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CRP, C-Peptide, and Risk of First-Time Cardiovascular Events and Mortality in Early Type 2 Diabetes: A Danish Cohort Study

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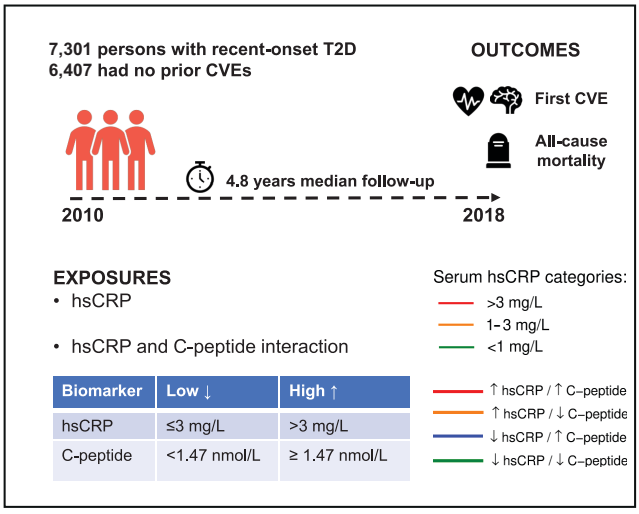
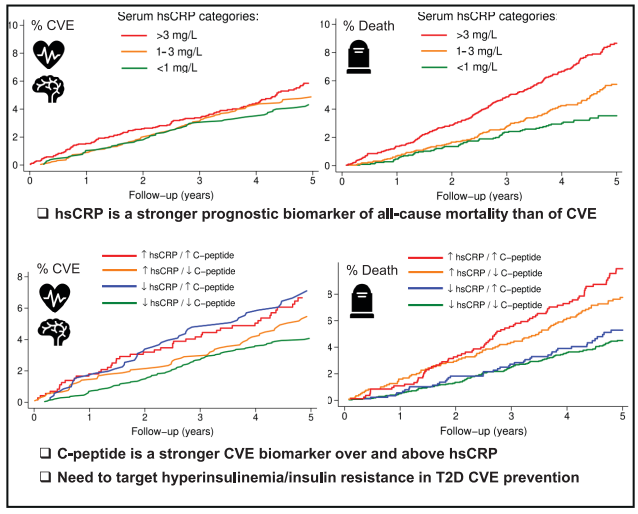
CRP, C-peptide, and risk of first-time cardiovascular event (CVE) and mortality in early type 2 diabetes (T2D) in a Danish cohort. ↑, high; ↓, low.





ARTICLE HIGHLIGHTS

- The relationship of low-grade inflammation and hyperinsulinemia/insulin resistance with cardiovascular events (CVEs) and mortality in recent-onset type 2 diabetes is unknown.
- This study evaluated the association of hs-CRP and C-peptide with CVEs and all-cause mortality.
- High hs-CRP was associated with modestly increased CVE risk and highly increased mortality, and high C-peptide marked substantially increased CVE risk, with elevated hs-CRP carrying limited additional prognostic information.
- Low-grade inflammation primarily reflects all-cause mortality in patients with recent-onset type 2 diabetes, whereas hyperinsulinemia/insulin resistance is a stronger CVE biomarker.

CRP, C-Peptide, and Risk of First-Time Cardiovascular Events and Mortality in Early Type 2 Diabetes: A Danish Cohort Study

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OBJECTIVE

We investigated the relationship between hs-CRP, a marker of low-grade inflammation, alone or in combination with C-peptide, a marker of hyperinsulinemia/insulin resistance, and risk for cardiovascular events (CVEs) and mortality in patients recently diagnosed with type 2 diabetes (T2D).

RESEARCH DESIGN AND METHODS

In patients with recent-onset T2D, we measured serum hs-CRP (*n* = 7,301) and C-peptide (*n* = 5,765) in the prospective Danish Centre for Strategic Research in Type 2 Diabetes cohort study. Patients with no prior CVE (*n* = 6,407) were followed until first myocardial infarction, stroke, coronary revascularization, or cardiovascular death, and all patients (*n* = 7,301) were followed for all-cause mortality. We computed adjusted hazard ratios (aHRs) by Cox regression and tested for the interaction between hs-CRP and C-peptide.

RESULTS

During follow-up (median 4.8 years), high (>3 mg/L) versus low (<1 mg/L) hs-CRP was associated with increased CVE risk (aHR 1.45 [95% CI 1.07–1.96]) and with even greater risk of all-cause mortality (2.47 [1.88–3.25]). Compared with patients with low hs-CRP (≤3 mg/L) and low C-peptide (<1,470 pmol/L), those with high levels of both biomarkers had the highest CVE (1.61 [1.10–2.34]) and all-cause mortality risk (2.36 [1.73–3.21]). Among patients with high C-peptide, risk of CVEs did not differ by low or high hs-CRP, whereas risk of all-cause mortality did.

CONCLUSIONS

The finding of high hs-CRP as a stronger prognostic biomarker of all-cause mortality than of CVEs may facilitate improved early detection and prevention of deadly diseases besides CVEs. Conversely, elevated C-peptide as a strong CVE biomarker supports the need to target hyperinsulinemia/insulin resistance in T2D CVE prevention.

Inflammation is pivotal in the development of many diseases and conditions, including insulin resistance, atherosclerosis, and cardiovascular disease (CVD) (1–3). A common biomarker of low-grade inflammation is CRP, an acute-phase protein produced by hepatocytes in response to cytokine production (primarily tumor necrosis factor and interleukin-6) in inflamed tissues (4,5). More than a decade ago, general population studies in Denmark showed that hs-CRP levels >3 mg/L vs. <1 mg/L

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were associated with a 30–60% increased risk of cardiovascular events (CVEs) and a twofold increased risk of all-cause mortality (6,7).

In cohort studies of patients with type 2 diabetes (T2D) and a history of CVEs, high hs-CRP levels have been associated with a 40–80% higher risk of CVEs compared with low levels (8,9). Results of a study of 4,168 U.S. patients with T2D (or metabolic syndrome) and a history of CVEs suggested little change in the association between hs-CRP and CVEs in the past 20–30 years, despite contemporary aggressive cardiovascular prevention efforts (9). In contrast to general population studies (7), that investigation uncovered no association between hs-CRP and all-cause mortality (9).

Of note, studies of inflammation and CVEs in patients with T2D have focused mainly on secondary CVD prevention (8,9). Whether the same associations identified in these reports apply to patients with T2D and no history of CVEs (i.e., the primary CVD prevention setting) is unknown. It is pharmacologically plausible that via anti-inflammatory and other actions, contemporary aggressive preventive therapy could ameliorate the association between low-grade inflammation and risk of a first CVE. In accordance with this notion, new data suggest that individuals with recent-onset T2D and no history of CVEs have only a modestly increased risk of myocardial infarction (29%) and cardiovascular death (35%) compared with the general population (10).

Breakthroughs in T2D substratification (phenotyping) suggest that the highest risk of CVEs and mortality may occur in a subgroup of patients with severe hyperinsulinemia and insulin resistance (11,12). A bidirectional association between inflammation and insulin resistance has been established (13), yet little is known about the dual effects of inflammation and hyperinsulinemia/insulin resistance on the risk of a first incident CVE and mortality in T2D. We examined the relationship between low-grade inflammation (expressed as hs-CRP levels) alone and in combination with hyperinsulinemia/insulin resistance (expressed as C-peptide levels) and risk of CVEs and mortality in a clinical prevention setting in a contemporary prospective cohort of patients with recent-onset T2D and no hospital history of CVEs.

RESEARCH DESIGN AND METHODS

Study Cohort

The Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort, established in 2010, is an ongoing, nationwide cohort study of patients recently diagnosed with T2D (14). General practitioners and hospital physicians throughout Denmark identify patients with newly or recently diagnosed T2D. After written informed consent, participants undergo a short interview and a physical examination (15). Fasting blood and urine samples are collected from each patient at enrollment and stored in a biobank (16). The eligible study population for this study included all patients enrolled by December 2016 (n = 7,588). Because of withdrawal of consent or lack of serum samples, measurements of serum hs-CRP were available for 7,301 patients (Supplementary Fig. 1). C-peptide was measured in participants enrolled by June 2015, and the measurements were thus available in a subcohort consisting of the first 5,765 DD2 participants (Supplementary Fig. 1).

Ethics

The DD2 study has been approved by the Danish Data Protection Agency (record number 2008-58-0035) and by the Regional Committees on Health Research Ethics for Southern Denmark (record number S-20100082).

Outcomes

Outcomes were CVEs (17) (a composite of first occurrence of myocardial infarction, ischemic stroke, coronary revascularization, or cardiovascular death), each individual CVE subtype, and all-cause mortality. For CVE outcomes, the main analysis was conducted among CVE-naive patients, i.e., in the 6,407 (88%) of the 7,301 patients with serum hs-CRP measurements who had no prior CVE recorded (Supplementary Fig. 1). We obtained all diagnoses and procedures for CVEs (primary and secondary discharge diagnoses) from the Danish National Patient Registry, which holds discharge diagnoses from all inpatient hospitalizations in Denmark since 1977 and all emergency department and hospital outpatient clinic visits since 1995 (18). Previous validation studies have found high validity of CVE diagnoses and procedures, i.e., positive predictive values of $\geq$97% for myocardial infarction, stroke, percutaneous coronary intervention, and coronary artery bypass graft surgery (19,20). Exact dates of death were retrieved from the Danish Civil Registration System (21). Information on cardiovascular, respiratory, and cancer deaths (as the immediate or underlying cause of death) was obtained from the Danish Registry of Causes of Death (22,23). Supplementary Table 1 shows all codes used in the study.

Serum hs-CRP Levels

Serum hs-CRP levels were measured using an in-house time-resolved immunofluorometric assay based on commercially available monoclonal antibodies (MAB17071 and BAM17072; R&D Systems, Minneapolis, MN) and calibrated against recombinant human CRP (World Health Organization International Standard 85/506; National Institute for Biological Standards and Control) as previously described (24). The intra- and interassay coefficients of variation were both $<$6%. Serum hs-CRP levels were categorized as low ($<$1.0 mg/L), intermediate (1.0–3.0 mg/L), or high ($>$3.0 mg/L), and in alternative analyses, as low ($\leq$3.0 mg/L) versus high ($>$3.0 mg/L) (6,25) or low ($<$2.0 mg/L) versus high ($\geq$2.0 mg/L) (26) based on common cutoffs.

Serum C-Peptide Levels

Fasting serum C-peptide levels were measured using a C-peptide assay (Roche Diagnostics, Mannheim, Germany) (12). Serum C-peptide levels were categorized as low ($<$1,470 pmol/L) or high ($\geq$1,470 pmol/L), corresponding to the upper limit of the normal range for the method used for measuring C-peptide (27).

Covariates

From the DD2 cohort questionnaire and linked medical and administrative registries, we extracted baseline data on potential confounders, as well as on likely intermediate factors (28). Potential confounders, i.e., covariates that, on the basis of prior knowledge (29), were likely to be associated with our main exposure (hs-CRP) and outcomes (CVEs and mortality) without being on the causal pathway between the exposure and outcomes, were as follows: age in years (continuous variable), sex (male or female), T2D duration (time in years since T2D diagnosis), obesity measures (waist circumference in cm [continuous variable] and

BMI in kg/m^2 [continuous variable]), and lifestyle measures (physical activity [minimum of 30 min activity reported 0, 1, 2, or ≥3 days/week]), alcohol consumption [≤14 or >14 alcoholic drinks/week for women and 21 drinks/week for men], and tobacco smoking [never, former, or current]) (Supplementary Table 1). As potential mediators (28), i.e., covariates likely on the causal pathway from hs-CRP to outcomes, we considered the following: systolic and diastolic blood pressure (continuous variables), comorbidities (modified Charlson comorbidity index [categorical variable]), fasting blood glucose (mmol/L), HbA_{1c} (%), estimated glomerular filtration rate (mL/min/1.73 m^2), urine albumin-to-creatinine ratio (mg/g), total cholesterol (mmol/L), LDL cholesterol (mmol/L), HDL cholesterol (mmol/L), triglycerides (mmol/L), mannose-binding lectin levels (μg/L), and use of antidiabetes, lipid-lowering, antihypertensive, or antithrombotic drugs.

Assessment and categorization of covariates in the DD2 cohort have been described in detail previously (14,30), and all covariates, definitions, and codes are listed in Supplementary Table 1. In brief, baseline data on obesity measures and lifestyle factors either were based on interview and clinical examination data as part of the DD2 enrollment process (15) or were ascertained by linkage with a nationwide quality-of-care database, the Danish Diabetes Database for Adults (14). Comorbidities were ascertained through a complete hospital contact history for each patient since 1994 from the Danish National Patient Registry. Use of medical drugs (i.e., filled drug prescriptions 1 year prior to enrollment) were ascertained from nationwide prescription databases (14).

Statistical Analyses

We used Stata 14.2 statistical software for all analyses. Patients with T2D were followed from the study enrollment date until an event, death, migration, or the end of the study period (10 August 2018), whichever came first. For outcomes including specific causes of death, follow-up ended 28 December 2016, the time of most recent data availability in the Danish Registry of Causes of Death. For all other outcomes, data were available until 10 August 2018, at which time the follow-up was terminated. Patients with any CVE prior to enrollment (n = 894) were excluded from the main analyses of the composite CVE outcome. Patients with an individual subtype of CVEs prior to enrollment (n = 434 myocardial infarction, n = 248 ischemic stroke, and n = 602 coronary revascularization) were excluded from the analyses for the relevant subtype of CVEs. We did not consider recurrent events.

To examine the association between serum hs-CRP as a continuous variable and risk of outcomes, we applied restricted cubic spline models with 5 df (Supplementary Fig. 2). Cumulative incidence of all-cause mortality was plotted using the Kaplan-Meier method. The cumulative incidence of CVEs, with noncardiovascular death as a competing risk, was plotted using the Stata stcompet command (Fig. 1). Incidence and mortality rates were calculated using the Stata stptime command.

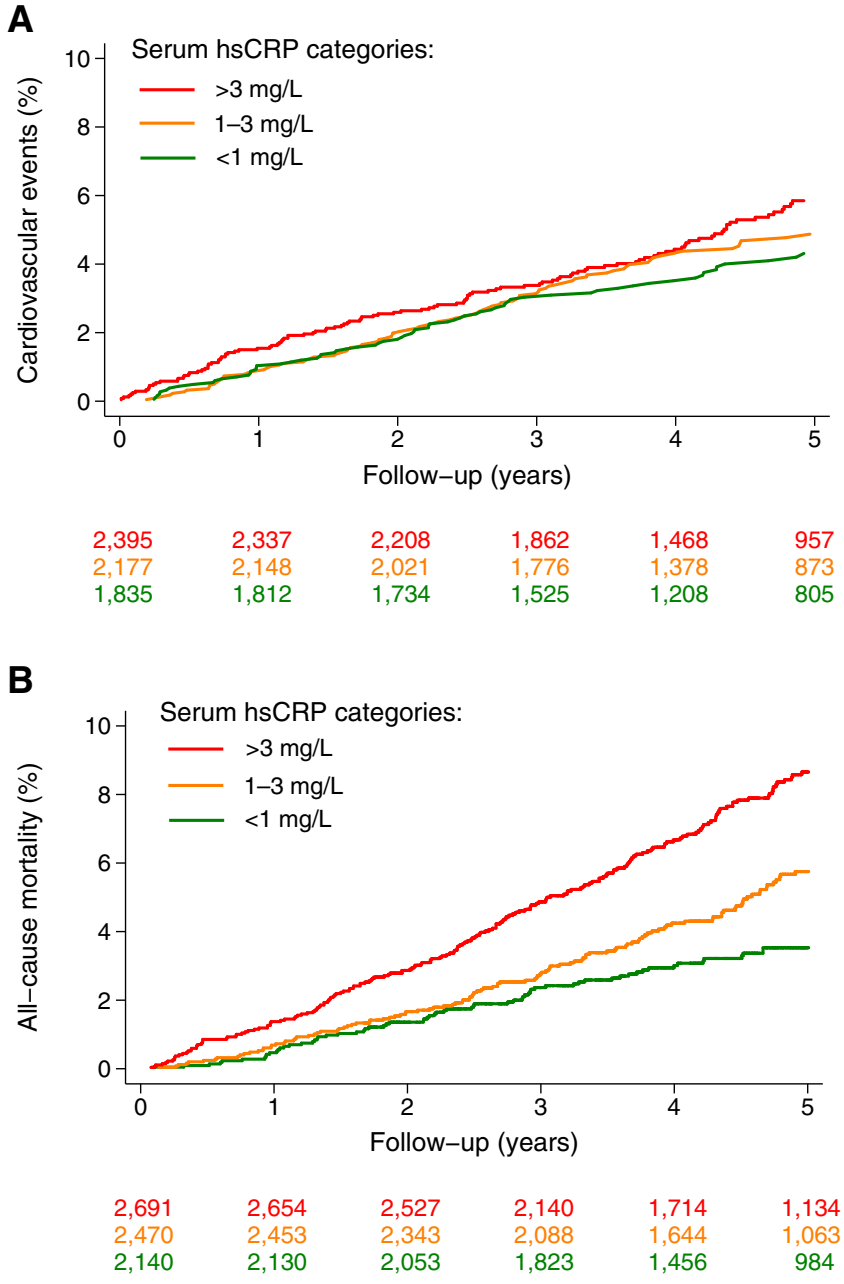

Figure 1—Cumulative incidence of first CVE and all-cause mortality by serum hs-CRP category. Cumulative incidence plots of first CVE (n = 6,407 patients with no prior history of CVE) (*A*) and all-cause mortality (n = 7,301 patients) (*B*) by serum hs-CRP categories. Cumulative incidence estimates are based on time from study enrollment to the first CVE, with noncardiovascular death as a competing risk (*A*).

Hazard ratios (HRs) and 95% CIs were calculated using Cox regression analysis. We graphically verified the proportional hazards assumption by plotting −ln(survival probability) against ln(analysis time), with no major violations detected. In the main analysis, we adjusted HRs for potential confounders (see *Covariates*). Missing covariates (n <5 to 3,964 [0.0–54.3%]) (Supplementary Table 2) were treated with multiple imputation to maximize precision and avoid selection bias, as previously described (17).

To evaluate the joint effects of inflammation (hs-CRP) and hyperinsulinemia/insulin resistance (C-peptide), we used several approaches. First, for hs-CRP alone, we further adjusted for C-peptide levels (Fig. 2). Second, we stratified the association between hs-CRP and outcomes according to low (<1,470 pmol/L) and high (≥1,470 pmol/L) C-peptide levels (Supplementary Figs. 9 and 10). Third, we evaluated a potential statistical interaction by introducing an interaction term (hs-CRP * C-peptide) into the Cox proportional hazard regression model and comparing the adjusted models with and without interaction terms by using a likelihood ratio test. Fourth, we examined the potential synergistic biological interaction between hs-CRP and C-peptide (31) by categorizing patients into four groups at baseline, including low levels of both hs-CRP and C-peptide, high hs-CRP but low C-peptide, high C-peptide but low hs-CRP, and high levels of both hs-CRP and C-peptide, according to clinically relevant cutoffs (<3 or ≥3 mg/L for hs-CRP and <1,470 or ≥1,470 pmol/L for C-peptide) (Fig. 3). The interaction analyses were conducted among patients with complete measurements of both biomarkers (5,700 for mortality outcome analyses and 4,970 CVE-naive patients for CVE outcome analyses) (Supplementary Fig. 1).

We performed a number of other additional and sensitivity analyses. First, we additionally adjusted hs-CRP for likely mediators of inflammation (see *Covariates*). Second, we performed a complete case analysis (i.e., no multiple imputation). Third, we repeated our analyses after stratifying all DD2 participants according to whether hs-CRP levels were above or below commonly used cutoffs of 3 mg/L or 2 mg/L, respectively (26). Fourth, because deaths from cancer and respiratory diseases are important contributors to all-cause mortality, in addition to cardiovascular death, we examined the association between hs-CRP and these specific mortality outcomes. Finally, to evaluate attrition, we assessed baseline characteristics comparing the 7,301 patients with available hs-CRP measurements with the 5,765 patients with available C-peptide measurements.

RESULTS

Baseline Characteristics

The study included 7,588 DD2 participants with recently diagnosed T2D, of whom 7,301 (96%) had serum hs-CRP and 5,765 (76%) had serum C-peptide measurements available (Supplementary Fig. 1). The cohort was followed for a median of 4.7 years (interquartile range [IQR]

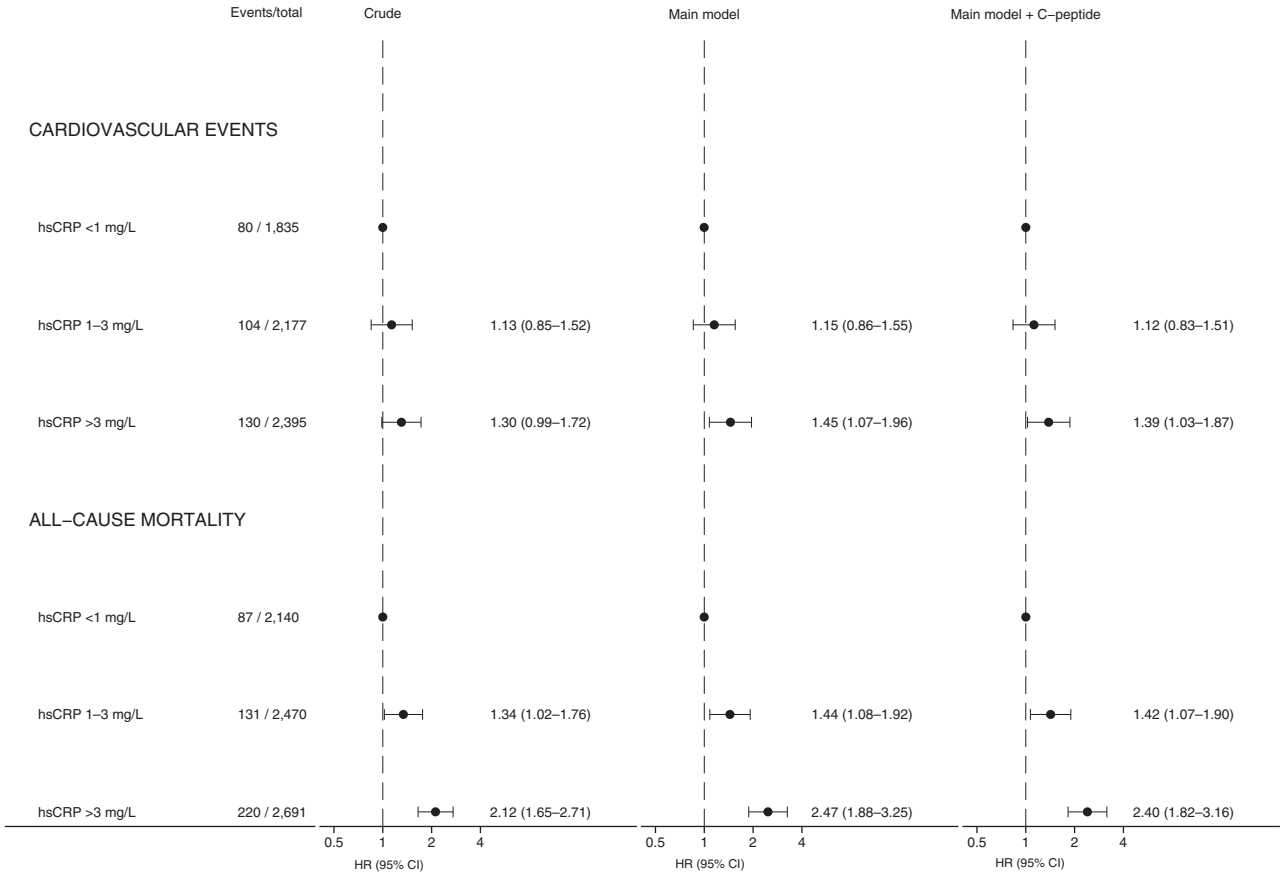

Figure 2—HRs for first CVE and all-cause mortality by serum hs-CRP category. Main model was adjusted for age, sex, T2D duration, waist circumference, BMI, physical activity, alcohol consumption, and smoking. Main model + C-peptide was additionally adjusted for C-peptide levels. Missing covariates were treated with multiple imputation (see *Statistical Analysis*).

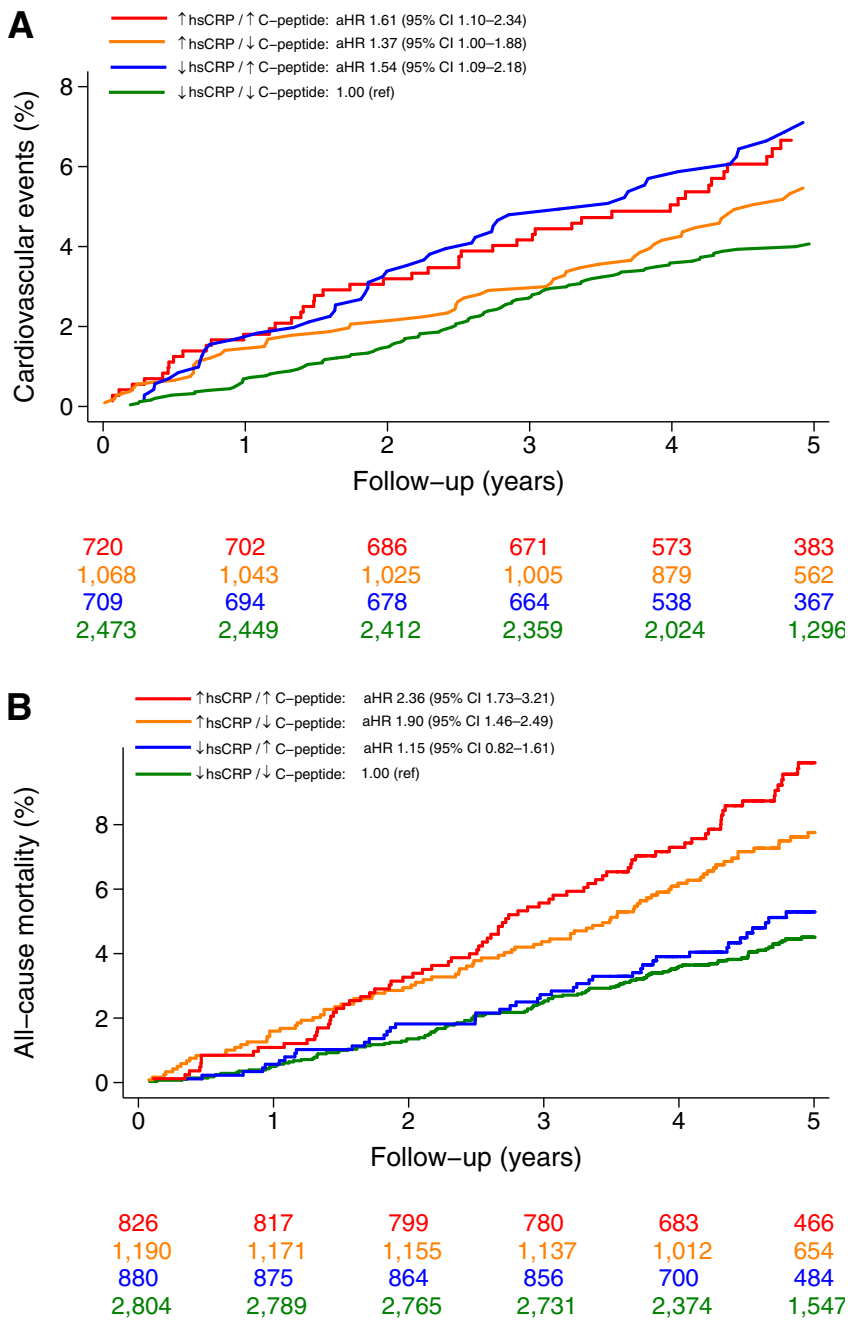

Figure 3—Joint effects of hs-CRP and C-peptide on first CVE and all-cause mortality. Cumulative incidence of first CVE (n = 4,970 patients with available measurements of both hs-CRP and C-peptide levels and no prior history of CVE) (*A*) and all-cause mortality (n = 5,700 patients with available measurements of both hs-CRP and C-peptide levels) (*B*). Patients were stratified by clinical cutoffs (<3 mg/L and ≥3 mg/L for hs-CRP and <1,470 pmol/L and ≥1,470 pmol/L for C-peptide) into four groups: low levels of both hs-CRP and C-peptide, high hs-CRP but low C-peptide, high C-peptide but low hs-CRP, and high levels of both hs-CRP and C-peptide. HRs adjusted for age, sex, T2D duration, BMI, waist circumference, physical activity, alcohol consumption, and smoking. ↑, high; ↓, low; aHR, adjusted hazard ratio; ref, reference.

3.4–5.7 years) for CVEs and 4.8 years (IQR 3.6–5.8 years) for all-cause mortality. A total of 314 patients developed CVEs between 2010 and 2018, and 438 patients died.

Table 1 presents baseline characteristics by serum hs-CRP category levels (<1.0, 1.0–3.0, and >3.0 mg/L). Overall, the high hs-CRP level was associated with obesity, less physical activity, smoking, increased comorbidity, dyslipidemia, and high serum C-peptide levels. The results were similar in the subcohort of patients with available measurements of both C-peptide and hs-CRP levels (Supplementary Table 3).

Serum hs-CRP Levels and Risk of CVEs

In spline models, the association between serum hs-CRP levels and CVEs showed a sharply increasing risk from 0 to 1 mg/L, followed by a plateau from 1 to 3 mg/L and linearly increasing risk levels at >3 mg/L (Supplementary Fig. 2*A*). The cumulative incidence curve for the high hs-CRP (>3 mg/L) was only slightly above the curve for the low hs-CRP (<1 mg/L) category (Fig. 1*A*). Incidence rates are shown in Supplementary Table 4.

High hs-CRP was associated with an increased risk of CVEs. The crude HRs -for intermediate and high versus low hs-CRP levels were 1.13 (95% CI 0.85–1.52) and 1.30 (0.99–1.72), respectively (Fig. 2). Corresponding adjusted estimates were 1.15 (0.86–1.55) and 1.45 (1.07–1.96) (Fig. 2). Adjustment for C-peptide levels slightly attenuated the risk estimates (Fig. 2). Additional adjustment for a range of potential mediators of inflammation further attenuated the HR estimates toward 1 (1.06 [0.78–1.43] and 1.19 [0.87–1.62], respectively). The risk estimates for the individual CVE subtypes were consistent with the composite CVE outcome (Supplementary Figs. 3–8). However, the risk estimates for cardiovascular mortality were higher and similar to those for all-cause mortality (see below and Supplementary Figs. 3–4, 7).

Serum hs-CRP Levels and Risk of All-Cause Mortality

In spline models, increasing serum hs-CRP levels showed a clear linear association with all-cause mortality (Supplementary Fig. 2*B*). In accordance with this finding, the cumulative incidence curves clearly increased across increasing hs-CRP categories (Fig. 1*B*). Incidence rates are shown in Supplementary Table 4.

High hs-CRP was associated with increased risk of all-cause mortality. The crude HRs for intermediate and high versus low hs-CRP levels were 1.34 (95% CI 1.02–1.76) and 2.12 (1.65–2.71), respectively (Fig. 2). The results were robust to adjustments for confounders, as well as further adjustments for potential mediators (1.30 [0.95–1.76] and 1.98 [1.48–2.66], respectively) (Fig. 2). Results for respiratory and cancer mortality were consistent with the analyses of all-cause mortality, as well

Table 1—Characteristics of DD2 cohort members at baseline by serum hs-CRP category

	Serum hs-CRP category		
	Low (<1 mg/L)	Intermediate (1–3 mg/L)	High (>3 mg/L)
Total patients	2,140 (29.3)	2,470 (33.8)	2,691 (36.9)
Age, years	63.3 (55.0–69.4)	62.4 (53.9–68.9)	60.5 (51.3–67.8)
Sex, men	1,420 (66.4)	1,483 (60.0)	1,371 (51.0)
Diabetes duration, years	1.5 (0.5–3.0)	1.3 (0.4–3.0)	1.0 (0.2–2.7)
Waist circumference, cm	100 (92–108)	106 (97–115)	112 (102–122)
Waist-to-hip ratio	0.97 (0.91–1.02)	0.98 (0.92–1.04)	0.99 (0.92–1.05)
BMI, kg/m^2	27.9 (25.2–31.0)	30.3 (27.1–33.8)	32.8 (29.0–37.6)
Physical activity,[a] days/week	5 (3–8)	5 (3–8)	4 (2–7)
High alcohol consumption[b]	128 (6.0)	172 (7.0)	175 (6.5)
Smoking			
Never	866 (53.2)	858 (46.4)	800 (41.6)
Former	523 (32.1)	647 (35.0)	678 (35.3)
Current	240 (14.7)	346 (18.7)	443 (23.1)
Systolic blood pressure, mmHg	130 (124–140)	130 (124–140)	130 (124–140)
Diastolic blood pressure, mmHg	80 (73–85)	80 (75–85)	80 (75–87)
Charlson comorbidity index score[c]			
0	1,562 (73.0)	1,725 (69.8)	1,735 (64.5)
1–2	487 (22.8)	622 (25.2)	775 (28.8)
3	91 (4.2)	123 (5.0)	181 (6.7)
Antidiabetes drug use	1,828 (85.4)	2,105 (85.2)	2,272 (84.4)
Lipid-lowering drug use	1,622 (75.8)	1,809 (73.2)	1,709 (63.5)
Antihypertensive drug use	1,463 (68.4)	1,818 (73.6)	1,960 (72.8)
Antithrombotic drug use	680 (31.8)	705 (28.5)	715 (26.6)
Fasting blood glucose, mmol/L	7.0 (6.2–7.9)	7.2 (6.4–8.3)	7.2 (6.5–8.5)
HbA_{1c}, %	6.5 (6.1–7.0)	6.6 (6.2–7.2)	6.7 (6.2–7.5)
C-peptide, pmol/L	964 (733–1,304)	1,167 (891–1,556)	1,329 (1,010–1,734)
Albumin-to-creatinine ratio, mg/g	8 (4–18)	9 (4–22)	10 (4–27)
eGFR, mL/min/1.73 m^2	88 (75–98)	88 (74–98)	90 (75–100)
Total cholesterol, mmol/L	4.3 (3.6–5.0)	4.4 (3.7–5.1)	4.4 (3.8–5.2)
LDL cholesterol, mmol/L	2.1 (1.6–2.7)	2.2 (1.7–2.8)	2.3 (1.8–3.0)
HDL cholesterol, mmol/L	1.3 (1.0–1.6)	1.2 (1.0–1.2)	1.1 (1.0–1.4)
Triglycerides, mmol/L	1.4 (1.0–2.1)	1.7 (1.2–2.4)	1.8 (1.3–2.6)
Serum MBL, μg/L	763 (228–1,645)	741 (227–1,551)	708 (217–1,693)

Data are median (IQR) and *n* (%). Number of participants varied depending on availability of data (missing covariates are listed in Supplementary Table 2). eGFR, estimated glomerular filtration rate; MBL, mannose-binding lectin. [a]Days per week with a minimum of 30 min of physical activity. [b]High alcohol consumption was defined as >14 alcoholic drinks/week for women and 21 alcoholic drinks/week for men. [c]Excludes diabetes.

as with cardiovascular mortality (data not shown).

Joint Effects of Serum hs-CRP and C-Peptide Levels on Risk of CVE and All-Cause Mortality

For hs-CRP alone, further adjustment for C-peptide levels provided results similar to the main analyses for all outcomes (Fig. 2 and Supplementary Figs. 5–8). Stratification according to C-peptide levels (<1,470 or ≥1,470 pmol/L) showed that high hs-CRP (>3 mg/L vs. <1 mg/L) was associated with an increased risk of CVEs in patients with low C-peptide (HR 1.55 [95% CI 1.06–2.29]) (Supplementary Fig. 9) but not in patients with high C-peptide levels (1.01 [0.57–1.79]) (Supplementary Fig. 10). In contrast, high hs-CRP (>3 mg/L) was associated with a more than two-fold increased risk of all-cause mortality, regardless of (low or high) C-peptide levels (Supplementary Figs. 9–10). We found no evidence of statistical interaction between hs-CRP and C-peptide (likelihood ratio *P* for interaction > 0.05).

Based on cutoffs ≤3 mg/L and >3 mg/L for hs-CRP and <1,470 pmol/L and ≥1,470 pmol/L for C-peptide, patients with high levels of both biomarkers had the highest risks of CVEs (adjusted HR 1.61 [95% CI 1.10–2.34]) (Fig. 3*A*) and all-cause mortality (2.36 [1.73–3.21]) (Fig. 3*B*). In the presence of high C-peptide, risk of CVE did not differ by high and low hs-CRP levels (1.61 [1.10–2.34] and 1.54 [1.09–2.18], respectively), whereas the risk of all-cause mortality did (2.36 [1.73–3.21] and 1.15 [0.82–1.61], respectively).

Sensitivity Analyses

Overall, the sensitivity analyses (complete case analysis and hs-CRP cutoffs at 2 mg/L and 3 mg/L) yielded results similar to the main analyses (Supplementary Figs. 11–15 and Supplementary Table 4).

CONCLUSIONS

In this contemporary prospective cohort study of 7,588 patients in a clinical prevention setting with recently diagnosed T2D and no history of CVEs, we found that high hs-CRP was a much weaker prognostic biomarker of CVEs than of all-cause mortality. Furthermore, the risk of CVEs seemed to be more closely related to C-peptide than to hs-CRP, whereas we observed the opposite pattern for all-cause mortality. Among patients with high C-peptide, who had substantially increased CVE risk, elevated hs-CRP carried limited additional prognostic information.

Besides vascular complications, T2D is associated with multiple nonclassical comorbidities, including liver, pulmonary, and neurocognitive diseases, as well as cancer and infections (32). Our finding of hs-CRP being a stronger risk marker of all-cause as opposed to CVE mortality may accordingly guide clinicians to improve early detection and prevention of serious diseases other than CVD in T2D. Conversely, the finding of C-peptide as a strong marker of CVE risk may improve CVD risk stratification in early T2D. This

result is valuable in clinical practice, as elevated C-peptide can identify patients with T2D who are at risk for future CVE despite a normal hs-CRP. Our findings are in line with emerging understanding of significant CVD benefits associated with interventions that reduce hyperinsulinemia/insulin resistance in T2D. These interventions include nonpharmacological means, such as physical activity, healthy nutrition, and weight loss, and pharmacological treatment; as for the latter, metformin, sodium–glucose cotransporter 2 inhibitors, and glucagon-like peptide 1 receptor agonists, may be particularly beneficial (33).

Our findings are in agreement with Danish general population studies showing that high hs-CRP is less strongly associated with risk of CVEs than with all-cause mortality (6,7). In contrast, a large (n = 160,309) individual participant meta-analysis found that high hs-CRP was associated with similar risks of CVEs and mortality in individuals without a history of CVEs, regardless of diabetes status (34). However, the meta-analysis used logarithmically transformed hs-CRP units scaled to 1 SD, so we cannot make direct comparisons of risk estimates with our study. Likewise, we are unable to directly compare our findings with those of previous cohort studies of patients with T2D, although those studies, in agreement with ours, did show an association between high hs-CRP and increased mortality risk (9,35–37).

Nevertheless, we report risk estimates for high hs-CRP and CVE risk that are directly comparable and similar to those of the Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) trial of 5,380 patients with T2D (adjusted HRs 1.42–1.45) (8). The patients in our study had no history of CVEs, whereas all the patients in the EXAMINE trial had a recent diagnosis of acute coronary syndrome (8). Collectively, these findings suggest that hs-CRP, as a prognostic biomarker for CVE risk, does not differentiate between individuals with or without T2D, regardless of CVE history.

A U.S. study of 4,168 patients with a history of CVEs and either T2D or metabolic syndrome found a clear association between hs-CRP and major adverse CVEs (highest quartile hs-CRP $\geq$3.42 mg/L vs. lowest quartile hs-CRP $\leq$0.73 mg/L; HR 1.79 [95% CI 1.28–2.50]), but no material association between the same hs-CRP quartiles and risk of all-cause mortality (HR 1.14 [95% CI 0.71–1.85]) (9). This pattern is in stark contrast to our findings of a much stronger association of high hs-CRP with risk of all-cause mortality than with CVE risk. A possible explanation for this discrepancy could be the primary intervention setting of our study, whereas the high-risk patients in the U.S. study reflected a secondary intervention setting. Thus, hs-CRP might be a better risk marker of all-cause mortality in the general population than in individuals with high CVE risk (25,34,38).

It is now generally accepted that increased CRP is not a causal risk factor for CVD but rather a marker of underlying inflammation, as seen in adipose tissue dysfunction (caused by obesity and sedentary lifestyle), atherosclerosis, and cancer (6,7). Elevated levels of inflammation have been observed in patients with T2D and severe insulin resistance (12,34). Even in healthy individuals, elevated CRP is associated with insulin resistance (10), and the association between inflammation and insulin resistance is likely bidirectional (13). We recently observed that a distinct hyperinsulinemic T2D phenotype characterized by insulin resistance and high β-cell function was associated with increased preexisting CVD and higher levels of inflammation at T2D onset (12) and a higher rate of subsequent CVEs and all-cause mortality (39).

Here, we provide novel evidence of a potential joint effect of inflammation and hyperinsulinemia/insulin resistance on the risk of incident CVE and mortality in patients with recent-onset T2D, as those with high levels of both biomarkers (hs-CRP and C-peptide) had the highest risk estimates. However, in patients with hyperinsulinemia and insulin resistance (high C-peptide), the risk of CVEs did not differ by hs-CRP levels, whereas the risk of all-cause mortality did. This finding may indicate that hs-CRP holds limited additional prognostic information in individuals who are already at high baseline CVE risk due to hyperinsulinemia and insulin resistance. On the other hand, hs-CRP is a strong prognostic biomarker of all-cause mortality regardless of hyperinsulinemia/insulin resistance.

The main strengths of this study include the cohort size of patients with recently diagnosed T2D and no history of CVEs, assessment of detailed information on clinical and lifestyle factors, and linkage with high-quality population-based health registries (14–16,18,21–23). These resources provided almost 100% completeness for serum hs-CRP levels and complete follow-up for outcome events.

Limitations include potential survival and referral biases. Survival bias could decrease participation of individuals with a very severe T2D phenotype or high cardiovascular risk and would likely bias results toward 1. Severe selection problems in our study are less likely because age, characteristics, and comorbidities in the DD2 cohort have been documented to be very similar to those of patients treated in routine clinical practice in Denmark, where treatment with glucose-lowering drugs is initiated right after a T2D diagnosis (14,40). Another limitation is a potential misclassification of registry diagnoses in our cohort. However, the positive predictive values for cardiovascular diagnoses and procedures in the Danish national registries are high (19,20). An additional concern is that we had only one baseline hs-CRP value and no follow-up values. However, hs-CRP levels are relatively stable over time and demonstrate almost no circadian variation (25). A further potential limitation is that imperfectly measured or unmeasured variables (e.g., diet, ethnicity) may have led to residual confounding in our study. Finally, >90% of Denmark's population is White, and our results may not necessarily apply to other individuals of other ethnicities.

In conclusion, this large, contemporary, prospective study of patients with recent-onset T2D shows that high hs-CRP is a modest prognostic biomarker of first incident CVEs and a strong biomarker of all-cause mortality. Patients with high C-peptide levels have substantially increased CVE risk, with elevated hs-CRP carrying limited additional prognostic information. The findings provide novel insights into how better to target early detection and prevention of CVE versus other serious diseases in T2D in a more differential and efficacious manner.

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