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C-peptide predicts all-cause and cardiovascular death in a cohort of individuals with newly diagnosed type 2 diabetes. The Skaraborg diabetes register

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ABSTRACT

Aims: To study the association between baseline level of C-peptide and all-cause death, cardiovascular death and cardiovascular complications among persons with newly diagnosed type 2 diabetes.

Methods: The Skaraborg Diabetes Register contains data on baseline C-peptide concentrations among 398 persons <65 years with newly diagnosed type 2 diabetes 1996–1998. National registries were used to determine all-cause death, cardiovascular death and incidence of myocardial infarction and ischemic stroke until 31 December 2014. The association between baseline C-peptide and outcomes were evaluated with adjustment for multiple confounders by Cox regression analysis. Missing data were handled by multiple imputation.

Results: In the imputed and fully adjusted model there was a significant association between 1 nmol/l increase in C-peptide concentration and all-cause death (HR 2.20, 95% CI 1.49–3.25, $p < 0.001$, number of events = 104), underlying cardiovascular death (HR 2.69, 1.49–4.85, $p = 0.001$, $n = 35$) and the composite outcome of underlying cardiovascular death, myocardial infarction or ischemic stroke (HR 1.61, 1.06–2.45, $p = 0.027$, $n = 90$).

Conclusions: Elevated C-peptide levels at baseline in persons with newly diagnosed type 2 diabetes are associated with increased risk of all-cause and cardiovascular mortality. C-peptide might be used to identify persons at high risk of cardiovascular complications and premature death.

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1. Introduction

Diabetes is one of the most common chronic diseases globally and is associated with severe micro- and macrovascular complications, and premature death [1]. From 1980 to 2014, age-standardized diabetes prevalence in adults has increased worldwide from 4.3% to 9.0% in men, and from 5.0% to 7.9% in women. If the trend continues the prevalence of diabetes is expected to be 12.8% in men and 10.4% in women in 2025 and the number of adults with diabetes will surpass 700 million [2]. Rising prevalence and decreasing age at diagnosis will lead to an increase in diabetes complications and the WHO projects that diabetes will be the seventh leading cause of death in 2030 [3].

Several cardiovascular risk factors such as high blood pressure, hyperglycaemia, dyslipidaemia, obesity and inactivity have been extensively studied among individuals with type 2 diabetes [4–6]. Worse prognosis has also been seen at younger age at diagnosis [7,8] and novel subgroups of adult-onset diabetes have been suggested in relation to age, insulin resistance and other clinical characteristics [9]. Yet considerable gaps remain in our understanding of the development of complications in type 2 diabetes.

Biomarkers have attracted increased attention for early identification of people at risk of type 2 diabetes and at risk of cardiovascular complications of type 2 diabetes [10]. However, studies in people with newly diagnosed type 2 diabetes are scarce. Identifying high risk individuals at an early stage of disease could possibly provide an opportunity to initiate more aggressive treatment and monitoring to avoid or post-poned complications.

C-peptide is a useful clinical indicator of insulin secretion [11], and represents a possible biomarker for predicting cardiovascular complications among individuals with newly diagnosed type 2 diabetes. However, studies of the association between C-peptide concentrations and complications in type 2 diabetes are still limited and contradictory. In a cross-sectional study from Turkey including 318 individuals with type-2 diabetes with mean duration of 9.1 years, a comparison of plasma C-peptide levels in patients with and without chronic complications in a logistic regression model showed an association of elevated C-peptide levels with macrovascular complications [12]. In another longitudinal study from Germany 3316 individuals without previously known diabetes undergoing coronary angiography were followed for a mean time of 7.6 years, the results showed an association of elevated C-peptide levels with all-cause and cardiovascular mortality [13]. On the other hand, in another study comparing C-peptide levels and the presence of diabetes complications in 77 individuals with type 2 diabetes treated only with oral hypoglycemic agents and a mean diabetes duration of 12.8 years, C-peptide levels did not differ in patients with or without diabetic complications [14]. None of those studies were based on individuals with newly diagnosed type 2 diabetes.

In a previous study of 399 participants with newly diagnosed type 2 diabetes with a follow-up time of 9 years, we

showed an association between elevated C-peptide and all-cause death, where individuals in the highest quartile had a significant 2.75-fold higher risk of all-cause death compared with those in the lowest quartile. There were no significant associations with cardiovascular death, incidence of cardiovascular events or the development of retinopathy [15]. However, due to the limited cohort size, associations with secondary outcomes may have been undetected due to low power. Therefore, the aim of this study was to use a longer 18-year follow-up period to evaluate C-peptide concentration at the time of diagnosis of type 2 diabetes as a possible biomarker to detect individuals at high risk of all-cause death, cardiovascular death and cardiovascular complications.

2. Material and methods

2.1. Study setting and participants

The participants in this cohort study comprised a subgroup of individuals registered in the Skaraborg diabetes register. The county of Skaraborg is a rural district in southern Sweden with a population of approximately 280 000 inhabitants in 1995. The Skaraborg diabetes register was established in 1991 [16] before the Swedish National diabetes register was established, and was active until 2004. Individuals with type 1 and 2 diabetes, but not gestational diabetes were registered. Registration was carried out at hospitals and diabetes outpatient clinics in primary healthcare, and the type of diabetes was determined clinically by the reporting physician according to prevailing World Health Organization guidelines [17]. The completeness of the Skaraborg diabetes register was 88.4% in 1995 for all individuals with diabetes and 97% for individuals treated with insulin or oral glucose-lowering drugs [16]. From 1996 to August 1998 all individuals registered in the Skaraborg diabetes register aged <65 years at the time of diagnosis were invited to participate in an additional study of islet antibodies and β -cell function [18]. Among those individuals who agreed to be tested, C-peptide was analysed at time of diagnosis and a biobank was established, being used for later additional laboratory analyses. In 2012 we analyzed creatinine by an enzymatic method and cystatin C and C-reactive protein (CRP) with an immunoturbidimetric assay in plasma from the biobank at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. In this study we included all individuals with type 2 diabetes, for whom blood samples were available at diagnosis ($n = 398$).

2.2. Diagnosis registers

Information on mortality and cardiovascular complications was retrieved from two national registers kept by the National Board of Health and Welfare using the unique Swedish personal identification number. The National Patient Register includes information about in-patient care [19]. The National Cause of Death Register contains data on virtually all deaths in Sweden, indicating the underlying cause of death which is defined by the World Health Organization as “the disease

or injury which initiates the train of morbid events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury”, as well as contributing causes of death which are assigned without priority order [20].

2.3. Study outcomes and follow-up

The primary study outcome was all-cause death. Secondary outcomes were underlying cardiovascular cause of death, contributing cardiovascular cause of death, underlying cancer cause of death, contributing cancer cause-of death, non-cardiovascular non-cancer death, fatal and non-fatal myocardial infarction, fatal and non-fatal ischemic stroke and a composite endpoint comprised of fatal and non-fatal myocardial infarction, fatal and non-fatal ischemic stroke and cardiovascular death. All outcomes were defined according to the International Statistical Classification of Diseases and Related Health Problems (ICD) version 9 and 10, see footnote Table 2. Study entry was defined as date of diagnosis of type 2 diabetes and the participants were followed until the first occurrence of: study outcome, death or end of study 31 December 2014.

2.4. Statistics

Continuous variables were described as mean \pm standard deviation (SD), or as median with interquartile range if skewed. Categorical variables were described as frequencies and percentages. Study outcomes were described as frequencies and event rates with 95% confidence intervals.

Missing covariate data were handled by multiple imputation with chained equations, creating 30 imputed datasets [21]. Non-normally continuous variables were ln-transformed before inclusion in the imputation model, and were exponentiated back to their original scale for analysis. The imputation model included baseline variables: age at diagnosis, sex, smoking, body mass index (BMI), systolic blood pressure, antihypertensive treatment, hemoglobin A1c (HbA1c), C-peptide, estimated glomerular filtration rate (eGFR), c-reactive protein, total cholesterol, previous myocardial infarction or ischemic stroke, as well as the event status and the Nelson-Aalen cumulative hazard for each endpoint analysed.

Cox regression proportional hazards models were used to calculate hazard ratios (HR) to investigate the association between baseline C-peptide concentrations as a linear continuous variable and the time from diabetes diagnosis to study outcomes. For maximal power in the analyses we didn't categorize C-peptide in quartiles, and as C-peptide was non-normally distributed we decided not to convert to Z-score. The linear functional form of C-peptide was assessed graphically by Martingale residuals. In the first model, crude unadjusted hazard ratios were calculated. In a second model, hazard ratios were adjusted for age and sex. In a third model, analyses were made for all participants without missing covariate data (complete cases) with further adjustment for smoking, BMI, systolic blood pressure, antihypertensive treatment, HbA1c, eGFR, c-reactive protein, total cholesterol and

previous myocardial infarction or ischemic stroke. In a fourth final model, hazard ratios were calculated using imputed data with the same adjustments as in model 3. Subgroup analyses were performed for individuals without previous myocardial infarction or ischemic stroke. The assumption of proportional hazards was confirmed by evaluating Schoenfeld residuals graphically and by the Stata *phptest* command.

All statistical analyses were performed with SPSS version 23 and Stata version 15.1 software. A two-sided p-value of <0.05 was considered statistically significant.

2.5. Ethics

The regional ethical review board in Gothenburg approved the study (Diary numbers 208-06 and T632-16).

3. Results

3.1. Study cohort

A total of 398 individuals <65 years old with clinical new-onset type 2 diabetes 1996–1998 and available fasting C-peptide at baseline were included in the study, see flow-chart Fig. 1. Baseline characteristics are presented in Table 1. The mean age at diagnosis was 52.4 (SD \pm 8.7) years and the proportion of men was 59.8%. The fasting C-peptide concentration ranged between 0.1 and 4.0 nmol/l, with median value 0.88 nmol/l. Twelve of the individuals had a record of previous myocardial infarction or ischemic stroke. Baseline characteristics of the 386 individuals without prior myocardial infarction or ischemic stroke were similar as in the total cohort, see Table 1.

3.2. Follow-up and study outcomes

The median follow-up time until death or end of study was 17.0 years and ranged between 0.2 and 18.9 years. The number of study outcome events and event incidence rates are presented in Table 2. The primary study outcome, all-cause death, occurred among 104 individuals (26.1%) during 6135 person-years of follow up. Causes of deaths were evenly distributed among underlying cardiovascular- (33.6%), cancer- (30.8%) and non-cardiovascular non-cancer deaths (35.6%). Cardiovascular disease as contributing cause of death was recorded for 58 individuals (55.8% of all deaths). Myocardial infarction occurred among 51 individuals (12.8%), ischemic stroke among 40 individuals (10.1%) and the composite endpoint of myocardial infarction, ischemic stroke or cardiovascular death among 90 individuals (22.6%). The number of events among individuals without prior myocardial infarction or ischemic stroke are presented in Table 2.

3.3. Association between C-peptide and study outcomes

Crude and adjusted HR regarding study outcomes per 1 nmol/l increase of C-peptide concentration are presented in Table 3. We saw a significant association between C-peptide concentration and the primary study outcome of all-cause death in the fully adjusted complete cases model (HR 2.90, 95% CI

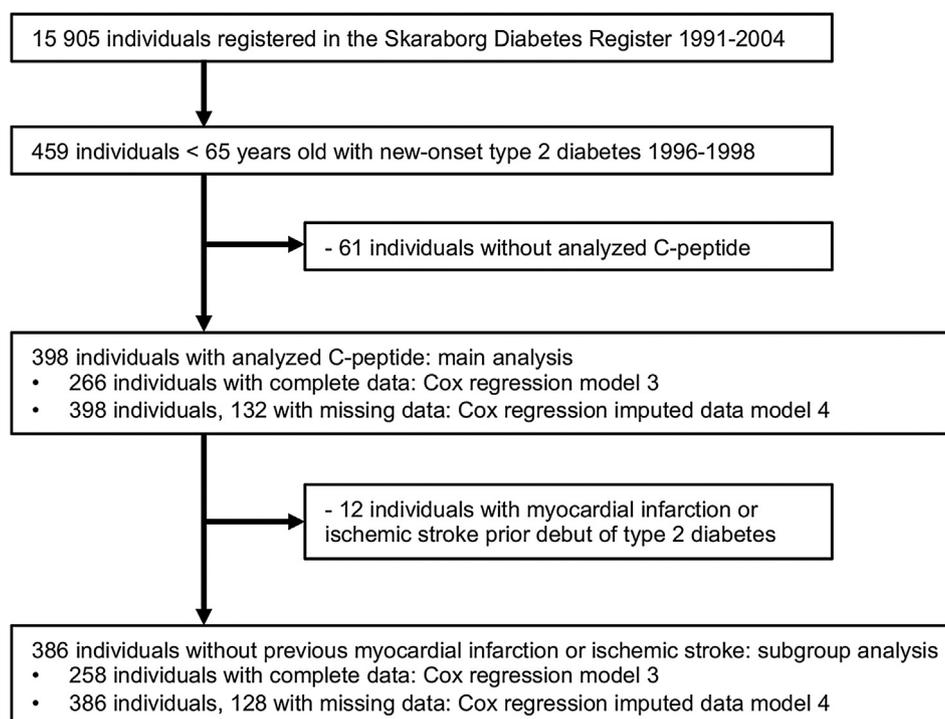


Fig. 1 – Flowchart depicting inclusion of study participants and performed analyses.

Table 1 – Baseline characteristics of the study population with new-onset type 2 diabetes 1996–1998.

	All individuals	Individuals without previous CV event ^b
Number of individuals in study population	398	386
Men	238 (59.8%)	226 (58.6%)
Age at diagnosis (years)	52.4 ± 8.7	52.4 ± 8.7
Current smoking	102 (25.6%)	100 (25.9%)
Body mass index (kg/m ²)	31.3 ± 5.6	31.2 ± 5.6
Systolic blood pressure (mmHg)	140.6 ± 19.7	140.4 ± 19.5
Diastolic blood pressure (mmHg)	83.1 ± 9.7	83.1 ± 9.8
Antihypertensive treatment	119 (29.9%)	113 (29.3%)
HbA1c, NGSP (%)	7.8 ± 1.7	7.8 ± 1.8
HbA1c, IFCC (mmol/mol)	61.6 ± 19.1	61.6 ± 19.1
C-peptide (nmol/l) ^a	0.88 (0.62–1.16)	0.87 (0.62–1.15)
Minimum	0.1	0.1
Maximum	4.0	4.0
Creatinine (μmol/l) ^a	67 (58–81)	67 (58–80)
Cystatin C (mg/l) ^a	0.85 (0.73–1.05)	0.85 (0.72–1.05)
eGFR (ml/min/1.73 m ²)	103.9 ± 38.2	104.2 ± 38.5
CRP (mg/l) ^a	3.4 (1.7–6.6)	3.4 (1.7–6.7)
Cholesterol (mmol/l)	5.8 ± 1.4	5.8 ± 1.3
Previous CV event ^b	12 (3.0%)	–

Values are expressed as mean ± SD or frequencies (%) if not otherwise specified.
 Missing data among all individuals (and individuals without previous CV event) for current smoking: n = 6 (5); systolic and diastolic blood pressure: n = 13 (12); HbA1c: n = 9 (9); creatinine, cystatin C, eGFR and CRP: n = 2 (2); cholesterol: n = 120 (117).
 CV, cardiovascular; HbA1c, hemoglobin A1c; NGSP, National glycohemoglobin standardization program; IFCC, International federation of clinical chemistry; eGFR, estimated glomerular filtration rate; CRP, c-reactive protein.

^a Median (interquartile range).
^b Myocardial infarction or ischemic stroke.

1.81–4.66, $p < 0.001$) and in the final fully adjusted imputed model (HR 2.20, 1.49–3.25, $p < 0.001$). The increased risk of mortality was mainly driven by cardiovascular disease, showing significantly increased HR in all models for underlying as

well as contributing cardiovascular causes of death. In the final fully adjusted imputed model the HR for cardiovascular disease as underlying cause of death was 2.69 (1.49–4.85, $p = 0.001$). There was no significant association between C-

Table 2 – Number of outcome events and event incidence rates per 1000 person-years among individuals with new-onset type 2 diabetes 1996–1998. Follow-up until 31 December 2014.

	All individuals (n = 398)			Individuals without previous CV event ^a (n = 386)		
	Number of events	Incidence rate	95% CI	Number of events	Incidence rate	95% CI
All-cause death	104	16.9	14.0–20.5	96	16.1	13.2–19.6
CV underlying death	35	5.7	4.1–7.9	29	4.9	3.4–7.0
CV contributing death	58	9.5	7.3–12.2	51	8.5	6.5–11.2
Cancer death	32	5.2	3.7–7.4	32	5.4	3.8–7.6
Cancer contributing death	36	5.9	4.2–8.1	35	5.9	4.2–8.2
Non-CV non-cancer death	37	6.0	4.4–8.3	35	5.9	4.2–8.2
Myocardial infarction	51	8.7	6.6–11.4	49	8.5	6.5–11.3
Ischemic stroke	40	6.8	5.0–9.2	35	6.1	4.4–8.5
CV death, myocardial infarction or ischemic stroke	90	14.8	12.0–18.1	81	13.6	10.9–16.9

CV, cardiovascular; 95% CI, 95% confidence interval.
CV death: ICD 10: I, ICD 9: 390–459; cancer death: ICD 10: C, ICD 9: 140–239; non-CV non-cancer death: ICD 10: all codes except C and I, ICD 9: all codes except 390–459 or 140–239; myocardial infarction: ICD 10: I21, ICD 9: 410; ischemic stroke: ICD 10: I63, ICD 9: 433, 434.
^a Myocardial infarction or ischemic stroke.

Table 3 – Association between linear continuous C-peptide concentration and death, myocardial infarction, ischemic stroke and composite endpoint among 398 individuals with debut of type 2 diabetes 1996–1998. Follow-up until 31 December 2014.

	Model 1 (crude)			Model 2 (adjusted for age and sex)			Model 3 (fully adjusted, complete cases) ^{a,b}			Model 4 (fully adjusted, imputed variables) ^a		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
All cause death	2.01	1.45–2.79	<0.001	2.16	1.55–3.01	<0.001	2.90	1.81–4.66	<0.001	2.20	1.49–3.25	<0.001
CV underlying death	2.86	1.85–4.43	<0.001	3.31	2.06–5.32	<0.001	4.26	2.00–9.04	<0.001	2.69	1.49–4.85	0.001
CV contributing cause of death	2.62	1.82–3.79	<0.001	2.96	2.00–4.36	<0.001	3.30	1.76–6.21	<0.001	2.31	1.43–3.72	0.001
Cancer death	1.45	0.72–2.91	0.30	1.48	0.74–2.97	0.27	2.19	0.76–6.27	0.14	1.90	0.88–4.12	0.10
Cancer contributing cause of death	1.43	0.74–2.77	0.29	1.49	0.77–2.89	0.24	2.45	0.93–6.48	0.071	1.98	0.95–4.13	0.069
Non-CV non-cancer death	1.58	0.84–2.95	0.16	1.72	0.90–3.27	0.098	3.32	1.30–8.48	0.012	1.94	0.92–4.08	0.081
Myocardial infarction	1.68	1.04–2.72	0.036	1.72	1.05–2.80	0.030	1.27	0.62–2.62	0.51	1.47	0.84–2.58	0.17
Ischemic stroke	1.56	0.85–2.86	0.16	1.68	0.91–3.11	0.099	1.36	0.60–3.06	0.46	1.22	0.62–2.39	0.56
CV death, myocardial infarction or ischemic stroke	1.78	1.21–2.60	0.003	1.87	1.28–2.74	0.001	1.96	1.23–3.12	0.005	1.61	1.06–2.45	0.027

CV, cardiovascular; HR, hazard ratio; 95% CI, 95% confidence interval.

Statistically significant results (p-value of <0.05) in bold.

^a Adjusted for age, sex, smoking, systolic blood pressure, HbA1c, antihypertensive treatment, BMI, CRP, eGFR, cholesterol, and previous myocardial infarction or ischemic stroke.^b Complete data was available for 266 cases.

peptide and cancer death either as underlying or contributing cause of death. The risk of non-cardiovascular non-cancer death was significantly increased in the fully adjusted complete cases model, but not in the final fully adjusted imputed model. We saw a significant association between C-peptide and risk of the composite endpoint (myocardial infarction, ischemic stroke or cardiovascular death) in all models with HR 1.61 (1.06–2.45, $p=0.027$) in the final fully adjusted imputed model. For the risk of myocardial infarction, no significant association could be detected in the fully adjusted models. Concerning the risk of ischemic stroke, no significant association was found to C-peptide. The subgroup analyses of individuals with no record of previous myocardial infarction or ischemic stroke are presented in supplementary Table 1. Similar results were found as in the total cohort with respect to significance and magnitude.

4. Discussion

In this 18-year follow-up prospective study with 398 individuals <65 years old with newly diagnosed type 2 diabetes in 1996–1998 we saw a highly significant association between C-peptide concentration at diagnosis and all-cause and cardiovascular death. The risk of all-cause death was 2.2-fold increased per 1 nmol/l increase in C-peptide concentration when adjusted for multiple risk factors, and the corresponding adjusted risks of death coded as either underlying or contributing cardiovascular disease were 2.7 and 2.3-fold. Of the 104 individuals who died during follow up, approximately one third (34%) died of underlying cardiovascular disease and the majority (56%) had a contributing cardiovascular cause of death. We also saw a significant but weaker 1.6-fold adjusted association between C-peptide concentration and the composite endpoint of myocardial infarction, ischemic stroke and underlying cardiovascular death, but no association could be detected in the adjusted models regarding myocardial infarction or ischemic stroke per se.

Our results are consistent with several previous studies. In an US prospective study of 1370 individuals with type 1 or type 2 diabetes with a mean diabetes duration of 15.3 years and a follow up of 16 years, C-peptide was significantly associated with all-cause and ischemic heart disease mortality after controlling for potential confounders (age, sex, BMI, duration of diabetes, systolic blood pressure, history of cardiovascular disease, diabetic retinopathy status, smoking, time since last meal, exogenous insulin use, and HbA1c) [22]. An Italian longitudinal study of 431 individuals with stable atherosclerosis, with or without diabetes, and a mean follow-up of 56 months showed that C-peptide was associated with cardiovascular mortality independently of known diabetes status, even after adjustment for age, sex, diabetes treatment, estimated glomerular filtration rate [23]. Also in our own previous study with shorter follow-up time we could show an elevated all-cause mortality for individuals with higher C-peptide levels [15]. However, there are also some studies not providing evidence of association between C-peptide and cardiovascular disease and cardiovascular mortality. For example, in an Italian retrospective cohort study with median follow-up of 14 years 2113 individuals with type

2 diabetes with an approximate diabetes duration of 9 years were studied. After adjusting for multiple confounders, no association could be found between higher baseline C-peptide levels and cardiovascular disease, all-cause mortality or mortality due to cancer, diabetes or cardiovascular diseases [24]. This study could however show that higher C-peptide levels were associated with a reduced risk of incident microvascular complications (retinopathy, nephropathy and neuropathy). This shows that the role of C-peptide in the development of diabetes complications is rather complex and not easy to interpret having in mind the coexistence of multiple cardiovascular risk factors.

C-peptide levels and cardiovascular complications and mortality have also been studied in non-diabetes populations. A retrospective cohort study of 5,153 non-diabetes individuals between 40 and 74 years old and a follow-up time of 14.4 years showed that C-peptide levels significantly related to cardiovascular and overall death [25]. Possible explanations discussed were that high C-peptide levels reflect insulin-resistance, that high C-peptide levels predict future worsening of the current risk factors or cardiovascular disease, or the existence of other significant predictors of cardiovascular death that could not be accounted for [25].

As mentioned one possible pathophysiological mechanism of the increased risk of cardiovascular mortality associated with elevated levels of C-peptide could be that elevated C-peptide levels mirror high insulin concentration and insulin resistance which is part of the metabolic syndrome which is a cardiovascular risk factor [26]. It should be noted that the association between elevated C-peptide levels and the increased risk of cardiovascular mortality was independent of BMI, a measurement for obesity which is also a part of the metabolic syndrome. The association remained significant also after adjustment for several other cardiovascular risk factors suggesting that C-peptide could be an independent risk factor for elevated cardiovascular mortality in our study population. Moreover, it is now accepted that insulin resistance is not only a consequence of obesity and metabolic syndrome but also an independent cardiovascular risk factor [27]. Experimental studies have shown that loss of insulin signalling in the endothelium plays an important role in the development of endothelial dysfunction [28]. This suggests the importance of not only treating elevated blood glucose levels, but also of seeking to improve the insulin sensitivity pharmacologically [29].

The lack of significant association between elevated C-peptide concentration and myocardial infarction or stroke in the adjusted models was somewhat unexpected, but possibly related to modest effect sizes and the rather small number of events due to the small population size, especially for myocardial infarctions where we saw a significant association in the crude, and age and sex adjusted models. In addition, silent myocardial infarctions may have passed unrecognized.

We did not study microvascular complications such as retinopathy or renal failure. However, it is interesting to note that higher C-peptide levels appear to be associated with reduced risk of incident microvascular diabetes complications as shown for example in the Italian study described in detail above [24] for both retinopathy, neuropathy and

nephropathy. This association has also been noted among individuals with type 1 diabetes, indicating a protective effect of preserved β -cell function regarding the development of microvascular complications [30]. C-peptide has been discussed as a possible therapeutic candidate in patients with type 1 diabetes and its protective effect has been suggested to be mediated through cell membrane interaction, intracellular mechanisms, anti-inflammatory and circulatory effects [31].

This suggests a complex role of C-peptide as a biomarker for diabetes complications [32]. Also, novel clinical phenotypes of adult-onset diabetes with different panoramas of complications have been proposed partly based on level of insulin resistance and β -cell function [9]. Positive effects of intensive blood glucose-lowering therapy on mortality and macrovascular complications in people with type 2 diabetes are still controversial. While some studies have reported short term [33] but not long term benefits [34], others have found that mortality might even increase [35]. This suggests that the intensity of antidiabetic treatment needs to be individualized and that it may be most beneficial when initiated early in the course of the disease.

A particular strength of the study is the focus on persons with newly diagnosed type 2 diabetes. This means both avoiding the risk of survival bias at study inclusion and also having a study population ideal for early detection of individuals at high risk, providing valuable information to initiate individualized treatment at an early stage. Another strength is the population-based design of the study that allows for real life analyses. The high capture rate of the Skaraborg diabetes register minimizes the risk of patient selection bias and increases the likelihood that persons in the study cohort are representative to those usually met and treated in primary care. Also, the biomarker C-peptide we studied is already available and used in clinical practice at relative low cost.

Our study has some limitations. First, the sample size was small, resulting in limited power to detect significant associations. For example, in a post-hoc power analysis we estimated that at least 228 myocardial infarctions among 1779 individuals would be needed to detect the 1.47 hazard ratio seen in the current study's fully adjusted model assuming no correlation between c-peptide and other covariates, 80% power and a two-sided 5%-level significance test. Alternatively, the hazard ratio was estimated to have to be minimum 2.26 given the current 51 myocardial infarctions among 398 individuals. Second, since this is an observational study we cannot determine causality between C-peptide levels and study outcomes. Third, only persons <65 years at diagnosis were included whereas many persons with type 2 diabetes mellitus are older at diagnosis. Fourth, the study's long follow-up is a strength, but also means that medical treatment used during the study period differs compared to potentially more beneficial contemporary treatment. Moreover we do not have any specific data on lipid or blood pressure lowering agents used or use of glucose lowering agents that could affect the endogenous insulin production and therefore the c-peptide levels. Fifth, Skaraborg county is mainly a rural area populated by people of European descent and it is possible our findings could be different in another setting. Finally, although adjusted for multiple confounders we cannot

exclude residual confounding since certain clinical data were not available, e.g. insulin levels and socioeconomic status.

In conclusion, we were able to show that elevated C-peptide levels in persons with newly diagnosed type 2 diabetes are associated with increased risk of all-cause and cardiovascular mortality. Currently C-peptide is mainly used to distinguish between different types of diabetes. Our results suggest that C-peptide might also be used to identify persons with newly diagnosed type 2 diabetes at high risk of cardiovascular complications and premature death.

We recommend further studies focusing on detecting the exact mechanism by which C-peptide could contribute to cardiovascular and all-cause death in individuals with type 2 diabetes and on providing possible therapeutic implications.

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Conflict of interest

There is no conflict of interest for any of the authors with respect to this article.

Author contribution

MP contributed to the design of the research questions, with the data collection, the analysis and interpretation of the data, and writing and critical review of the manuscript. TA contributed with the data collection, the analysis and interpretation of the data, and writing and critical review of the manuscript. KBB contributed to the design of the research questions, assistance with the data collection, the analysis and interpretation of the data and a critical review of the manuscript. OM contributed to the design of the research questions, assistance with the analysis and interpretation of the data and a critical review of the manuscript. KR contributed with assistance with the analysis and interpretation of the data and a critical review of the manuscript. JC contributed to the design of the research questions, assistance with the analysis and interpretation of the data and a critical

review of the manuscript. All authors approved the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.diabres.2019.03.014>.

REFERENCES

- [1] Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. *Lancet Diabetes Endocrinol* 2014;2:634–47. [https://doi.org/10.1016/S2213-8587\(14\)70102-0](https://doi.org/10.1016/S2213-8587(14)70102-0).
- [2] NCD Risk Factor Collaboration. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 2016;387:1513–30. [https://doi.org/10.1016/S0140-6736\(16\)00618-8](https://doi.org/10.1016/S0140-6736(16)00618-8).
- [3] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006;3:e442. <https://doi.org/10.1371/journal.pmed.0030442>.
- [4] UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ*. 1998;317:703–13. <https://doi.org/10.1136/bmj.317.7160.703>.
- [5] Krentz AJ. Lipoprotein abnormalities and their consequences for patients with type 2 diabetes. *Diabetes Obes Metab* 2003;5 (Suppl 1):S19–27. <https://doi.org/10.1046/j.1462-8902.2003.0310.x>.
- [6] Sullivan PW, Morrato EH, Ghushchyan V, Wyatt HR, Hill JO. Obesity, inactivity, and the prevalence of diabetes and diabetes-related cardiovascular comorbidities in the U.S., 2000–2002. *Diabetes Care* 2005;28:1599–603. <https://doi.org/10.2337/diacare.28.7.1599>.
- [7] Huo L, Magliano DJ, Ranciere F, Harding JL, Nanayakkara N, Shaw JE, et al. Impact of age at diagnosis and duration of type 2 diabetes on mortality in Australia 1997–2011. *Diabetologia* 2018;61:1055–63. <https://doi.org/10.1007/s00125-018-4544-z>.
- [8] Andersson T, Hjerpe P, Carlsson AC, Pivodic A, Wändell P, Manhem K, et al. Mortality trends and cause of death in patients with new-onset type 2 diabetes and controls: a 24-year follow-up prospective cohort study. *Diabetes Res Clin Pract* 2018;138:81–9. <https://doi.org/10.1016/j.diabres.2018.01.038>.
- [9] Ahlqvist E, Storm P, Karajamaki A, Martinell M, Dorkhan M, Carlsson A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol* 2018;6:361–9. [https://doi.org/10.1016/S2213-8587\(18\)30051-2](https://doi.org/10.1016/S2213-8587(18)30051-2).
- [10] Nowak C, Carlsson AC, Ostgren CJ, Nystrom FH, Alam M, Feldreich T, et al. Multiplex proteomics for prediction of major cardiovascular events in type 2 diabetes. *Diabetologia* 2018;61:1748–57. <https://doi.org/10.1007/s00125-018-4641-z>.
- [11] Leighton E, Sainsbury CA, Jones GC. A practical review of C-peptide testing in diabetes. *Diabetes Ther* 2017;8:475–87. <https://doi.org/10.1007/s13300-017-0265-4>.
- [12] Sari R, Balci MK. Relationship between C peptide and chronic complications in type-2 diabetes mellitus. *J Natl Med Assoc* 2005;97:1113–8.
- [13] Marx N, Silbernagel G, Brandenburg V, Burgmaier M, Kleber ME, Grammer TB, et al. C-peptide levels are associated with mortality and cardiovascular mortality in patients undergoing angiography: the LURIC study. *Diabetes Care* 2013;36:708–14. <https://doi.org/10.2337/dc12-1211>.
- [14] Mavrakanas T, Frachebois C, Soualah A, Aloui F, Julier I, Bastide D. C-peptide and chronic complications in patients with type-2 diabetes and the metabolic syndrome. *Presse Med* 2009;38:1399–403. <https://doi.org/10.1016/j.lpm.2009.02.023>.
- [15] Pikkemaat M, Melander O, Molstad S, Garberg G, Bostrom KB. C-peptide concentration, mortality and vascular complications in people with Type 2 diabetes. The Skaraborg Diabetes Register. *Diabet Med* 2015;32:85–9. <https://doi.org/10.1111/dme.12608>.
- [16] Berger B, Stenstrom G, Chang YF, Sundkvist G. The prevalence of diabetes in a Swedish population of 280,411 inhabitants. A report from the Skaraborg Diabetes Registry. *Diabetes Care* 1998;21:546–8. <https://doi.org/10.2337/diacare.21.4.546>.
- [17] World Health Organization. Diabetes mellitus. Report of a WHO Study Group. World Health Organ Tech Rep Ser 1985;727:1–113.
- [18] Berger B, Borg H, Fernlund P, Stenstrom G, Sundkvist G. Islet antibodies associated with pancreatic B-cell dysfunction at and 3 years after diagnosis of diabetes in subjects aged 35–64 years old: degree of impairment less severe than in those aged 0–34 years old. *Diabet Med* 2006;23:1180–5. <https://doi.org/10.1111/j.1464-5491.2006.01972.x>.
- [19] Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011;11:450. <https://doi.org/10.1186/1471-2458-11-450>.
- [20] Brooke HL, Talbäck M, Hörnblad J, Johansson LA, Ludvigsson JF, Druid H, et al. The Swedish cause of death register. *Eur J Epidemiol* 2017;32:765–73. <https://doi.org/10.1007/s10654-017-0316-1>.
- [21] White IR, Royston P, Wood AM. Multiple imputation using chained equations: issues and guidance for practice. *Stat Med* 2011;30:377–99. <https://doi.org/10.1002/sim.4067>.
- [22] Hirai FE, Moss SE, Klein BE, Klein R. Relationship of glycemic control, exogenous insulin, and C-peptide levels to ischemic heart disease mortality over a 16-year period in people with older-onset diabetes: the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). *Diabetes Care* 2008;31:493–7. <https://doi.org/10.2337/dc07-1161>.
- [23] Cardellini M, Farcomeni A, Ballanti M, Morelli M, Davato F, Cardolini I, et al. C-peptide: a predictor of cardiovascular mortality in subjects with established atherosclerotic disease. *Diab Vasc Dis Res* 2017;14:395–9. <https://doi.org/10.1177/1479164117710446>.
- [24] Bo S, Gentile L, Castiglione A, Prandi V, Canil S, Ghigo E, et al. C-peptide and the risk for incident complications and mortality in type 2 diabetic patients: a retrospective cohort study after a 14-year follow-up. *Eur J Endocrinol* 2012;167:173–80. <https://doi.org/10.1530/EJE-12-0085>.
- [25] Patel N, Taveira TH, Choudhary G, Whitlatch H, Wu WC. Fasting serum C-peptide levels predict cardiovascular and overall death in nondiabetic adults. *J Am Heart Assoc* 2012;1: e003152. <https://doi.org/10.1161/jaha.112.003152>.
- [26] Reaven GM. The insulin resistance syndrome: definition and dietary approaches to treatment. *Annu Rev Nutr* 2005;25:391–406. <https://doi.org/10.1146/annurev.nutr.24.012003.132155>.
- [27] Patel TP, Rawal K, Bagchi AK, Akolkar G, Bernardes N, Dias Dda S, et al. Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. *Heart Fail Rev* 2016;21:11–23. <https://doi.org/10.1007/s10741-015-9515-6>.

- [28] Paneni F, Costantino S, Cosentino F. Role of oxidative stress in endothelial insulin resistance. *World J Diabetes* 2015;6:326–32. <https://doi.org/10.4239/wjd.v6.i2.326>.
- [29] Paneni F, Costantino S, Cosentino F. Insulin resistance, diabetes, and cardiovascular risk. *Curr Atheroscler Rep* 2014;16:419. <https://doi.org/10.1007/s11883-014-0419-z>.
- [30] Panero F, Novelli G, Zucco C, Fornengo P, Perotto M, Segre O, et al. Fasting plasma C-peptide and micro- and macrovascular complications in a large clinic-based cohort of type 1 diabetic patients. *Diabetes Care* 2009;32:301–5. <https://doi.org/10.2337/dc08-1241>.
- [31] Wahren J, Kallas A, Sima AA. The clinical potential of C-peptide replacement in type 1 diabetes. *Diabetes* 2012;61:761–72. <https://doi.org/10.2337/db11-1423>.
- [32] Leberherz C, Marx N. C-Peptide and its career from innocent bystander to active player in diabetic atherogenesis. *Curr Atheroscler Rep* 2013;15:339. <https://doi.org/10.1007/s11883-013-0339-3>.
- [33] Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Advance Collaborative Group, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008;358:2560–72. <https://doi.org/10.1056/NEJMoa0802987>.
- [34] Zoungas S, Chalmers J, Neal B, Billot L, Li Q, Hirakawa Y, et al. Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. *N Engl J Med* 2014;371:1392–406. <https://doi.org/10.1056/NEJMoa1407963>.
- [35] Gerstein HC, Miller ME, Byington RP, Goff Jr DC, Bigger JT, Action to Control Cardiovascular Risk in Diabetes Study Group, et al. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*. 2008;358:2545–59. <https://doi.org/10.1056/NEJMoa0802743>.