Total and cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype in individuals with diabetes - two general population studies

Associate Professor, Chief Physician, Christina Ellervik, MD, PhD;
Professor, Chief Physician, Thomas Mandrup-Poulsen, MD, DMsci;
Professor, Chief Physician, Anne Tybjærg-Hansen, MD, DMSci;
Professor, Chief Physician, Børge G. Nordestgaard, MD, DMSci.

Running title: Mortality by transferrin saturation in diabetes

Departments of Clinical Biochemistry, Næstved Hospital (CE), Rigshospitalet (ATH), and Herlev Hospital (BGN); all Copenhagen University Hospitals, Denmark. Department of Biomedical Sciences (TMP), and Department of Clinical Medicine (CE, ATH, BGN); all Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; and Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden (TMP).

Word count: Abstract (226), Text (3876). 3 Figures, 1 Table. 1 Supplementary Table.

Correspondence: Christina Ellervik, M.D., PhD, Associate Professor, Chief Physician, Department of Clinical Biochemistry (Section on Thrombosis and Hemostasis), Næstved Hospital, Copenhagen University Hospital, Herlufsvænge 14C stuen, DK-4700 Næstved, Denmark.
Phone: +45 2446 4663. Fax: +45 5651 3107. E-mail: christina@ellervik.dk
Objective: Mortality is increased in patients with hereditary hemochromatosis, in individuals from the general population with increased transferrin saturation (TS), and also in patients with diabetes type 1 and increased TS from a highly specialised diabetes clinic. Thus, we have recommended targeted screening for TS in specialised diabetes clinics. Whether mortality is also increased in individuals ascertained from the general population with diabetes and increased TS is unknown.

Research design and methods: In two Danish population studies (N=84,865), we examined mortality according to baseline TS and hemochromatosis genotype (HFE) C282Y/C282Y in individuals with diabetes (type 1, N=118; type 2, N=3228; total, N=3346).

Results: The cumulative survival was reduced in individuals with diabetes with TS ≥ 50% vs. <50% (log-rank; P<0.0001), with median survival age of 66 and 79 years, respectively. The hazard ratio (HR) for TS ≥ 50% vs. <50% was 2.0 (95% CI: 1.3-2.8; P=0.0004) for total mortality overall (and similar for men and women separately); 2.6 (1.3-5.4; P=0.008) for neoplasms and 3.4 (2.0-6.0; P=0.00002) for endocrinological causes. A stepwise increased risk of total mortality was observed for stepwise increasing levels of TS (log-rank; P=0.0001), with a HR for TS ≥ 70% vs. TS < 20% of 4.8 (2.0-12; P=0.0006). The HR for total mortality in individuals with diabetes for C282Y/C282Y vs. wildtype/wildtype was 3.3 (1.04-10; P=0.04), and for (C282Y/C282Y & TS ≥ 50%) vs. (wildtype/wildtype & TS < 50%) was 6.0 (1.5-24; P=0.01). Six percent of these premature deaths can possibly be avoided by early screening for TS or HFE genotype.

Conclusions: Individuals with diabetes, ascertained in the general population, with increased TS or HFE genotype have a 2-6-fold increased risk of premature death.
Hereditary hemochromatosis is an autosomal recessive disease, characterised by lifelong iron accumulation in various organs e.g. the endocrine pancreas and the liver(1). 83% of hereditary hemochromatosis is explained by homozygosity for a $G \rightarrow A$ substitution at nucleotide 845 in codon 282 changing a cysteine to tyrosine (C282Y) in the \textit{HFE} gene located at chromosome 6p21.3(2). The protein HFE is a transmembrane glycoprotein(2) located at the basolateral membrane of crypt enterocytes that inhibits iron export into the circulation. Homozygosity for C282Y prevents formation of a disulfide bond in the protein and prevents cell-surface expression of the protein(3) leading to unregulated absorption of iron and iron overload(1).

There is evidence that hemochromatosis genotype (\textit{HFE}) C282Y/C282Y(4,5), as well as iron overload independent of \textit{HFE} genotype(6), both confer risk of diabetes. There is also evidence for increased risk of premature death due to organ damage in patients with clinically overt hereditary hemochromatosis(7-11), in individuals from the general population with increased transferrin saturation (TS)(12) independent of \textit{HFE} genotype(13), and in patients with diabetes and increased TS or \textit{HFE} genotype from a highly specialised diabetes clinic, the Steno Diabetes Centre(14). In contrast, there is no evidence for increased risk of premature death in individuals with \textit{HFE} genotype C282Y/C282Y in population-based studies(13,15-17) or in patients with type 2 diabetes of mixed ethnicity(18). Importantly however, early detection and treatment of iron overload before the development of diabetes and cirrhosis can prevent excess mortality(10,19) and can restore normal life expectancy(7-10). Also, a recent study showed that patients with diabetes on maintenance hemodialysis with serum ferritin levels above 700 ng/mL had slightly increased risk of 1-year mortality(20).

A recent study in patients with hereditary hemochromatosis demonstrated a decline in diabetes prevalence in those patients diagnosed after determining that they carried the \textit{HFE} gene compared to those diagnosed before(21) suggesting that awareness of hemochromatosis in general
and development of diabetes in those patients in particular will translate into a greater life expectancy. Furthermore, we demonstrated a decline in mortality in patients with Type 1 diabetes offered targeted screening for TS(14). Thus, we have recommended targeted screening for TS in specialised diabetes clinics(4,14).

Whether mortality is also increased in Caucasian individuals ascertained from the general population with diabetes and increased TS or \textit{HFE} genotype C282Y/C282Y is unknown. If this was the case, however, recommendation on targeted screening could also cover individuals with diabetes in the general population, when these individuals see their general practitioner for regular diabetes check-ups, that is, if they have not already previously been diagnosed with hemochromatosis.

Therefore, in this study, we investigate total and cause-specific mortality according to increased transferrin saturation or \textit{HFE} genotype C282Y/C282Y in Caucasian Danish individuals with diabetes ascertained from two general population studies.

**Research design and methods**

Using two similar, but independent Caucasian Danish population-based follow-up studies, The Copenhagen General Population Study (CGPS, N=2971) 2003-2007 examination and The Copenhagen City Heart Study (CCHS, N=375) 1991-1994(12), we included 3346 individuals with any diabetes from a total population size of 84,865 individuals. Information of prevalent diabetes was obtained from The National Danish Patient Registry (Type 1 diabetes: ICD-8 (249), ICD-10 (E10); Type 2: ICD-8 (250), ICD-10 (E11, E13, E14) and from information on self-reported diabetes and anti-diabetic medication. Individuals with undiagnosed diabetes but a non-fasting blood glucose above 11mmol/L were also included as having Type 2 diabetes (N=141). In total, 118 individuals had Type 1 diabetes, and 3228 individuals had Type 2 diabetes. Individuals in the two
studies were ascertained and examined similarly(12) with questionnaire and health examination. The studies were approved by Herlev Hospital and Danish ethical committees (KF-100.2039/91, KF-01-144/01, H-KF-01-144/01). Written informed consent was obtained from all participants in both studies; there were no overlap of individuals between the two studies. The studies complied with the Declaration of Helsinki.

Transferrin saturation (TS)

TS (in %) was determined as iron concentration (in µmol/L) divided by 2×transferrin concentration (in µmol/L)x100. Transferrin was measured by turbidimetry and iron by colorimetry (Konelab, Helsinki, Finland). A threshold level of TS ≥ 50% was chosen as suggestive of increased TS, in accordance with accepted clinical practice(22-24). To explore a graded relationship, transferrin saturation was divided into 7 categories: TS<20%, TS≥20% but TS<30%, TS≥30% but TS<40%, TS≥40% but TS<50%, TS≥50% but TS<60%, TS≥60% but TS<70%, and TS≥70%. Median transferrin saturation was 22% (interquartile range(IQR): 17%-28%; range:2%-98%). All individuals with diabetes had a transferrin saturation determined.

Genotyping

Genotyping of the CCHS for C282Y (single nucleotide polymorphism database(dbSNP): rs1800562), a G/A nucleotide change at position 845 in the HFE gene(2), and H63D (dbSNP: rs1799945), a C/G nucleotide change at position 187 in the HFE gene(2), was by allele specific amplification(25), and restriction enzyme digestion to confirm genotyping(2,4). The amplification refractory mutation system (ARMS) simultaneously detects both HFE mutations C282Y and H63D including sense and antisense primers for C282Y, H63D, and human growth hormone as internal
amplification control(25). Genotyping of the CGPS was by a TaqMan assay (Applied Biosystems, Foster City, Calif)(14). Of 1865 individuals with diabetes HFE genotypes were available.

Other characteristics

Individuals were questioned about alcohol consumption, smoking habits, medication, and physical activity. Body mass index was calculated as weight in kilograms divided by squared height in meters. Plasma total cholesterol was measured enzymatically(26). Diabetic microvascular complications were not recorded; however, information on ischemic heart disease(IHD) (ICD8: 410-414, ICD10: I20-I25) and ischemic cerebrovascular disease (ICVD) (ICD8: 432-435, ICD10: I63-I64, G45) from The National Danish Patient Registry and on levels of plasma-creatinin >90 mmol/L for women and >100 mmol/L for men as proxies for renal impairment)(27) were available.

Endpoints

Using the Central Person Registry Number, a number unique to every person living in Denmark, information on total and cause-specific mortality was obtained from time of blood sampling through linkage to the Danish Civil Registration System(28) until June 7, 2011, and to the National Danish Causes of Death Registry (NDCDR)(29) until December 31, 2009, due to delay in this registry. The NDCDR contains information on all underlying and contributing causes of death; until 2007, the coding was done by the Danish National Board of Health based on paper-based death certificates completed by physicians in hospitals, general practice, or forensic medicine(29); after 2007, it is the physician who verifies the death and issues the electronic death certificate and who also classifies the causes of death according to the International Classification of Diseases (ICD) coding(29). The NDCDR has consistently used ICD codes and since 1994 the ICD-10 codes. The following ICD-10 codes were used for cause-specific deaths in this study: neoplasms (C00-D48; N=128); liver cancer
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(C220, C221, C223, C229; N=6); endocrinological (endocrine, nutritional and metabolic diseases E00-E90; N=58); and cardiovascular (diseases of the circulatory system I00-I99; N=141). The follow-up in the CGPS, was from 2003-2011 through June 2011 (median and interquartile range(IQR): 4(2-6) years), whereas follow-up in the CCHS was from 1991-1994 through June 2011 (median(IQR): 10(5-17) years). Follow-up information was acquired for all participants. During a median of 4 years of follow-up (max18 years), 541 individuals with diabetes died.

**Statistics**

Stata/SE 11.0 statistical software package (Stata Corp., College Station, TX) was used. Mann-Whitney U tests and Pearson \( \chi^2 \) tests were used for continuous and categorical variables, respectively. Two-sided \( P \)-values \(<0.05\) were considered significant. *A priori* we stratified main analyses by gender, because penetrance of clinically manifest hemochromatosis differs markedly between the two genders(1). In explorative analysis we also stratified participants into 7 groups of TS levels as described above.

Cumulative survival was plotted with the use of Kaplan-Meier curves as a function of age and differences between TS levels or *HFE* genotype were examined by log-rank tests. Cox proportional hazards regression was used to estimate hazard ratios with 95% confidence intervals. The assumption of proportional hazards was tested with the use of Schoenfeld residuals, and no violations were observed. Interaction of TS levels or *HFE* genotype with risk factors on mortality was evaluated by including 2-factor interaction terms, 1 at a time, in the multifactorial Cox regression model. No significant or clinically relevant interactions were observed.

Crude hazard ratios included adjustments for age and gender. For TS, multifactorially adjusted hazard ratios included age, gender, alcohol consumption (intake of \( \leq 7 \) drinks/week vs. >7 drinks/week), smoking habits (current vs. non-smoker; packyears of smoking: 0, 0< packyears \( \leq 10, \) and >10 packyears; one packyear is equivalent to smoking 20 cigarettes/day for 365 days/year),
leisure time physical activity (almost completely inactive, some activity, regular activity, regular hard physical training)(30), body mass index (<25 kg/m$^2$ vs. ≥25 kg/m$^2$), plasma cholesterol (<5 mmol/L vs. ≥5 mmol/L), antihypertensive medication (yes vs. no), plasma creatinin (women: ≤90 & >90 mmol/L, men: ≤100 & >100 mmol/L), and history of IHD/ICVD(yes/no). For $HFE$ genotype, multifactorial adjustment only included age, gender, smoking, leisure time physical activity, and plasma cholesterol; alcohol consumption, body mass index, antihypertensive medication, plasma creatinin, and history of IHD/ICVD were not significant confounders in these analyses.

Population attributable risk was estimated as $[f(HR-1)]/[1+f(HR-1)]$, where $f$ is the frequency of TS ≥50% in the population, and HR is the hazard ratio for total mortality(31).

For each analysis, we calculated the hazard ratio that could be detected with 80% power assuming a 2-sided P<0.05 using NCSS Pass software (NCSS, Kaysville, UT). Study power was too small to study type 1 diabetes alone, or to stratify results for gender or cause-specific mortality for $HFE$ genotype since only 11 individuals with diabetes were C282Y/C282Y. Likewise, it was not possible to present data for cause-specific mortality due to liver cancer specifically since only 6 individuals with diabetes experienced this event.

**Results**

Table 1 lists characteristics of participants at study entry. Those who died were more often men, were older at baseline, were diagnosed with diabetes at an older age, had a shorter diabetes duration, had higher tobacco pack-years, were more often current smokers, were more physically inactive, more often had a history of IHD/ICVD(only CGPS), and more often had elevated plasma creatinin compared to those who survived (Table 1). There were no differences in type of diabetes, body mass index, plasma cholesterol, antihypertensive medication or alcohol consumption.

*Transferrin saturation*
The cumulative survival was reduced in individuals with diabetes with TS≥50% versus <50% (log-rank \(P<0.0001\)), and overall median survival time was 66 years (TS≥50%) and 79 years (TS<50%). Crude hazard ratios for total mortality for TS≥50% vs <50% were 2.0 (95% CI 1.3–2.8; \(P=0.0004\)) overall, 1.8 (1.2–2.8; \(P=0.003\)) in men, and 3.2 (1.2–8.8; \(P=0.02\)) in women (Figure 1); and 1.4(0.9-2.2;\(P=0.1\)) in CCHS and 2.6(1.3-5.0;\(P=0.006\)) in CGPS, respectively. Multifactorially adjusted analyses, analysis for type 2 diabetes only, and analysis excluding \(HFE\) genotypes (C282Y/C282Y and C282Y/H63D showed similar results(Figure 1). Crude hazard ratios for cause-specific death due to neoplasms was 2.6(1.3-5.4; \(P=0.008\)), whereas death due to cardiovascular causes was not different by TS≥50% vs <50% (Figure 1). Crude hazard ratios for cause-specific death (Figure 1) due endocrinological causes was 3.4(2.0-6.0;\(P=0.0002\)); thus in individuals with diabetes and TS≥50% 26% had an endocrinological cause of death, whereas in individuals with TS<50% only 5% had an endocrinological cause of death. The 163 registered individuals who died from endocrinological causes, all died from diabetes, but one also had primary adrenocortical insufficiency noted on the death certificate.

A stepwise increased risk of total mortality was observed for stepwise increasing levels of TS (log-rank \(P=0.0001\)) with the highest risk conferred by TS≥70% vs TS<20% with a hazard ratio of 4.8 (2.0–12; \(P=0.0006\)) overall (Figure 2). Analyses in men and women separately (Figure 2), multifactorially adjusted analyses (Figure 2), and analysis for type 2 diabetes only or analysis excluding \(HFE\) genotypes C282Y/C282Y and C282Y/H63D showed similar results (two latter not shown). The population-attributable risk of total mortality overall among individuals with diabetes was 2% for TS≥50%.

\(Hemochromatosis\) genotype(\(HFE\))
The crude hazard ratio for total mortality in individuals with diabetes and HFE genotype C282Y/C282Y vs. wild type/wild type was 3.3(1.04-10; P=0.04) overall (Figure 3); results were similar in individuals with type 2 diabetes only. The crude hazard ratio for total mortality in individuals with (C282Y/C282Y or TS≥50%) vs. (wild type/wild type and TS<50%) was 2.1(1.4-3.0; P=0.0009). Also, the crude hazard ratios for total mortality in individuals with (C282Y/C282Y and TS<50%), (wild type/wild type and TS≥50%), and (C282Y/C282Y and TS≥50%) vs. (wild type/wild type and TS<50%) were 2.0(0.3-14; P=0.5), 2.0(1.02-3.7; P=0.04) and 6.0(1.5-24; P=0.01), respectively (trend: P=0.003; TS-genotype interaction: p=0.05). Multifactorially adjusted hazard ratios showed similar results. Hazard ratios for other HFE genotypes than C282Y/C282Y were not significant (Supplementary Table 1). The population-attributable risk of total mortality overall among individuals with diabetes was 2% for C282Y/C282Y, 6% for C282Y/C282Y or TS≥50%, and 2% for C282Y/C282Y and TS≥50%.

**Conclusions**

In two homogenous Caucasian Danish population-based follow-up studies comprising 84,865 individuals we identified 3,346 individuals with prevalent diabetes, with the majority having type 2 diabetes. We showed that individuals with diabetes with the threshold TS≥50% vs. TS<50% have an increased risk of premature death overall and that individuals with diabetes had a stepwise increased risk of total mortality for stepwise increasing levels of TS, with the highest risk conferred for TS≥70%; results were similar in men and women and for type 2 diabetes alone. Risk was independent of HFE genotype since risk was still increased when excluding HFE genotypes. Also, individuals with the threshold TS≥50% vs. TS<50% had an increased risk of cause-specific death from neoplasms and endocrinological diseases, which were diabetes related diagnoses. Moreover,
elevated TS and C282Y/C282Y both increased risk of premature death independently, but the joint
effect of exposure to transferrin saturation and C282Y/C282Y was higher than the sum of both
effects. We calculated population attributable risk showing that six percent of premature deaths
among individuals with diabetes in the general population could potentially be avoided by early
screening for TS or HFE genotype. Thus, TS and C282Y/C282Y independently and in combination
increase risk of premature death 2-6-fold in individuals with diabetes from a general population
study. These are novel findings.

Our findings that elevated TS increases the risk of premature death in individuals with type 2
diabetes underscore the results from our previous paper in patients with late-onset type 1 diabetes
from a highly specialised diabetes clinic(14). Both of these studies were based on a baseline-TS-test
at a random time-point in life and not an early TS-test. In the same recent paper we also showed that
awareness of iron overload in a diabetes clinic with early measurement of TS may reduce mortality
in patients with late-onset type 1 diabetes relative to that of the background diabetic population(14);
furthermore, we showed that patients with type 2 diabetes who have an early measurement of TS
have a mortality similar to the background diabetic population. However, for that patient cohort we
lacked a control group who had a random TS-test for comparison, which is provided in the present
study. Since the organ-manifestations of iron overload include almost any organ(1), awareness of
iron overload with early measurements of iron indices may increase survival. This is supported by a
study of health check-ups and family screening where subjects offered early detection of iron
overload had improved survival compared to that of the background population(32). The reason for
the improved survival in subjects offered early TS-test is likely conferred by early diagnosis and
treatment of iron overload or other conditions, but we have no data to demonstrate the reasons for
specific health benefits of early measurement of TS. In our study, we did not measure ferritin but
another study in patients with diabetes on maintenance hemodialysis with serum ferritin levels
above 700 ng/mL had slightly increased 1-year mortality(20). Whether the biochemical testing
should be TS, ferritin or both needs to be resolved(33). The dose-response relationship
with increased risk of total mortality for stepwise increasing levels of TS has also been shown in
two previous population-based studies(12); however, risk in the population cohorts increased from
TS≥40%, whereas in this study based on individuals with diabetes risk increased from TS≥30%.
Thus, it could be speculated that in individuals with diabetes the TS cut-off should be even lower
than 50%. To support this, it has been shown that iron depletion in patients with diabetes
ameliorates HbA1c levels, insulin secretion, insulin resistance(34), and vascular dysfunction(35).

In this study of individuals with mainly type 2 diabetes and in a previous study of
patients with late-onset type 1 diabetes(14), we have shown that HFE genotype C282Y/C282Y
alone or combined with elevated TS increases risk of premature death; this is in accordance with the
fact that patients with iron overload and manifest organ dysfunction have increased mortality(8-10).
In population-based studies C282Y/C282Y confer risk of diabetes(4,5) but not risk of premature
death(13,15,17); thus, it could be speculated that development of organ dysfunction is needed
before the genotypic effect confers risk of premature death; however, the joint effect of HFE
genotype with transferrin saturation or ferritin has not been studied in population-based mortality
studies of C282Y/C282Y.

Our study contrasts that of another Australian study that could not show evidence of
increased mortality according to iron overload or HFE genotype in patients with type 2
diabetes(18); however, that study was of only 1,265 patients of mixed ethnicity and a shorter
follow-up and less power, and thus not comparable to our study, which is larger and of homogenous
ethnicity. It has previously been shown, that ethnicity matters in terms of the risk conferred by iron
overload and HFE genotype(5).
Remarkably, those who died in the study had diabetes diagnosed later in life than those who survived, but also had shorter diabetes duration; thus, this group of individuals may be susceptible to factors increasing their sensitivity to the detrimental effects of diabetes or may have had undiscovered and thus untreated diabetes for a longer time than those with an earlier diagnosis.

We did not have sufficient power to exclude a modestly increased risk of total mortality in the CCHS alone or of cardiovascular disease overall conferred by elevated TS; furthermore, we did not have sufficient power to exclude a modestly increased risk of total mortality conferred by other HFE genotypes than C282Y/C282Y; however, these genotypes have not previously been associated with increased mortality in individuals with diabetes(14).

The correctness of the underlying and contributing causes of death relies on the codes and on the physicians who have filled in the death certificates(29). Before 2007, it was the National Board of Health in Denmark who interpreted the written information on underlying and contributing causes of death on paper-based death certificates issued by physicians and translated this information to ICD-codes; this practice may have resulted in misinterpretations(29). After 2007, electronic coding is done by the physician who issues the electronic death certificate and thus the coding relies on the diagnostic accuracy by the attending physicians(29). However, both systems share some general limitations. Differences in the causes of death may be due to new diagnostic techniques, increased focus on special diseases, and less focus on ill-defined diseases(29). Furthermore, in 1990 the legislation on autopsies on persons who died a natural death in Denmark was changed from a practice where a previous consent from the person who died or consent from the family was not needed to a practice where either of these consents was required. Thus, autopsy rates in Denmark has since declined and is low (below 10%)(29). A recent meta-analysis on the discrepancy between clinical and autopsy diagnoses estimates that 30% of the diagnoses on the death certificates are incorrect(36). Furthermore, in Denmark, causes of death are
not regularly validated\(^{(29)}\) as opposed to e.g. Finland where a validation report estimated that of 7\% questionable death certificates, half of them were re-assigned to a different ICD-code\(^{(37)}\). Thus, correctness of causes of death is crucial for mortality statistics and health surveillance but also for research purposes. However, total mortality in Denmark is based on the Danish Civil Registration System which updates vital status continuously and is thus considered complete for Danish residents\(^{(28)}\); however, for those persons who have emigrated or disappeared, death is only registered if the Danish authorities are informed about their death or the death occurred in Denmark\(^{(28)}\).

The pathogenetic link between iron overload and increased mortality may be exerted through iron-catalysed formation of hydroxyl radicals via the Fenton reaction and ensuing tissue oxidative stress\(^{(38)}\). A recent study showed increased mortality in patients with diabetes with C282Y/C282Y or iron overload and elevated urinary excretion of oxidised RNA\(^{(39)}\). Thus, oxidative stress is linked to decreased survival.

This study is a genetic epidemiological association study of markers of mortality, and as such does not provide proof of causality as would have been possible in an intervention study. Two recent papers have reviewed the principles of screening according to the WHO-guidelines in context of hereditary hemochromatosis \(^{(40,41)}\), and conclude that generalised population screening in primary care is generally not recommended; however, there may be a role for focused screening in Caucasian men\(^{(40)}\) as 84\% of men above 55 years have elevated ferritin and 37\% have ferritin above 1000\(\mu\)g/L which is a generally accepted threshold for organ impairment\(^{(41)}\). The reasons for the conclusions are among others that the gold standard measure for the screening has not been clarified yet (TS, ferritin, or both?; and/or genetic screening?); that the biological variability of TS is high; that the biochemically measured iron overload (in case of ferritin overload) may have many
other causes(42) that do not justify phlebotomy intervention(40); and that penetrance of homozygosity for C282Y/C282Y is low(40).

In a study of patients with late-onset Type 1 diabetes(4), the positive and negative predictive values of TS≥50% for detecting C282Y/C282Y were 26% and 100%, respectively; and the sensitivity and specificity were 100% and 96%, respectively. For comparison, in the present study among patients with type 2 diabetes, the positive and negative predictive values of TS≥50% for detecting C282Y/C282Y were 12% and 99%, respectively; and the sensitivity and specificity were 64% and 97%, respectively. Likewise, in the general population from which the patients with diabetes in this study were identified, the positive and negative predictive values of TS≥50% for detecting C282Y/C282Y were 12% and 99%, respectively; and the sensitivity and specificity were 70% and 99%, respectively. Thus the specificities were comparable and high and corresponded to those reported in The HEmochromatosis and IRon overload Screening study (HEIRS)(40). The sensitivity was highest among patients with late-onset type 1 diabetes; but in the general population overall and in patients with type 2 diabetes sensitivities corresponded to that in HEIRS but were relatively low limiting the role of TS as a screening test. The positive predictive values were low like in HEIRS(40); thus, TS≥50% reflects more than just homozygosity for C282Y/C282Y.

In current clinical practice, targeted screening for iron overload or HFE genotype has mainly been offered to patient-populations who have already developed organ-manifestations of iron overload. However, the results from the present study together with other survival-studies (8,10,14,20,21,32,43,44) add up to the conclusion that with manifest organ-disease the prognosis of survival is low. Thus, early detection of iron overload before organ-manifestations is desirable in the future. Prerequisites are awareness among physicians and decision makers in health politics. However, cost-effectiveness analyses on screening still only recommend targeted screening and not population-screening for hemochromatosis (45,46).
In summary, individuals with diabetes ascertained in the general population with increased TS or $HFE$ genotype have a 2-6-fold increased risk of premature death.

**Acknowledgments**

Authors have no conflict of interest to disclose.

CE is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

*Authors’ contribution*

Study design: CE, TMP, ATH, BGN

Data collection: ATH, BGN

Data analysis: CE

Data interpretation: CE, TMP, ATH, BGN

Writing the manuscript: CE

Editing the manuscript: CE, TMP, ATH, BGN

Figures, Tables: CE
References


Legends

Figure 1. Total and cause-specific mortality by transferrin saturation $\geq 50\%$ vs. $<50\%$.

Power is 80% to detect a given hazard ratio. *Individuals with C282Y/C282Y and C282Y/H63D excluded from analyses. See text on statistics for adjustments. Exp: exposed.

Figure 2. Total mortality by stepwise increasing transferrin saturation.

Power is 80% to detect a given hazard ratio. See text on statistics for adjustments. *There were no deaths among women with TS $\geq 70\%$. Exp: exposed.

Figure 3. Total mortality by hemochromatosis genotype ($HFE$) C282Y/C282Y.

Power is 80% to detect a given hazard ratio. *P=0.046. See text on statistics for adjustments. Exp: exposed.
Table 1. Baseline characteristics of participants with diabetes in two population-based follow-up studies.

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<td>Current smoker, %</td>
<td>20</td>
<td>27**</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>Plasma cholesterol&gt;5 mmol/L, %</td>
<td>44</td>
<td>39</td>
<td>88</td>
<td>84</td>
</tr>
<tr>
<td>Anti-hypertensive medication, %</td>
<td>60</td>
<td>65</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>Alcohol&gt;84g/week (i.e.&gt;7 units/week), %</td>
<td>50</td>
<td>46</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>Physically inactive², %</td>
<td>11</td>
<td>20***</td>
<td>13</td>
<td>22*</td>
</tr>
<tr>
<td>IHD or ICVD, %</td>
<td>16</td>
<td>32***</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Elevated plasma creatinin§, %</td>
<td>11</td>
<td>30***</td>
<td>30</td>
<td>45*</td>
</tr>
</tbody>
</table>

CGPS: Copenhagen General Population Study. CCHS: Copenhagen City Heart Study. IHD: ischemic heart disease. ICVD: ischemic cerebrovascular disease. Variables expressed as median (± interquartile range) or proportion. Statistical comparisons were made using two-sided Mann-Whitney U test and Pearson’s χ² test as appropriate. *p<0.05, **p<0.01, ***p<0.001. ¹1 Pack-year is equivalent to smoking 20 cigarettes each day for one year. ²Physically activity was leisure time physical activity (almost completely inactive, some activity, regular activity, regular hard physical training). § women:>90μmol/L , men: >100 μmol/L.
### Mortality by TS>=50% vs. <50%

<table>
<thead>
<tr>
<th>Total mortality</th>
<th>Person-time (y)</th>
<th>Failures (N)</th>
<th>Incidence rate (95%)</th>
<th>Adjusted hazard ratio (95% CI)</th>
<th>Power 00%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexp/Exp</td>
<td>Unexp/Exp</td>
<td>Events/1000 per year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>14521/452</td>
<td>511/30</td>
<td>3532-38/66/46-95</td>
<td>1.7 (1.2-2.5); p=0.004</td>
<td>2.2</td>
</tr>
<tr>
<td>Men</td>
<td>8011/370</td>
<td>328/26</td>
<td>41 (37-46)/71 (48-103)</td>
<td>1.5 (1.0-2.3); p=0.05</td>
<td>2.4</td>
</tr>
<tr>
<td>Women</td>
<td>6510/62</td>
<td>183/4</td>
<td>28 (24-32)/49 (18-130)</td>
<td>3.8 (1.4-11); p=0.01</td>
<td>4.3</td>
</tr>
<tr>
<td>CCHS</td>
<td>3681/266</td>
<td>264/21</td>
<td>72 (64-81)/79 (51-121)</td>
<td>1.1 (0.7-1.7); p=0.7</td>
<td>5.2</td>
</tr>
<tr>
<td>CGPS</td>
<td>10840/187</td>
<td>247/9</td>
<td>23 (20-26)/48 (25-92)</td>
<td>2.5 (1.2-4.9); p=0.01</td>
<td>3.6</td>
</tr>
<tr>
<td>Type 2, only</td>
<td>14077/453</td>
<td>504/30</td>
<td>36 (33-39)/66 (46-95)</td>
<td>1.7 (1.2-2.5); p=0.004</td>
<td>2.2</td>
</tr>
<tr>
<td>Genotypes excluded*</td>
<td>11428/326</td>
<td>431/25</td>
<td>38 (34-41)/77 (52-113)</td>
<td>1.9 (1.2-2.8); p=0.003</td>
<td>2.4</td>
</tr>
</tbody>
</table>

### Cause-specific mortality

<table>
<thead>
<tr>
<th></th>
<th>Person-time (y)</th>
<th>Failures (N)</th>
<th>Incidence rate (95%)</th>
<th>Adjusted hazard ratio (95% CI)</th>
<th>Power 00%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexp/Exp</td>
<td>Unexp/Exp</td>
<td>Events/1000 per year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplasms</td>
<td>12200/302</td>
<td>120/8</td>
<td>9.8 (8.2-12)/26 (13-53)</td>
<td>2.6 (1.3-6.0); p=0.007</td>
<td>3.2</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>12433/304</td>
<td>135/6</td>
<td>11 (9.2-13)/20 (9-44)</td>
<td>1.3 (0.6-3.1); p=0.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Endocrinological death</td>
<td>12504/306</td>
<td>149/14</td>
<td>12 (10-14)/38 (23-65)</td>
<td>4.2 (1.7-11); p=0.002</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Figure 1
107x78mm (600 x 600 DPI)
Total mortality
by stepwise increasing transferrin saturation (%)

<table>
<thead>
<tr>
<th>Group</th>
<th>Person-time(year)</th>
<th>Failure(N)</th>
<th>Incidence rate(95%)</th>
<th>Events/1000 person-years</th>
<th>Adjusted hazard ratio(95% CI)</th>
<th>Power 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS&gt;20</td>
<td>5098/8563</td>
<td>143/211</td>
<td>28(24-33)(36-41)</td>
<td>1.1(0.9-1.4) p=0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS=30 &amp; &lt;40</td>
<td>5098/2737</td>
<td>143/119</td>
<td>28(24-33)(36-52)</td>
<td>1.2(0.9-1.5) p=0.02</td>
<td></td>
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</tr>
<tr>
<td>TS=40 &amp; &lt;50</td>
<td>5098/823</td>
<td>143/38</td>
<td>28(24-33)(34-63)</td>
<td>1.2(0.8-1.5) p=0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS=50 &amp; &lt;60</td>
<td>5098/274</td>
<td>143/16</td>
<td>28(24-33)(36-95)</td>
<td>1.5(0.9-2.6) p&lt;0.1</td>
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</tr>
<tr>
<td>TS=60 &amp; &lt;70</td>
<td>5098/98</td>
<td>143/9</td>
<td>28(24-33)(49-190)</td>
<td>2.4(1.2-4.7) p=0.02</td>
<td></td>
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</tr>
<tr>
<td>TS=70+</td>
<td>5098/83</td>
<td>143/5</td>
<td>28(24-33)(60-255)</td>
<td>3.5(1.4-8.6) p=0.008</td>
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</tr>
</tbody>
</table>

Men

<table>
<thead>
<tr>
<th>Group</th>
<th>Person-time(year)</th>
<th>Failure(N)</th>
<th>Incidence rate(95%)</th>
<th>Events/1000 person-years</th>
<th>Adjusted hazard ratio(95% CI)</th>
<th>Power 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS&gt;20</td>
<td>2134/3335</td>
<td>77/138</td>
<td>36(29-45)(36-49)</td>
<td>1.1(0.9-1.5) p=0.3</td>
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<td></td>
</tr>
<tr>
<td>TS=30 &amp; &lt;40</td>
<td>2134/1995</td>
<td>77/83</td>
<td>36(29-45)(35-54)</td>
<td>1.0(0.7-1.4) p=0.9</td>
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<td></td>
</tr>
<tr>
<td>TS=40 &amp; &lt;50</td>
<td>2134/617</td>
<td>77/30</td>
<td>36(29-45)(34-69)</td>
<td>1.1(0.7-1.7) p=0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS=50 &amp; &lt;60</td>
<td>2134/236</td>
<td>77/14</td>
<td>36(29-45)(59-100)</td>
<td>1.3(0.7-2.3) p=0.4</td>
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<tr>
<td>TS=60 &amp; &lt;70</td>
<td>2134/90</td>
<td>77/5</td>
<td>36(29-45)(78-194)</td>
<td>1.9(0.9-4.1) p=0.1</td>
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<tr>
<td>TS=70+</td>
<td>2134/45</td>
<td>77/5</td>
<td>36(29-45)(110-265)</td>
<td>3.0(1.5-10) p=0.005</td>
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</tbody>
</table>

Women

<table>
<thead>
<tr>
<th>Group</th>
<th>Person-time(year)</th>
<th>Failure(N)</th>
<th>Incidence rate(95%)</th>
<th>Events/1000 person-years</th>
<th>Adjusted hazard ratio(95% CI)</th>
<th>Power 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS&gt;20</td>
<td>2963/2509</td>
<td>66/73</td>
<td>22(17-28)(29-36)</td>
<td>1.1(0.8-1.5) p=0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TS=30 &amp; &lt;40</td>
<td>2963/931</td>
<td>66/36</td>
<td>22(17-28)(43-60)</td>
<td>1.6(1-2.5) p=0.03</td>
<td></td>
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</tr>
<tr>
<td>TS=40 &amp; &lt;50</td>
<td>2963/206</td>
<td>66/8</td>
<td>22(17-28)(39-77)</td>
<td>1.1(0.5-2.5) p=0.7</td>
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<td></td>
</tr>
<tr>
<td>TS=50 &amp; &lt;60</td>
<td>2963/37</td>
<td>66/2</td>
<td>22(17-28)(53-212)</td>
<td>3.2(0.8-14) p=0.1</td>
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</tr>
<tr>
<td>TS=60+</td>
<td>2963/44</td>
<td>66/2</td>
<td>22(17-28)(48-1180)</td>
<td>6.4(1.5-27) p=0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2
108x79mm (600 x 600 DPI)
Figure 3

110x82mm (600 x 600 DPI)
Supplementary Table 1. Risk of total mortality according to hemochromatosis genotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Alive (N)</th>
<th>Dead (N)</th>
<th>Crude Hazard ratio (95% CI)</th>
<th>P-value</th>
<th>Adjusted Hazard ratio (95% CI)</th>
<th>P-value</th>
<th>Power 80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type/wild type</td>
<td>958</td>
<td>298</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H63D/wild type</td>
<td>242</td>
<td>96</td>
<td>1.1(0.9-1.5)</td>
<td>0.2</td>
<td>1.1(0.9-1.4)</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>22</td>
<td>6</td>
<td>0.8(0.4-1.8)</td>
<td>0.6</td>
<td>0.8(0.4-1.8)</td>
<td>0.5</td>
<td>2.9</td>
</tr>
<tr>
<td>C282Y/wild type</td>
<td>146</td>
<td>56</td>
<td>1.2(0.9-1.5)</td>
<td>0.3</td>
<td>1.1(0.9-1.5)</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>21</td>
<td>9</td>
<td>0.9(0.5-1.8)</td>
<td>0.8</td>
<td>0.8(0.4-1.6)</td>
<td>0.6</td>
<td>2.9</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>8</td>
<td>3</td>
<td>3.3(1.04-10)</td>
<td>0.04</td>
<td>3.2(1.02-10)</td>
<td>0.05*</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Crude hazard ratio is age- and gender-adjusted. Adjusted hazard ratio is multifactorially adjusted for age, gender, smoking habits, leisure time physical activity, cholesterol. Power is 80% to detect a given hazard ratio. *P=0.046.