, 268 patients (52.2%) either persisted with low CRP levels or decreased CRP between baseline and second-year of follow-up, whereas 245 patients (47.8%) either increased or persisted with high CRP levels. Table 1 outlines the baseline characteristics of all 616 patients and of those with baseline high ($\geq$3.0 mg/L) and low serum CRP levels. High CRP was significantly associated with a higher proportion of young people, higher proportion of women, and higher proportion of obese people. Patients with high CRP levels had equal prevalences of chronic diabetic complications, except for a lower prevalence of retinopathy, used insulin more frequently, and had higher mean first-year HbA$_{1c}$, serum triglycerides, and estimated glomerular filtration rate than those with low CRP levels. They had higher clinic diastolic blood pressure and 24-hour systolic blood pressure levels, but were on similar antihypertensive treatment, except for a borderline greater use of β-blockers.

During a median follow-up of 8.4 years (maximum 11 years), which corresponds to 4650 patient-years until death or end of follow-up, there were 131 total CVEs (21%, crude incidence of 2.99 per 100 patient-years during 4377 patient-years of follow-up), and 89 major CVEs (14%, incidence of 1.96 per 100 patient-years during 4539 patient-years of follow-up). There were 129 all-cause

Table 1. Characteristics of All Diabetic Patients and Divided According to High or Low CRP at Baseline

| Variables	All Patients (n=616)	Low CRP (<3.0 mg/L) (n=323)	High CRP (≥3.0 mg/L) (n=293)	P Value
Age, y	60.1 (9.4)	61.1 (9.2)	58.9 (9.5)	0.005
Male sex, %	36.9	42.5	30.6	0.003
BMI, kg/m^2	29.6 (4.8)	28.7 (4.5)	30.5 (4.9)	<0.001
Smoking, current/past, %	45.1	46.2	44.0	0.63
Physical activity, %	22.6	24.6	20.3	0.21
Diabetes mellitus duration, y	8 (3–15)	8 (3–16)	8 (3–14)	0.55
Chronic diabetic complications, %				
Cerebrovascular disease	9.1	8.0	10.3	0.33
Coronary artery disease	15.7	14.2	17.5	0.27
Peripheral arterial disease	16.9	15.1	19.0	0.20
Retinopathy	32.6	36.4	28.6	0.037
Nephropathy	30.8	29.7	31.9	0.60
Peripheral neuropathy	28.6	30.7	26.2	0.24
Cardiovascular autonomic neuropathy	24.0	23.5	24.6	0.84
Diabetes mellitus treatment, %				
Metformin	87.5	85.8	89.3	0.22
Sulfonylureas	44.0	46.2	41.6	0.26
Insulin	47.9	40.9	55.7	<0.001
Aspirin	90.7	92.6	88.7	0.096
Arterial hypertension, %	86.5	84.3	89.0	0.098
Number of antihypertensive drugs	3 (1–3)	2 (1–3)	3 (1–4)	0.091
ACE inhibitors/AR blockers, %	83.8	83.2	84.4	0.74
Diuretics, %	67.7	66.0	69.5	0.38
Calcium channel blockers, %	31.2	30.8	31.6	0.86
β-Blockers, %	50.1	46.7	53.9	0.085
Clinic SBP, mm Hg	140 (19)	139 (18)	141 (20)	0.21
Clinic DBP, mm Hg	79 (11)	78 (10)	81 (11)	0.001
Ambulatory 24-hour SBP, mm Hg	129 (15)	127 (14)	130 (16)	0.012
Ambulatory 24-hour DBP, mm Hg	74 (10)	73 (9)	74 (10)	0.21
Dyslipidemia, %	87.5	86.8	88.3	0.63
Statins use, %	76.6	76.6	76.6	0.99
Laboratory variables				
Fasting glucose, mmol/L	8.9 (3.7)	8.7 (3.5)	9.2 (4.0)	0.14
Mean first-year HbA$_{1c}$, %	7.7 (1.5)	7.6 (1.4)	7.8 (1.6)	0.019
Triglycerides, mmol/L	1.56 (1.08–2.24)	1.46 (1.03–2.13)	1.64 (1.21–2.30)	0.005
HDL-cholesterol, mmol/L	1.14 (0.31)	1.14 (0.31)	1.11 (0.28)	0.23
LDL-cholesterol, mmol/L	2.79 (0.85)	2.74 (0.83)	2.84 (0.88)	0.13
Serum creatinine, μmol/L	79 (24)	80 (23)	79 (24)	0.60
Glomerular filtration rate, mL/min per 1.73 m^2	92 (34)	89 (29)	96 (37)	0.010
Albuminuria, mg/24 h	13 (7–41)	12 (7–38)	15 (7–42)	0.22
Number of total CVEs (incidence rate, per 100 patient-years of follow-up)	131 (2.99)	56 (2.33)	75 (3.80)	0.007

Table 1. Continued

Variables	All Patients (n=616)	Low CRP (<3.0 mg/L) (n=323)	High CRP (≥3.0 mg/L) (n=293)	P Value
Number of major cardiovascular events (incidence rate, per 100 patient-years of follow-up)	89 (1.96)	31 (1.24)	58 (2.84)	<0.001
Number of all-cause deaths (incidence rate, per 100 patient-years of follow-up)	129 (2.77)	58 (2.31)	71 (3.32)	0.040
Number of cardiovascular deaths (incidence rate, per 100 patient-years of follow-up)	53 (1.14)	23 (0.92)	30 (1.40)	0.12

Values are proportions, and means (SDs) or medians (interquartile range), except for incidence rates of end points that are absolute number (rate per 100 person-years). ACE indicates angiotensin-converting enzyme; AR, angiotensin II receptor; BMI, body mass index; CRP, C-reactive protein; CVEs, cardiovascular events; HbA$_{1c}$, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

deaths (2.77 per 100 patient-years), 53 from cardiovascular diseases (1.14 per 100 patient-years). Patients with high serum CRP at baseline had a greater incidence of CVEs and all-cause deaths during follow-up than those with lower CRP levels (Table 1 and Figure).
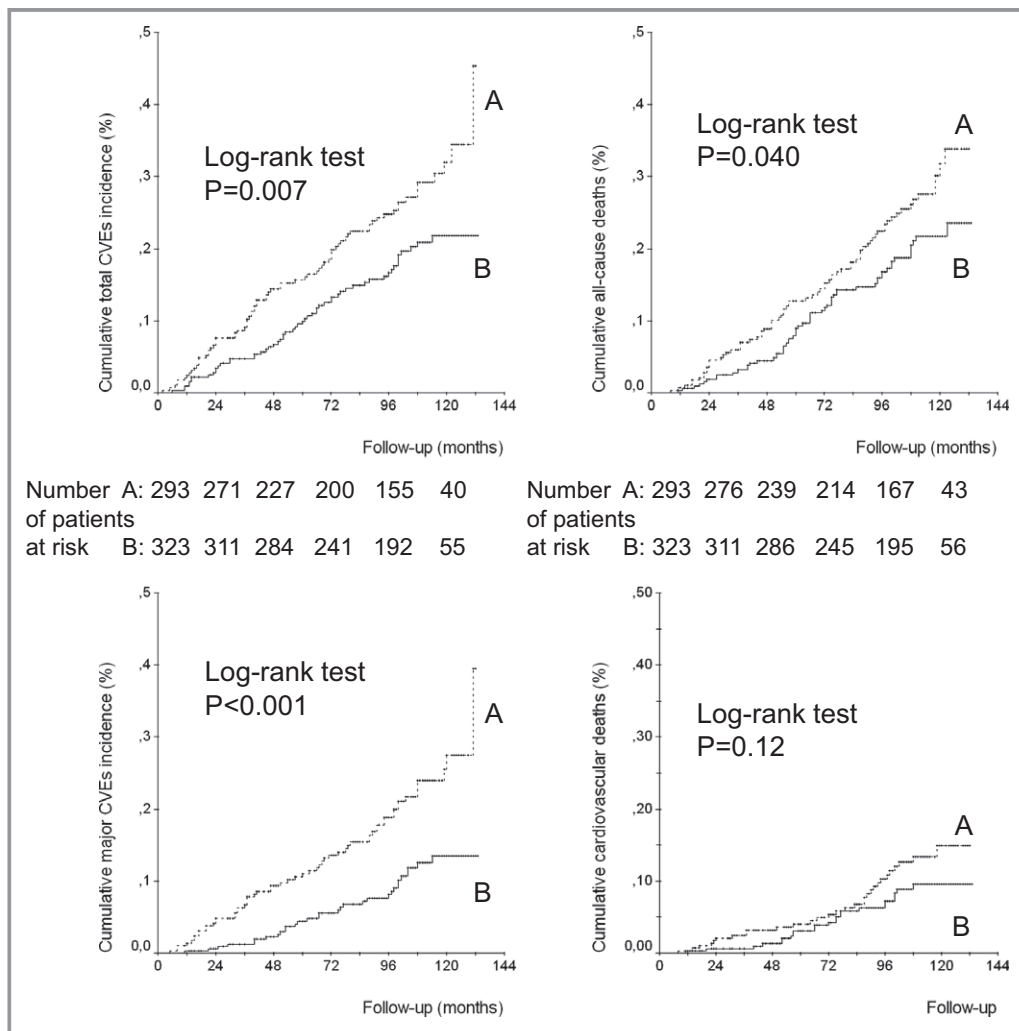
Figure. Kaplan–Meier curves of cumulative total cardiovascular events incidence (left upper panel), of major cardiovascular events (left lower panel), and of all-cause mortality (right upper panel) and cardiovascular mortality (right lower panel) in type 2 diabetic patients grouped according to baseline CRP ≥3.0 mg/L (curve A) or <3.0 mg/L (curve B). CRP indicates C-reactive protein; CVEs, cardiovascular events.

Survival Analyses

Tables 2 and 3 present the results of Cox survival analyses of different CRP cut-off values, as well as continuous CRP, collected at baseline and at the second year of follow-up, for the 4 primary end points. As a continuous variable ($\log_e$-transformed), both baseline and second-year CRP levels were significantly associated with total and major CVEs occurrence, but not with all-cause or cardiovascular mortality. Dichotomized at 3.0 mg/L, the classic cut-off value, a high CRP was also associated with total and major CVEs occurrence, except for the second-year CRP for total CVEs. Patients in the upper tertile subgroup of baseline and second-year CRP had increased risks of total and major CVEs, and also of all-cause mortality (for baseline CRP) in contrast to those in the lower tertile subgroup. No CRP parameter was associated with cardiovascular mortality, after full statistical adjustment. Furthermore, increasing CRP levels or persisting with high levels during the first 2 years of follow-up was associated with a nearly 2-fold excess risk of major CVEs, independent of baseline CRP values, in contrast to patients who decreased or persisted with low CRP levels.

According to AIC, the inclusion of baseline CRP ($\geq$3.0 mg/L) into a full model improved model prediction for total cardiovascular events (AIC reduction from 1532 to 1528) and for major CVEs (AIC reduction from 1025 to 1019), but not for all-cause or cardiovascular mortalities (AIC reduction from 1486 to 1485, and 607 to 607, respectively). Change in CRP levels during follow-up also improved model prediction to major CVEs occurrence (AIC reduction from 768 to 764).

In interaction analyses, the prognostic value of baseline CRP ($\geq$3.0 mg/L) was not influenced by age, sex, obesity, glycemic control, and presence of microvascular or macrovascular complications (all P value for interaction terms >0.20). Excluding patients with CVEs during the first 2 years of follow-up did not change the prognostic impact of baseline CRP, suggesting that there is no reverse causality between CRP and CVEs. Also, excluding patients with CRP levels >10.0 mg/L did not affect any of the analyses.

Discussion

This observational cohort study with high cardiovascular risk type 2 diabetic patients followed up for up to 11 years has 2 main findings. First, elevated baseline CRP levels were significantly associated with adverse cardiovascular outcomes independent of classic cardiovascular risk factors, including ambulatory BP, lipids, and HbA_{1c} levels. This effect was particularly pronounced for major CVEs (nonfatal acute myocardial infarctions and strokes plus cardiovascular deaths) occurrence, where a baseline CRP $\geq$3.0 mg/L nearly doubled the risk. Second, increasing CRP or persisting with elevated levels during the first 2 years of follow-up was additionally associated with higher risks of major CVEs occurrence, independent of baseline CRP. Overall, these findings suggest that monitoring CRP levels may help to refine cardiovascular risk stratification in high-risk patients with type 2 diabetes mellitus.

Table 2. Results of Cox Survival Analysis for the Prognostic Value of CRP for CVE Occurrence

CRP Parameters	All Cardiovascular Events (n=131)		Major Cardiovascular Events (n=89)	
	Age/Sex-Adjusted HR (95% CI)	Multivariate-Adjusted[§] HR (95% CI)	Age/Sex-Adjusted HR (95% CI)	Multivariate-Adjusted[§] HR (95% CI)
Baseline CRP measurement				
Continuous CRP (1-SD $\log_e$)	1.30 (1.09–1.55)[†]	1.22 (1.02–1.47)[‡]	1.52 (1.22–1.89)*	1.34 (1.07–1.68)[‡]
CRP $\geq$3.0 mg/L	1.72 (1.21–2.44)[†]	1.47 (1.03–2.10)[‡]	2.43 (1.57–3.76)*	1.89 (1.21–2.94)[†]
Upper tertile CRP ($\geq$4.8 mg/L) vs lower tertile (<1.6 mg/L)	1.84 (1.19–2.83)[†]	1.57 (1.01–2.46)[‡]	2.65 (1.55–4.52)*	1.94 (1.12–3.36)[‡]
Second-year CRP measurement				
Continuous CRP (1-SD $\log_e$)	1.32 (1.07–1.63)[†]	1.29 (1.04–1.61)[‡]	1.46 (1.13–1.89)[†]	1.37 (1.04–1.80)[‡]
CRP $\geq$3.0 mg/L	1.43 (0.95–2.15)	1.46 (0.95–2.22)	1.76 (1.07–2.91)[‡]	1.72 (1.01–2.92)[‡]
Upper tertile CRP ($\geq$4.5 mg/L) vs lower tertile (<1.8 mg/L)	1.88 (1.14–3.11)[‡]	1.84 (1.10–3.09)[‡]	2.83 (1.44–5.54)[†]	2.46 (1.23–4.94)[‡]
CRP change between first and second measurements				
Increased or persisted high (vs decreased or persisted low)	1.42 (0.95–2.12)	1.46 (0.97–2.20)	1.85 (1.12–3.04)[‡]	1.84 (1.10–3.06)[‡]

BMI indicates body mass index; CRP, C-reactive protein; CVEs, cardiovascular events; HbA_{1c}, glycated hemoglobin; HR, hazard ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[§]Adjusted for age, sex, diabetes mellitus duration, BMI, smoking, physical activity, arterial hypertension, number of antihypertensive drugs in use, 24-hour SBP, presence of microvascular and macrovascular complications, mean HbA_{1c}, HDL- and LDL-cholesterol, and use of aspirin, statins, and insulin during the first year of follow-up. CRP change was also adjusted for baseline values.

*P<0.001; [†]P<0.01; [‡]P<0.05.

Table 3. Results of Cox Survival Analysis for the Prognostic Value of CRP for All-Cause and Cardiovascular Mortality

CRP Parameters	All-Cause Mortality (n=129)		Cardiovascular Mortality (n=53)	
	Age/Sex-Adjusted HR (95% CI)	Multivariate-Adjusted* HR (95% CI)	Age/Sex-Adjusted HR (95% CI)	Multivariate-Adjusted* HR (95% CI)
Baseline CRP measurement				
Continuous CRP (1-SD $\log_e$)	1.31 (1.10–1.57) †	1.19 (0.99–1.44)	1.36 (1.03–1.79) ‡	1.13 (0.84–1.52)
CRP ≥3.0 mg/L	1.73 (1.21–2.45) †	1.38 (0.95–1.99)	1.87 (1.08–3.25) ‡	1.38 (0.77–2.46)
Upper tertile CRP (≥4.8 mg/L) vs lower tertile (<1.6 mg/L)	1.92 (1.27–2.91) †	1.54 (1.00–2.38) ‡	1.87 (0.97–3.58)	1.24 (0.62–2.48)
Second-year CRP measurement				
Continuous CRP (1-SD $\log_e$)	1.22 (0.99–1.51)	1.14 (0.91–1.42)	1.39 (1.01–1.93) ‡	1.30 (0.92–1.83)
CRP ≥3.0 mg/L	1.17 (0.77–1.79)	0.99 (0.64–1.55)	1.60 (0.84–3.05)	1.46 (0.74–2.86)
Upper tertile CRP (≥4.5 mg/L) vs lower tertile (<1.8 mg/L)	1.65 (0.98–2.78)	1.37 (0.79–2.37)	2.85 (1.20–6.80) ‡	2.39 (0.97–5.89)
CRP change between first and second measurements				
Increased or persisted high (vs decreased or persisted low)	1.05 (0.69–1.61)	0.90 (0.58–1.39)	1.90 (1.00–3.63) ‡	1.79 (0.93–3.47)

BMI indicates body mass index; CRP, C-reactive protein; HbA$_{1c}$, glycated hemoglobin; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; SBP, systolic blood pressure.

*Adjusted for age, sex, diabetes mellitus duration, BMI, smoking, physical activity, arterial hypertension, number of antihypertensive drugs in use, 24-hour SBP, presence of microvascular and macrovascular complications, mean HbA$_{1c}$, HDL- and LDL-cholesterol, and use of aspirin, statins, and insulin during the first year of follow-up. CRP change was also adjusted for baseline values.

†$P<0.01$.

‡$P<0.05$.

Some previous longitudinal studies have examined the prognostic value of CRP exclusively in patients with type 2 diabetes mellitus,[11–15,17,20,21] or in population-based samples with separate analyses for the subgroup with diabetes mellitus.[16,18,19,29] However, the results were controversial, with some studies demonstrating that CRP contributed to cardiovascular risk prediction,[11–17,29] whereas others showed no association with cardiovascular risk at all.[18–22] The reasons for these disparate findings are unclear, but might possibly be explained by different populations with variable sample sizes (ranging from 169[18] to 2381[14] diabetic patients) with higher[11,13–15,17,21,29] or lower[12,18–20,22] baseline cardiovascular risks (based on presence or absence of previous cardiovascular diseases at study entry) and different baseline CRP values, and with different cardiovascular outcomes assessed over variable follow-up periods (ranging from 2 years[29] to 11 years[17]). In general, studies that included diabetic populations with higher prevalence of previous cardiovascular diseases[14,15,17] tended to demonstrate the prognostic importance of CRP, whereas those that excluded patients with previous cardiovascular diseases[20,22] tended to show a nonsignificant prognostic value of CRP. Our study, mainly in middle-aged to elderly individuals, most with either micro- or macrovascular diabetic complications, supports this assumption. This suggests that CRP may be more useful on risk stratification for secondary than for primary cardiovascular disease prevention.

Otherwise, few studies addressed whether serial changes in CRP could provide additional prognostic information beyond baseline levels in patients with diabetes mellitus.[15,21,22] Only one of them (the Bypass Angioplasty Revascularization Investigation 2 Diabetes [BARI 2D] trial[15]), in type 2 diabetic patients with coronary artery disease, showed that changes in CRP during follow-up (evaluated as a time-varying covariate) were significantly associated with major CVEs occurrence. The other 2 studies,[21,22] both substudies of larger randomized trials (the Veterans Affairs Diabetes Trial [VADT][21] and the Collaborative Atorvastatin Diabetes Study [CARDS][22]), did not find any association with CVEs occurrence, neither with CRP measured at baseline, nor with changes in CRP during the first year of follow-up. While the CARDS included only patients without cardiovascular diseases, the VADT included nearly a third of patients with a history of previous cardiovascular diseases. However, the sample size of this VADT substudy[21] was relatively small (266 patients) with few CVEs (62 events); hence, it was possibly underpowered to reveal significant associations between CRP changes and cardiovascular prognosis. Therefore, our study confirms the BARI 2D study findings, by showing that increasing or persistently elevated CRP levels during the first 2 years of follow-up were associated with an 80% excess risk of future major CVEs incidence, independent of other classic cardiovascular risk factors.

In this regard, several interventions, such as weight loss,[30] physical activity (independent of weight loss),[31] strict metabolic control,[32] and some pharmacological treatments (such as with statins, aspirin, and thiazolidinediones)[33] have been demonstrated to reduce CRP levels in patients with diabetes mellitus. Not surprisingly, most of these

interventions have also been demonstrated to provide cardiovascular protection. Nevertheless, whether the beneficial effects of these interventions are mediated, at least partially, by CRP reduction, has never been demonstrated. Indeed, the true role of CRP in the atherosclerotic process has been increasingly debated,[34] with some experimental evidences suggesting an active role in atherosclerosis,[35,36] whereas other epidemiological studies using Mendelian randomization failed to demonstrating any causal association between CRP levels and vascular atherosclerotic diseases.[37]

This study has some limitations that warrant discussion. First, although we adjusted our analyses for baseline use of statins and aspirin (drugs that can influence CRP levels), we did not take into account drug changes during follow-up. Otherwise, the prevalence of statins and aspirin use at baseline was high (77% and 91%, respectively), leaving little room for further increases during follow-up. In the same way, we also did not consider other treatment changes and patients' adherence during follow-up. Second, we relied on 2 single CRP sample measurements (with a second sample collected only if CRP >10 mg/L) to assess CRP changes during the first 2 years of follow-up. Although it has been demonstrated that individual CRP levels were stable over long periods,[38] and that even extreme values still retained prognostic value,[39] few measurements may not have completely reflected actual CRP intra-individual variability and might have increased the possibility of measurement errors. Moreover, the "regression-to-the-mean" phenomenon[40] also might have affected CRP changes analysis. However, these effects would tend to bias the data towards the null hypothesis; hence, the excess risk associated with increasing CRP levels may be even higher than that we demonstrated here. Finally, as discussed before, our findings may not be generalized to younger, lower-risk patients with type 2 diabetes mellitus treated at primary care.

In conclusion, this prospective study provides evidence that baseline and serial changes in CRP levels represent important risk markers for future adverse cardiovascular outcomes, in addition to traditional cardiovascular risk factors, in high-risk patients with type 2 diabetes mellitus, and may be useful in refining cardiovascular risk stratification. Whether targeting reduction of specific inflammatory biomarkers, such as CRP,[34] will provide additional cardiovascular protection in particular subgroups of patients must await future interventional trials.[41–43]

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Disclosures

None.

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