# Association of glycated hemoglobin A1c levels with cardiovascular outcomes in the general population: Results from the BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) consortium

**Christoph Sinning** (✉ christoph.sinning@t-online.de)
University Heart & Vascular Center Hamburg https://orcid.org/0000-0002-7331-8840
**Nataliya Makarova**
University Heart & Vascular Center Hamburg
**Henry Völzke**
Universitätsmedizin Greifswald: Universitatsmedizin Greifswald
**Renate Schnabel**
University Heart & Vascular Center Hamburg
**Francisco Ojeda**
University Heart & Vascular Center Hamburg
**Marcus Dörr**
Universitätsmedizin Greifswald: Universitatsmedizin Greifswald
**Stephan B. Felix**
Universitätsmedizin Greifswald: Universitatsmedizin Greifswald
**Wolfgang Koenig**
Deutsches Herzzentrum München: Deutsches Herzzentrum Munchen
**Annette Peters**
Helmholtz Zentrum München Institute of Epidemiology: Helmholtz Zentrum Munchen Institute of Epidemiology
**Wolfgang Rathmann**
German Diabetes Center Düsseldorf
**Ben Schöttker**
Deutsches Krebsforschungszentrum
**Hermann Brenner**
Deutsches Krebsforschungszentrum
**Giovanni Veronesi**
University of Insubria: Universita degli Studi dell'Insubria
**Giancarlo Cesana**

University of Milano–Bicocca: Universita degli Studi di Milano-Bicocca
**Paolo Brambilla**
University of Milano–Bicocca: Universita degli Studi di Milano-Bicocca
**Tarja Palosaari**
National Institute of Health and Welfare Helsinki
**Kari Kuulasmaa**
National Institute of Health and Welfare Helsinki
**Inger Njølstad**
Arctic University of Norway Alta Campus: UiT Norges arktiske universitet - Campus Alta
**Ellisiv Bøgeberg Mathiesen**
Arctic University of Norway Alta Campus: UiT Norges arktiske universitet - Campus Alta
**Tom Wilsgaard**
Arctic University of Norway Alta Campus: UiT Norges arktiske universitet - Campus Alta
**Stefan Blankenberg**
Penn Heart and Vascular Center
**Stefan Söderberg**
Umea Universitet
**Marco M. Ferrario**
University of Insubria: Universita degli Studi dell'Insubria
**Barbara Thorand**
Helmholtz Zentrum Munchen Institut fur Diabetesforschung

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**Research Article**

**Keywords:** biomarkers, glycated hemoglobin A1c (HbA1c), cardiovascular risk, mortality, BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe), MORGAM (MONICA Risk Genetics Archiving and Monograph)

**Posted Date:** August 4th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-774608/v1



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**Version of Record:** A version of this preprint was published at Cardiovascular Diabetology on November 15th, 2021. See the published version at https://doi.org/10.1186/s12933-021-01413-4.

# Abstract

## Background

Biomarkers may contribute to improved cardiovascular risk estimation. Glycated hemoglobin $A_{1c}$ ($HbA_{1c}$) is used to monitor the quality of diabetes treatment. Its role for prediction of cardiovascular outcomes in the general population remains uncertain. This study aims to assess the role of $HbA_{1c}$ in predicting cardiovascular outcomes in the general population.

## Methods

Data from six prospective population-based cohort studies across Europe comprising 36,180 participants were analyzed. $HbA_{1c}$ was evaluated in conjunction with classical cardiovascular risk factors (CVRFs) for association with cardiovascular mortality, cardiovascular disease (CVD), and overall mortality in subjects without diabetes (N=32,477) and with diabetes (N=3,703).

## Results

Kaplan-Meier curves showed higher event rates with increasing $HbA_{1c}$ levels (log-rank-test: $p<0.001$). Cox regression analysis revealed significant associations between $HbA_{1c}$ (in mmol/mol) log-transformed and divided by interquartile range in the total study population and the examined outcomes. Thus, a hazard ratio (HR) of 1.12 (95% confidence interval (CI): 1.04–1.20, p=0.002) for cardiovascular mortality, 1.10 (95% CI: 1.04–1.16, $p<0.001$) for CVD, and 1.09 (95%CI: 1.05–1.14, $p<0.001$) for overall mortality can be reported. An increased risk of CVD was also observed in subjects without diabetes with increased $HbA_{1c}$ levels (HR 1.09; 95% CI: 1.01-1.16, p=0.021).

$HbA_{1c}$ cut-off values of 39.9 mmol/mol (5.8%), 36.6 mmol/mol (5.5%), and 38.8 mmol/mol (5.7%) for cardiovascular mortality, CVD, and overall mortality, respectively, show an increased risk for the outcome.

## Conclusions

$HbA_{1c}$ was demonstrated to be an independent prognostic biomarker for all investigated outcomes in the general European population. A mostly monotonically increasing relationship was observed between $HbA_{1c}$ levels and outcomes. Elevated $HbA_{1c}$ levels were also associated with the outcomes in participants without diabetes (i.e., $HbA_{1c}$ levels < 6.5% (<48mmol/mol)) underlining the importance of $HbA_{1c}$ levels in the overall population.

# Introduction

Prediction of cardiovascular outcomes in the general population is important for clinical decision-making, including the prescription of medication or targeting of lifestyle intervention strategies. Despite the identification of novel independent biomarkers, established risk prediction algorithms rely on a set of

traditional cardiovascular risk factors (CVRFs) such as age, sex, blood pressure, lipid levels, diabetes mellitus (DM), and smoking. The decision whether to include novel biomarkers in cardiovascular risk assessment remains a topic of intense debate and research [1]. Diabetes is regarded as a classical risk factor for cardiovascular disease (CVD) [2]. Due to the clinical need to identify novel risk factors to improve cardiovascular risk prediction, glycated hemoglobin or hemoglobin $A_{1c}$ ($HbA_{1c}$) may be a potential candidate [3]. The primary clinical use of $HbA_{1c}$ is as an indicator of the average blood glucose levels over the past three months, in addition to its use as a diagnostic and screening tool for DM [4, 5].

While associations between $HbA_{1c}$ levels and the risk of cardiovascular outcomes or overall mortality have been reported, [6–9] only few studies suggested that $HbA_{1c}$ may be associated with cardiovascular outcomes in an apparently healthy population [6, 10–14]. Recently published results underlined the additional use of $HbA_{1c}$ levels in middle-aged individuals without a history of CVD and $HbA_{1c}$ levels in the nondiabetic range [15]. Studies employing Mendelian randomization supported the role of a link between increasing $HbA_{1c}$ levels and an increased risk of coronary artery disease [16, 17]. In this context, $HbA_{1c}$ represents a promising indicator of increased risk and might be of importance in individuals without a diagnosis of diabetes [1].

In the present study, we evaluated the distribution of $HbA_{1c}$ levels in population-based cohorts across Europe. Furthermore, we analyzed the association of continuous $HbA_{1c}$ levels with cardiovascular mortality, CVD, and overall mortality. In addition, the association between $HbA_{1c}$ levels and time-to-event was analyzed in subgroups with and without diabetes and according to age. Finally, cut-offs for the dichotomization of $HbA_{1c}$ were determined for each outcome.

# Methods

# Study overview

The design and rationale of the Biomarker for Cardiovascular Risk Assessment across Europe (BiomarCaRE) project have been described previously [18]. Briefly, BiomarCaRE is based on the MORGAM (MONICA Risk Genetics Archiving and Monograph) Project. The MORGAM/BiomarCaRE Data Center in Helsinki harmonized individual data from 21 population-based cohort studies with central storage of selected biomaterial of more than 300,000 participants [18]. Using the harmonized database of the BiomarCaRE project (FP7/2007–2013) [18], we analyzed individual data of 36,180 study participants with available $HbA_{1c}$ levels.

# Study cohorts

The present analysis included six cohort studies from four European countries (Germany, Italy, Sweden, and Norway), namely the Cooperative Health Research in the Region of Augsburg (KORA) Study, the Study of Health in Pomerania (SHIP), and the Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten THerapie chronischer ERkrankungen in der älteren Bevölkerung (ESTHER)

Study, all from Germany, the MONICA Brianza Study from Italy, the Northern Sweden MONICA Study from Sweden, and the Tromsø Study from Norway. Each cohort is based on a well-defined population (see Supplementary Table S1).

For each cohort, the following harmonized variables were available at baseline: duration in years, age, sex, smoking status, body-mass-index (BMI), systolic blood pressure, diastolic blood pressure, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, $HbA_{1c}$, and history of DM. The history of DM was defined as documented or self-reported history of diabetes. This variable includes both types 1 and type 2 DM. Detailed definitions of this variable in each cohort are provided in Supplementary Table S1. Also participants not diagnosed with DM but with high $HbA_{1c}$ levels (> 48 mmol/mol/6.5% – a diagnostic criterion for clinical DM) were assigned to the DM group. Additional sub-classification into type 1 or type 2 DM was not possible with this dataset.

Smoking status was determined based on self-reports. BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, and HDL cholesterol parameters were measured (high blood pressure was defined either as systolic blood pressure > 140mmHg and/or diastolic blood pressure > 90mmHg) and it was recorded whether the patient was on antihypertensive medication. Prevalent CVD, like previous myocardial infarction or stroke, was assessed using the documented or self-reported history of myocardial infarction or stroke, including angina pectoris when the data did not permit its distinction from myocardial infarction. In MORGAM, prevalent heart failure was assessed with the item documented or self-reported history of heart failure.

# Study outcome

The following outcome measures were defined: (I) cardiovascular mortality, (II) CVD, and (III) overall mortality, defined as mortality due to any cause during follow-up. Follow-up commenced at the baseline examination date [19].

Cardiovascular mortality included death due to coronary heart disease or stroke. Cardiovascular disease, as an endpoint, was defined as the first fatal or non-fatal coronary event or likely cerebral infarction. Coronary events included acute definite or potential myocardial infarction or coronary death, unstable angina pectoris, cardiac revascularization, and unclassifiable death (i.e., death with insufficient evidence of coronary origin and no competing cause). In the MONICA/KORA Augsburg study, cardiac revascularization was not followed-up. In the MONICA/KORA Augsburg and MONICA Brianza studies, unstable angina pectoris was not assessed as an outcome but primarily included in the category “possible myocardial infarction” of the WHO MONICA classification used in these studies.

# Laboratory procedures

$HbA_{1c}$ was measured using whole blood. All $HbA_{1c}$ measurements were performed upon study entry to avoid glycation of blood samples during storage (except the Northern Sweden MONICA cohort measuring $HbA_{1c}$ levels in samples that had been stored at -80°C). Locally measured $HbA_{1c}$ values were transferred directly to the MORGAM Data Center (except for the SHIP study). The assays either reported their results

as percentages (%), following the National Glycohemoglobin Standardization Program (NGSP), or in units of mmol/mol if they had employed the International Federation of Clinical Chemistry (IFCC) consensus reference method. Data from cohorts which reported their values as percentages were converted to mmol/mol using the standard formula: IFCC = 10.93 * NGSP − 23.50.

# Statistical analyses

Unadjusted and age-adjusted Kaplan-Meier survival curves for cardiovascular mortality, CVD, and overall mortality were computed based on $HbA_{1c}$ tertiles. For the age-adjusted Kaplan–Meier survival analyses the three $HbA_{1c}$ tertiles were < 34.4 mmol/mol (5.3%), 34.4 mmol/mol (5.3%) − 38.8 mmol/mol (5.7%); and > 38.8 mmol/mol (5.7%). To adjust the survival curves for the age distribution in the data, the following procedures were applied: a) age was categorized using cut-offs 35, 40, 45, 50, 55, 60, 65, and 70 years; b) an individual belonging to a particular $HbA_{1c}$ tertile and to age category j was assigned a weight equal to $(n_j/N)/(n_{ij}/N)$, where N is the total sample size, $n_j$ is the number of individuals in age category j, and $n_{ij}$ is the number of individuals in age category j belonging to the $HbA_{1c}$ tertile; c) these weights were then applied to the observations for estimating the Kaplan-Meier curves. A log-rank test was used to compare the unadjusted survival curves. The adjusted survival curves we compared using a robust score test obtained from a weighted Cox model with $HbA_{1c}$ categorized using tertiles as the only predictor. Follow-up time quartiles were estimated by the Kaplan-Meier potential follow-up estimator [20].

Sex- and cohort-stratified Cox proportional hazards models for cardiovascular mortality, CVD, and overall mortality were computed using individual-level data from the available cohorts. For these analyses the $HbA_{1c}$ (in mmol/mol) was log-transformed, divided by the interquartile range (IQR) of the log-transformed values in the total study population (IQR = 0.213), and used as continuous variable. The Cox models for the three endpoints were adjusted for age (time scale), sex and cohort (strata), and CVRFs, smoking status (daily smoker yes/no), BMI, systolic blood pressure, DM (yes/no), and total cholesterol to HDL cholesterol ratio. Two separate extensions of the models were considered. In one extension, baseline age groups < 55, 55 − 64, ≥ 65 years at baseline and their interaction with log-transformed $HbA_{1c}$ was added to the models. In the second extension, an interaction between log-transformed $HbA_{1c}$ and the group indicator for diabetes was added. To assess the linearity assumption of log-$HbA_{1c}$ used in the previous Cox regressions, additional Cox models with log-$HbA_{1c}$/IQR were formulated using penalized cubic splines.

$HbA_{1c}$ cut-offs that intend to separate subjects into low- and high-risk groups were calculated for each endpoint using the method of Contal and O'Quigley [21]. This method examines a rescaled version of the log-rank test statistic for each possible cut-off and selects the cut-off that maximizes the rescaled log-rank test statistic. Equality of survival curves in the groups separated by the optimal cut-off values was tested using the methods described by Contal and O'Quigley [21].

Individuals with CVD at baseline were excluded in the survival analyses using CVD as endpoint. There were no exclusions based on prevalent disease for the two other endpoints.

A two-sided P-value of < 0.05 was considered statistically significant. Adjustment for multiple testing was not performed due to the exploratory nature of the analyses [22]. All statistical methods were implemented in R statistical software version 4.0.3 (www.R-project.org) [23].

## Results

# Baseline characteristics

The baseline characteristics for individuals with available $HbA_{1c}$ measurements for the entire cohort and for individuals with and without DM are shown in Table 1. The characteristics of each cohort are summarized in Supplementary Table S2.

For 36,180 subjects, $HbA_{1c}$ measurements and information regarding diabetes status were available. Men and women were represented almost equally (19,111 women; 52.8%). The median age was 57.4 years, the median BMI 26.4 kg/$m^2$, and the median systolic blood pressure 133.5 mmHg. At baseline, approximately 28% of the study cohort were daily smokers, 47.5% had high blood pressure or were taking antihypertensive medication, and 10.2% had a diagnosis of diabetes.

# Distribution of $HbA_{1c}$ levels in the cohort

The distribution of $HbA_{1c}$ levels and log-transformed $HbA_{1c}$ levels/IQR in the entire cohort and each cohort study are shown in Supplementary Figure S1. The median HbA1c was 36.6 mmol/mol (5.5%). The 25th and 75th percentiles were 32.2 mmol/mol (5.1%) and 39.9 mmol/mol (5.8%), respectively.

# $HbA_{1c}$ levels and association with cardiovascular mortality, cardiovascular disease, and overall mortality

The maximum follow-up time was 21.9 years. During the median follow-up time of 9.9 years, 1,392 cases of cardiovascular death, 2,711 cases of CVD, and 4,601 deaths due to any cause were observed. Further information on the median follow-up for each cohort is provided in Supplementary Table S3.

As illustrated in the age-adjusted Kaplan–Meier survival analyses for the three $HbA_{1c}$ tertiles < 34.4 mmol/mol (5.3%), 34.4 mmol/mol (5.3%) – 38.8 mmol/mol (5.7%); and > 38.8 mmol/mol (5.7%) the probability of all investigated outcomes increased with increasing $HbA_{1c}$ levels (Supplementary Figure S2). Adjusting the curves for age reduces the separation between the curves (Fig. 1).

# Cut-off value of $HbA_{1c}$ for risk estimation

We also calculated and analyzed the respective cut-offs for the different $HbA_{1c}$ tertiles. After applying the method used by Contal and O'Quigley [21], $HbA_{1c}$ cut-off values for cardiovascular mortality, CVD, and overall mortality were calculated, yielding 39.9 mmol/mol (5.8%), 36.6 mmol/mol (5.5%), and 38.8 mmol/mol (5.7%), respectively. These cut-offs indicate an increased risk for the outcome. This is shown

by the age-adjusted Kaplan-Meier curves for the three outcomes (Fig. 5). During follow-up, participants that were above the respective cut-off at baseline clearly have a higher risk for each of the outcomes. The unadjusted Kaplan-Meier curves are shown in Supplementary Figure S3.

# $HbA_{1c}$-associated risk in the overall cohort, age groups and individuals with and without DM

The fully adjusted hazard ratios (HRs) indicates associations with cardiovascular mortality, CVD, and overall mortality, with respective HRs of 1.12 (95% confidence interval (CI): 1.04 − 1.20, p = 0.002), 1.10 (95% CI: 1.04 − 1.16, p < 0.001), and 1.09 (95% CI: 1.05–1.14, p < 0.001) (Fig. 2). The calculated Cox proportional hazards models for cardiovascular mortality, CVD, and overall mortality are presented in Supplementary Table S4.

Following stratification according to age groups, the association between $HbA_{1c}$ and risk of cardiovascular mortality was strongest in individuals aged < 55 years (HR: 1.28, 95% CI: 1.09 − 1.51, p = 0.003) and the strength of the association declined with increasing age (p-value for interaction: 0.038). While the HRs for cardiovascular mortality and overall mortality were also highest for those aged < 55 years, we did not observe a significant interaction between age group and HbA1c for either of these two outcomes (Fig. 2).

While associations between $HbA_{1c}$ and time-to-event tended to be higher for individuals with DM (Fig. 3) the test for interaction was only statistically significant for the outcome of overall mortality (p = 0.041). In individuals with DM, we observed a HR of 1.15 (95% CI: 1.04—1.26; p = 0.004) for cardiovascular mortality, 1.12 (95% CI: 1.03—1.22; p = 0.008) for CVD, and 1.15 (95% CI: 1.08—1.22; p < 0.001) for overall mortality. In participants without diabetes, the respective HRs for cardiovascular mortality, CVD, and overall mortality were 1.09 (95% CI: 0.98—1.21; p = 0.13), 1.09 (95% CI: 1.01—1.16; p = 0.021), and 1.05 (95% CI: 1.00—1.11; p = 0.056).

# Dose-response relationships

Modelling the association of log-transformed $HbA_{1c}$/IQR and time-to-event using cubic splines indicates a slightly curved increasing association for the considered endpoints, with the exception of cardiovascular mortality where the curve decreases until approximately 33 mmol/mol (5.2 %) (Fig. 4). We therefore had to reject the hypothesis of linearity of log- $HbA_{1c}$ for all 3 endpoints (p = 0.021 for cardiovascular mortality, p = 0.046 for CVD, and p = 0.016 for overall mortality).

# Cut-off value of $HbA_{1c}$ for risk estimation

We also calculated and analyzed the respective cut-offs for the different $HbA_{1c}$ tertiles. After applying the method used by Contal and O'Quigley [21], $HbA_{1c}$ cut-off values for cardiovascular mortality, CVD, and

overall mortality were calculated, yielding 39.9 mmol/mol (5.8%), 36.6 mmol/mol (5.5%), and 38.8 mmol/mol (5.7%), respectively. These cut-offs indicate an increased risk for the outcome. This is shown by the age-adjusted Kaplan-Meier curves for the three outcomes (Fig. 5). During follow-up, participants that were above the respective cut-off at baseline clearly have a higher risk for each of the outcomes. The unadjusted Kaplan-Meier curves are shown in Supplementary Figure S3.

## Discussion

Based on a fairly harmonized large-scale assessment of $HbA_{1c}$ and cardiovascular outcome, the present study has several main findings. First, $HbA_{1c}$ levels were independently associated with overall-mortality, cardiovascular mortality, and cardiovascular disease. Second, $HbA_{1c}$ levels showed a mostly monotonically increasing association with all three outcomes. Third, the association of $HbA_{1c}$ with cardiovascular mortality was strongest in individuals under the age of 55 years. Fourth, subgroup analyses based on diabetes status demonstrated that the association between $HbA_{1c}$ and the examined outcomes tended to be stronger in persons with diabetes, although $HbA_{1c}$ was also significantly associated with CVD in persons without diabetes. Finally, $HbA_{1c}$ cut-off values were derived to define a threshold above which the risk of the examined outcomes is significantly elevated.

# $HbA_{1c}$ levels and the risk for cardiovascular outcomes

Although the presence of DM is a common risk factor for CVD, a continuous biomarker reflecting this risk factor is not currently used for risk prediction in the general population.[3] In the present prospective population-based study including 36,180 participants from six European countries, we demonstrated a mostly monotonically increasing risk for cardiovascular mortality, CVD, and overall mortality with increasing $HbA_{1c}$ levels. As reported previously [24], the association was mostly monotonic and not J-shaped, which is also supported by additional data [25]. Therefore, our findings underline previous statements from the American Diabetes Association [26] concerning the measurement of $HbA_{1c}$ for cardiovascular risk assessment as well as the recent European Society of Cardiology 2019 guidelines [3] on adults without a diagnosis of diabetes. In this context, neither guideline defines prediabetes as a self-contained clinical entity although individuals with prediabetes have an increased risk to develop diabetes or cardiovascular disease [3, 26, 27]. The American guidelines underline the fact that prediabetes is associated with cardiovascular risk factors like obesity, arterial hypertension, and dyslipidemia and further that levels in the prediabetes range are associated with the risk to develop diabetes. The increasing risk for developing diabetes with increasing $HbA_{1c}$ levels has been reported before [28]. Furthermore, the American guidelines [26, 27] also support the notion of including $HbA_{1c}$ determination in clinical practice for prevention purposes to reduce future CVD burden. The results of the present study support this notion of using $HbA_{1c}$ levels to identify individuals with an increased risk, e.g., classify them as prediabetic based on a cut-off value of > 38.8 mmol/mol (5.7%), especially since all our calculated cut-offs for subject differentiation were below the reported threshold to diagnose diabetes. The cut-off of >

38.8 mmol/mol (5.7%) is also in line with the threshold proposed by the American Diabetes Association to define prediabetes in individuals without a diagnosis of diabetes [26, 27]. A recent study in a smaller study population from Spain could also show the additional benefit of including $HbA_{1c}$ levels for the association with CVD [15].

The recent study by Welsh and colleagues using data from the UK only with a median follow-up of 8.9 years also showed an association between $HbA_{1c}$ levels and cardiovascular outcome [29]. The authors suggested that this risk may be increased due to a higher prevalence of CVRFs in the investigated population [29]. However, this interpretation stands in contrast to findings by a different article which showed that the cohort used in the Welsh et al. study had a lower prevalence of risk factors than the average UK population [30].

## Age-dependent effect of $HbA_{1c}$ and risk for cardiovascular outcome

An important finding was that the association between $HbA_{1c}$ and cardiovascular outcomes is strongest in individuals aged under 55 years. This could imply that young individuals with elevated $HbA_{1c}$ levels may carry an additional risk for cardiovascular outcomes. A possible causal link between $HbA_{1c}$ levels and an increased risk of coronary artery disease was proposed in Mendelian randomization studies [16, 17].

## $HbA_{1c}$ measurement and its implication regarding the defined outcome

In this large European general population sample the association of $HbA_{1c}$ with the risk of cardiovascular mortality, cardiovascular disease, and overall mortality was mostly monotonic, considering the broad range of $HbA_{1c}$ levels encountered in such large population-based studies [6, 24, 29, 31, 32]. Considering this association, the recent definition of prediabetes,[33] and the potentially higher risk of developing diabetes or cardiovascular disease, additional clinical evaluation might be warranted in individuals with a higher risk profile. In our study, CVD was associated with $HbA_{1c}$ in participants without diabetes. In addition, the calculated cut-offs showing an elevated risk for overall mortality in individuals with > 38.8 mmol/mol (5.7%) highlights the importance of elevated $HbA_{1c}$ levels and might indicate an increased risk for cardiovascular outcomes and overall mortality.

## Strengths and limitations

The present study has several strengths and limitations. An important strength is the considerable size of the dataset, with harmonized data from well-defined European population-based cohort studies with a long follow-up time.

Despite the well-defined dataset, we identified 717 individuals with $HbA_{1c}$ levels above 48 mmol/mol/6.5% that had not been diagnosed with diabetes. As the omission of such a sizable group

may introduce considerable errors into our findings, we decided to classify individuals with $HbA_{1c}$ levels above 48 mmol/mol/6.5% and without the diagnosis of diabetes as subjects with prevalent diabetes. An additional limitation is the heterogeneity of data. Several cohort studies whose data we used commenced in the 1980s and 1990s, when treatment options and guidelines differed substantially from today's. In population-based cohort studies with apparently healthy individuals, selection bias is a common problem.

Haemoglobinopathies, different ethnicities, and certain disease states like bleeding, transfusion, or hemodialysis can interfere with the measurement of $HbA_{1c}$ which may affect our results [33, 34].

Due to the absence of additional measures of dysglycemia like 2-hour post load glucose and fasting glucose we could not perform additional analyses to prove and to validate the prognostic impact of $HbA_{1c}$. These parameters were reported to have a prognostic impact and might be better for risk stratification than $HbA_{1c}$ [35, 36].

# Conclusion

The present study employed one of the largest population-based datasets with predominantly harmonized data on $HbA_{1c}$ from several European countries. $HbA_{1c}$ levels were positively associated with an increased risk for cardiovascular mortality, CVD, and overall mortality. There was a mostly monotonically increasing association between $HbA_{1c}$ levels and time-to-event regarding the defined endpoints cardiovascular mortality, cardiovascular disease, and overall mortality, emphasizing the potential use of $HbA_{1c}$ measurement as a biomarker in the general population. When including risk stratification, $HbA_{1c}$ levels could be particularly important in subjects with $HbA_{1c}$ levels > 38.8 mmol/mol (5.7%), indicating a potential prediabetic metabolism and potential risk of cardiovascular disease. In addition, the findings might be of importance in individuals younger than 55 years who showed a pronounced association of $HbA_{1c}$ levels and cardiovascular mortality. Further research and external validation in a clinical setting are required to define whether additional standardized measurement of $HbA_{1c}$ is necessary for cardiovascular risk assessment.

# Declarations

### Ethics approval and consent to participate:

The trial was conducted in accordance with the principles of the Declaration of Helsinki. Please also refer to the online supplement Table S1.

### Consent for publication:

Not applicable

### Availability of data and materials:

The data are not available in a public repository. Access to the data is dependent upon ethics approval and restricted by the legislation of the European Union and the countries providing data to the study. Furthermore, approval by the Principal Investigator of each cohort study and the MORGAM/ BiomarCaRE Steering Group is required to release the data. The MORGAM Manual at https://www.thl.fi/publications/morgam/manual/contents.htm gives more information on access to the data.

## Competing interests:

**Dr. Sinning** is deputy editor of the European Heart Journal – Case Reports and BioMed Central editorial board member for BMC Cardiovascular Disorders

**Dr. Söderberg** reports grants and other from Actelion Ltd, outside the submitted work.

**Dr. Schnabel** reports personal fees from BMS/Pfizer, outside the submitted work.

**Dr. Makarova** reports personal fees from Abbott Laboratories, outside the submitted work.

**Dr. Koenig** reports personal fees from AstraZeneca, personal fees from Novartis, personal fees from Pfizer, personal fees from The Medicines Company, personal fees from GSK, personal fees from DalCor, personal fees from Sanofi, personal fees from Berlin-Chemie, personal fees from Kowa, personal fees from Amgen, grants and non-financial support from Roche Diagnostics, grants and non-financial support from Beckmann, grants and non-financial support from Singulex, grants and non-financial support from Abbott, all outside the submitted work.

**Dr. Blankenberg** reports grants and personal fees from Abbott Diagnostics, grants and personal fees from Bayer, grants and personal fees from SIEMENS, grants from Singulex, grants and personal fees from Thermo Fisher, personal fees from Astra Zeneca, grants and personal fees from Bayer, personal fees from AMGEN, personal fees from Medtronic, personal fees from Pfizer, personal fees from Roche, personal fees from Siemens Diagnostics, personal fees from Novartis, personal fees from Abbott, all outside the submitted work.

All other authors have nothing to disclose in relation to the current study.

## Funding:

The MORGAM Project received funding from EU projects MORGAM (Biomed, BMH4-CT98-3183), GenomEUtwin (FP5, QLG2-CT-2002-01254), ENGAGE (FP7, HEALTH-F4-2007-201413), CHANCES (FP7, HEALTH-F3-2010-242244), BiomarCaRE (FP7, HEALTH-F2-2011-278913), euCanSHare (Horizon 2020, No. 825903), and AFFECT-EU (Horizon 2020, No. 847770), as well as from the Medical Research Council, London (G0601463, No. 80983: Biomarkers in the MORGAM Populations). This has supported central coordination, workshops, and part of the activities of the MORGAM Data Center, the MORGAM Laboratories, and the MORGAM Participating Centers.

## Author contributions:

C. Sinning: guarantor of the manuscript, literature search, study design, figures, data analysis, writing, data interpretation

N. Makarova: literature search, study design, figures, data analysis, writing, data interpretation

H. Völzke: data collection, data interpretation, writing

R. B. Schnabel: data interpretation, writing

N. Rübsamen: statistical analysis, data analysis, writing, data interpretation

F. Ojeda: statistical analysis, data analysis, writing, data interpretation

M. Dörr: writing, data interpretation, data collection

S. B. Felix: writing, data interpretation, data collection

W. Koenig: study design, writing, data interpretation, data collection

A. Peters: writing, data collection

W. Rathmann: writing, data interpretation

B. Schöttker: writing, data interpretation

H. Brenner: writing, data interpretation

G. Veronesi: writing, data interpretation, data collection

G. Cesana: writing, data interpretation, data collection

P. Brambilla: writing, data interpretation, data collection

T. Palosaari: writing, data interpretation, data collection

K. Kuulasmaa: writing, study design, data interpretation, data collection

I. Njølstad: writing, data interpretation, data collection

E. B. Mathiesen: writing, data interpretation, data collection

T. Wilsgaard: writing, data interpretation, data collection

S. Blankenberg: study design, data interpretation, writing, supervision of the study

S. Söderberg: study design, data interpretation, writing, supervision of the study

M. M. Ferrario: study design, data interpretation, writing, supervision of the study

B. Thorand: study design, data interpretation, writing, supervision of the study

C. Sinning and N. Makarova contributed equally as first authors.

S. Söderberg, M. M. Ferrario and B. Thorand contributed equally as last authors.

**Acknowledgments:**

Editorial assistance, in the form of language editing and correction, was provided by XpertScientific Editing and Consulting Services.

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# Tables

**Table 1** Baseline characteristics of the entire study population

| Baseline characteristics | All (N=36180) | No diabetes (N=32496) | Prevalent diabetes (N=3684) |
|---|---|---|---|
| Survey year | 1987-2012 | | |
| Examination age (years) | 57.4 (47.0, 65.1) | 56.4 (45.3, 64.5) | 64.0 (57.9, 69.0) |
| Male (%) | 17069 (47.2) | 15095 (46.5) | 1974 (53.6) |
| BMI (kg/m²) | 26.4 (23.8, 29.4) | 26.1 (23.6, 29.0) | 29.2 (26.4, 32.6) |
| Daily smoker (%) | 8243 (27.7) | 7602 (27.9) | 641 (25.7) |
| High blood pressure (%) | 17084 (47.5) | 14506 (44.9) | 2578 (70.6) |
| Systolic BP (mmHg) | 133.5 (120.0, 149.0) | 132.0 (120.0, 147.0) | 140.0 (130.0, 155.5) |
| Diastolic BP (mmHg) | 80.0 (74.0, 90.0) | 80.0 (74.0, 89.5) | 80.5 (76.0, 90.0) |
| Antihypertensive medication (%) | 7827 (21.7) | 6057 (18.7) | 1770 (48.5) |
| Diabetes (documented or self-reported) (%) | 3684 (10.2) | 0 (0) | 3684 (100) |
| Family history of CHD (%) | 4716 (18.6) | 4242 (18.7) | 474 (17.6) |
| History of MI (%) | 1417 (4.0) | 1060 (3.3) | 357 (10.0) |
| Prev. Stroke (%) | 862 (2.4) | 624 (1.9) | 238 (6.6) |
| History of heart failure (%) | 1454 (5.7) | 1046 (4.7) | 408 (13.2) |
| **Endpoint variables** | | | |
| Cardiovascular mortality (%) | 1392 (3.9) | 1080 (3.3) | 312 (8.5) |
| Cardiovascular disease (%) | 2339 (8.2) | 2043 (7.8) | 296 (12.1) |
| Overall mortality (%) | 4601 (12.7) | 3768 (11.6) | 833 (22.7) |

| Biomarker variables | | | |
|---|---|---|---|
| $HbA_{1c}$ (mmol/mol) | 36.6 (32.2, 39.9) | 35.5 (32.2, 38.8) | 50.8 (44.3, 59.6) |
| $HbA_{1c}$ (%) | 5.5 (5.1, 5.8) | 5.4 (5.1, 5.7) | 6.8 (6.2, 7.6) |
| Total cholesterol (mmol/L) | 5.9 (5.0, 6.7) | 5.9 (5.1, 6.8) | 5.7 (4.8, 6.5) |
| HDL cholesterol (mmol/L) | 1.4 (1.2, 1.7) | 1,4 (1.2, 1.7) | 1.2 (1.0, 1.4) |

Baseline characteristics are presented as absolute and relative frequencies for categorical variables, and quartiles (medians with 25th and 75th percentiles) for continuous variables as well as range in years for years of baseline examinations. BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction. The numbers provided for the cardiovascular disease endpoint are after excluding those individuals with history of cardiovascular disease.

# Figures

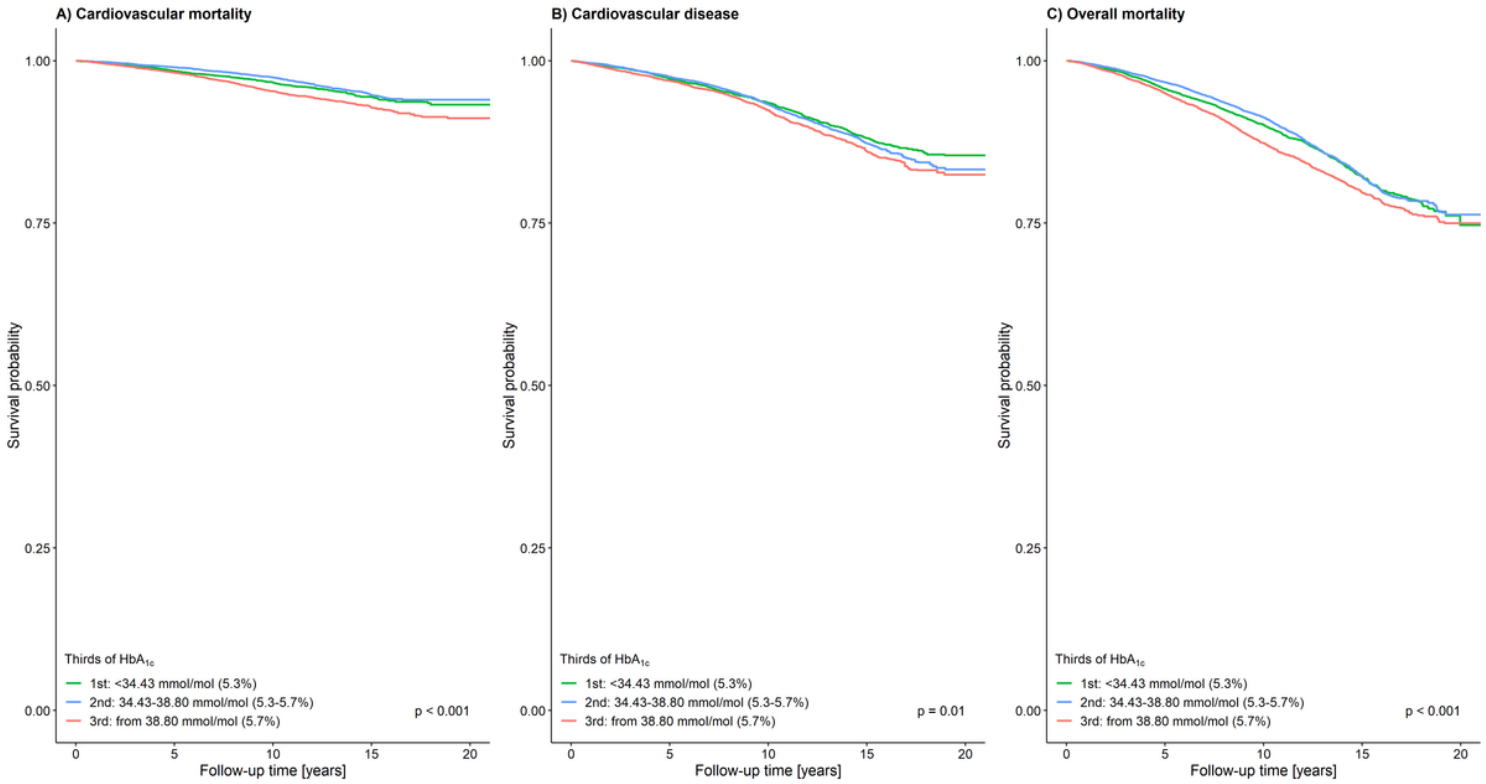
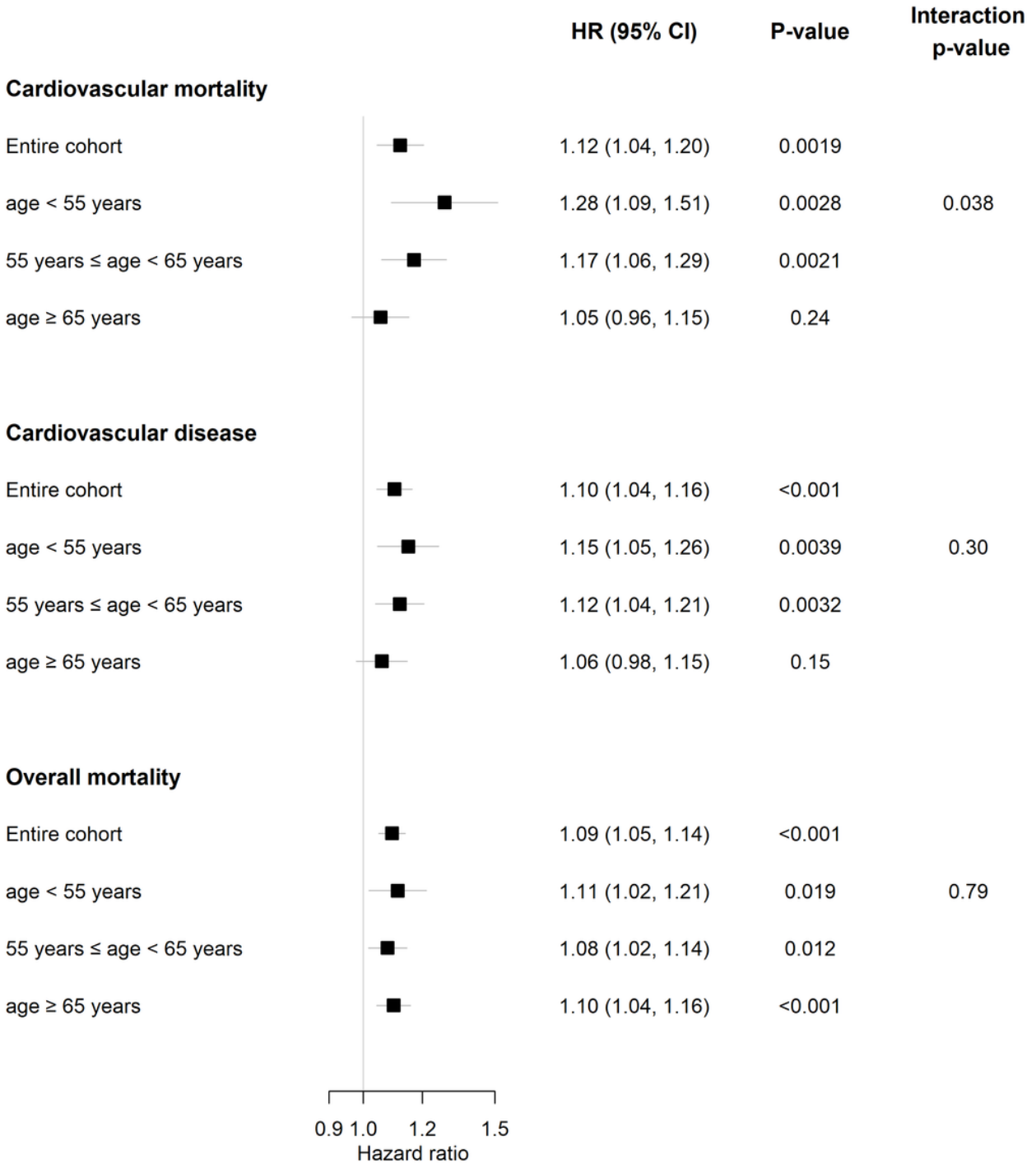



**Figure 1**

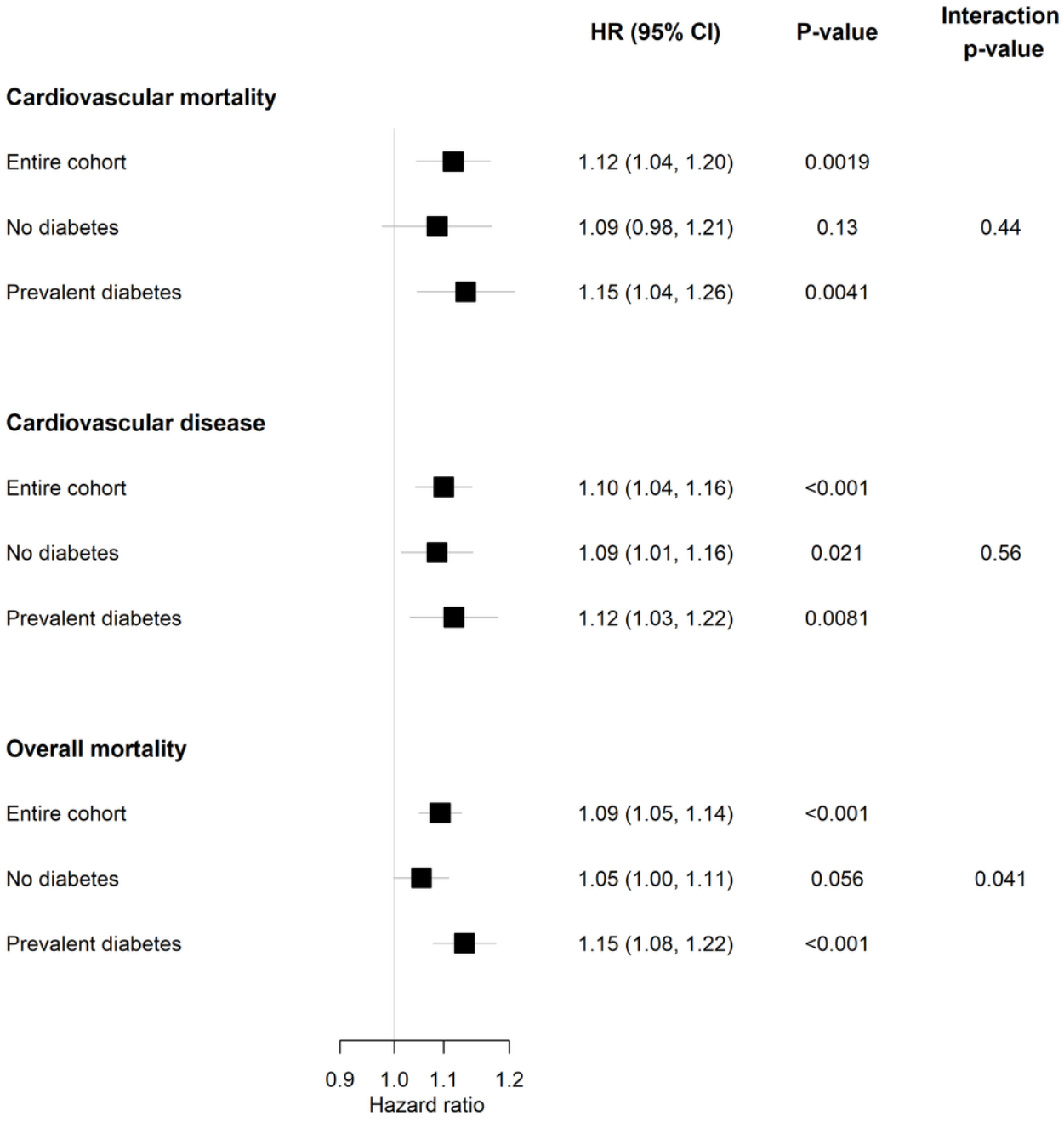
Age-adjusted Kaplan–Meier curves of (A) cardiovascular mortality, (B) cardiovascular disease, and (C) overall mortality for each HbA1c tertile.




## Figure 2

Hazard ratios for HbA1c and outcomes: cardiovascular mortality, cardiovascular disease, and overall mortality, stratified into age groups. HbA1c in mmol/mol was log-transformed and divided by the interquartile range (IQR) of the log-transformed values in the entire sample (IQR = 0.213). The Cox models for the three endpoints were adjusted for age (time scale), sex and cohort (strata), and CVRFs, smoking

status, BMI, systolic blood pressure, DM, and total cholesterol to HDL cholesterol ratio. The p-value for interaction is for an interaction between age groups and HbA1c




## Figure 3

Subgroup analysis comparing the association between HbA1c and time-to-event in individuals with and without DM. HbA1c in mmol/mol was log-transformed and divided by the interquartile range (IQR) of the log-transformed values in the entire sample (IQR = 0.213). The models include an interaction term between HbA1c and the subgroup indicator (DM yes/no). The Cox models for the three endpoints were adjusted for age (time scale), sex and cohort (strata), and CVRFs, smoking status, BMI, systolic blood

pressure, DM, and total cholesterol to HDL cholesterol ratio. The p-value for interaction is for an interaction between DM and HbA1c

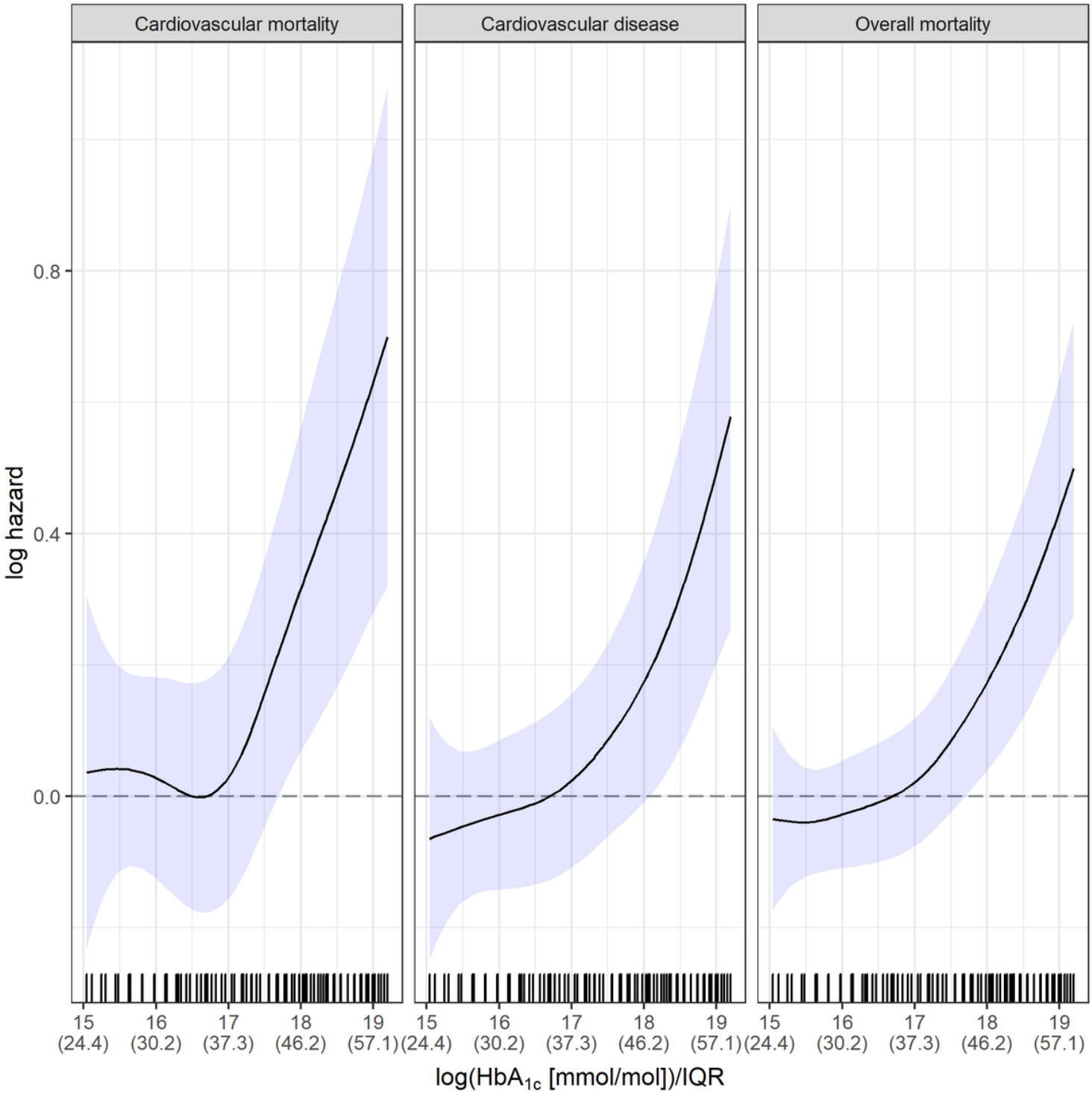



**Figure 4**

Penalised cubic splines for the association between HbA1c and time-to-event. Untransformed HbA1c in mmol/mol are given in parentheses on the x-axis. Log-transformed HbA1c/IQR was used. The natural logarithm was used. A reference value of 35 mmol/mol was used for HbA1c (yielding a reference value for log(HbA1c)/IQR of 16.7).

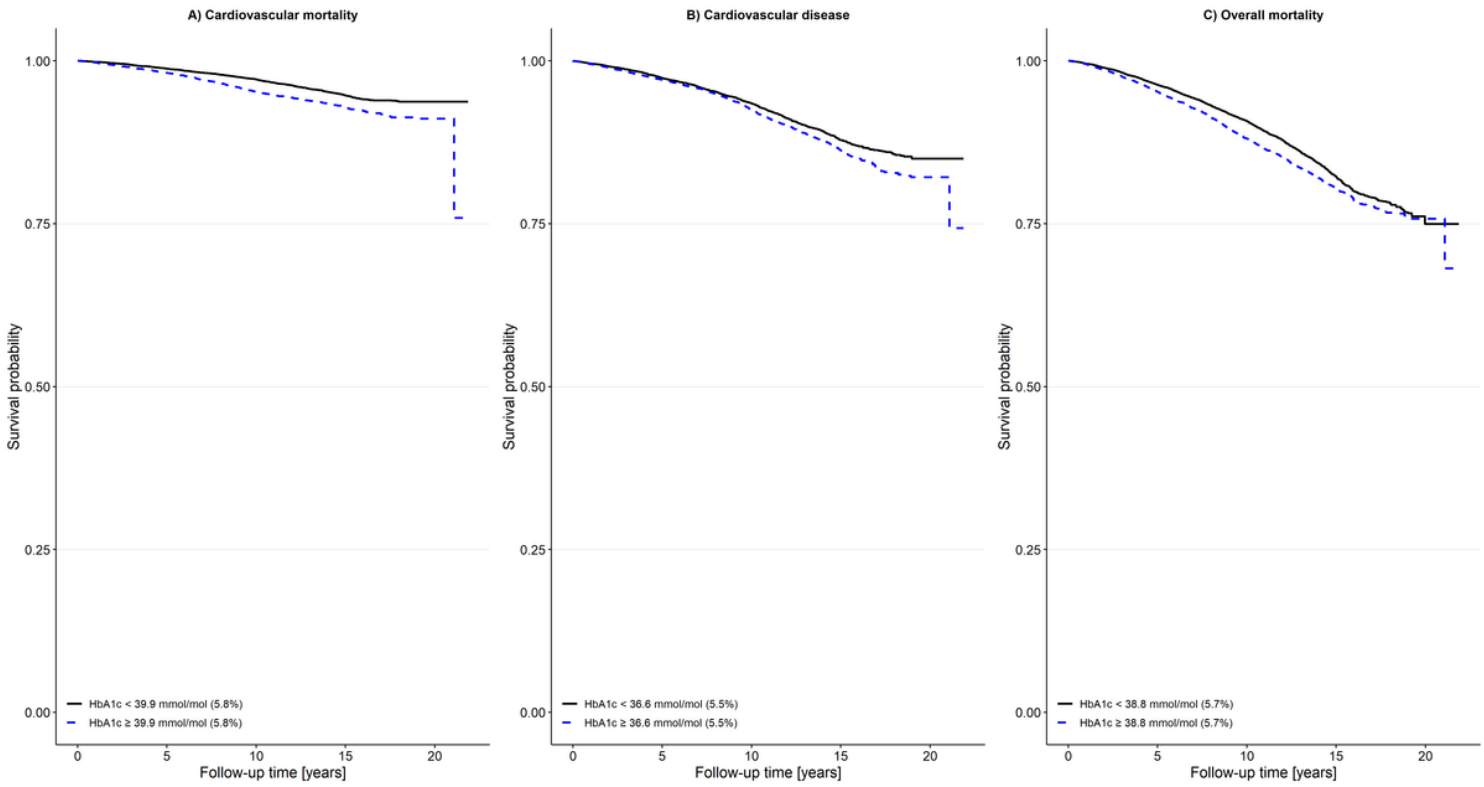



**Figure 5**

Age adjusted Kaplan–Meier curves for the outcomes (A) cardiovascular mortality, (B) cardiovascular disease, and (C) overall mortality based on the calculated cut-off values.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 20210801Supplementarymaterial.docx