

# Total Mortality by Elevated Transferrin Saturation in Patients With Diabetes

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**OBJECTIVE**—It is not known to what extent iron overload predicts prognosis in patients with diabetes after diagnosis or whether iron overload is a risk factor independent of the *HFE* genotype. We investigated total and cause-specific mortality according to increased transferrin saturation ( $\geq 50$  vs.  $< 50\%$ ), whether mortality is driven by the *HFE* genotype, and whether early measurement of transferrin saturation helps to predict mortality outcome.

**RESEARCH DESIGN AND METHODS**—Cohort 1 included patients with late-onset type 1 diabetes ( $n = 716$ ) with a cross-sectional measurement of transferrin saturation and *HFE* genotype. Cohort 2 included consecutively recruited patients with any diabetes ( $n = 6,120$ ), transferrin saturation measurement at referral, and *HFE* genotype if transferrin saturation was above 50%.

**RESULTS**—In cohort 1, the hazard ratio for total mortality was 2.3 (95% CI 1.3–3.9;  $P = 0.002$ ) and for cause-specific mortality by neoplasms was 5.8 (2.4–14;  $P = 0.00007$ ) in patients with transferrin saturation  $\geq 50$  vs.  $< 50\%$ . Excluding genotypes C282Y/C282Y and C282Y/H63D gave similar results. The hazard ratio for total mortality was 4.0 (1.2–13;  $P = 0.01$ ) and for cause-specific mortality by neoplasms was 13 (3.6–49;  $P = 0.0001$ ) in patients with C282Y/C282Y versus wild type. In cohort 2, total mortality was not different in patients with transferrin saturation  $\geq 50$  vs.  $< 50\%$ . In patients with late-onset type 1 diabetes and transferrin saturation  $\geq 50\%$ , the hazard ratio for total mortality was 0.4 (0.2–0.9;  $P = 0.03$ ) in cohort 2 versus cohort 1.

**CONCLUSIONS**—Increased transferrin saturation and *HFE* genotype C282Y/C282Y predict total mortality in patients with late-onset type 1 diabetes, and increased transferrin saturation after diagnosis is an independent risk factor. Early measurement of transferrin saturation in these patients leading to early intervention improves life expectancy.

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There is evidence for increased risk of premature death due to organ damage in individuals with iron overload in the general population (1) and in patients with hemochromatosis (2,3). Early detection and treatment of iron overload, before the development

of diabetes and cirrhosis, can prevent excess mortality (3,4). A recent study showed that diabetic patients with serum ferritin levels  $> 700$  ng/mL who were receiving maintenance hemodialysis had an increased risk of 1-year mortality (5).

In contrast, there is no evidence for increased risk of premature death in individuals with hemochromatosis (*HFE*) C282Y/C282Y versus wild type genotype in either population-based studies (6–9) or a previous study of patients with type 2 diabetes (10).

A recent study of patients with hereditary *HFE* demonstrated a decline in diabetes prevalence in patients diagnosed after determining that they carried the *HFE* gene compared with those diagnosed before this determination (11), suggesting that awareness of *HFE* in general and the development of diabetes in those patients in particular will translate into a greater life expectancy.

In 2001, in a study of *HFE* in patients with late-onset type 1 diabetes, to improve life expectancy in patients with diabetes secondary to hereditary hemochromatosis (12), we recommended measurement of transferrin saturation in all patients with onset of diabetes after 30 years of age followed by genetic testing for *HFE* in patients with a transferrin saturation above 50%. To assess this recommendation in an independent cohort, from 2001–2007 we measured transferrin saturation in consecutive patients with any type of diabetes (13) followed by genetic testing if the transferrin saturation was above 50%. We found that elevated transferrin saturation conferred a two- to threefold increased risk of developing any form of diabetes, as well as type 1 and type 2 diabetes separately, independent of *HFE* genotype (13). However, it is not known to what extent iron overload predicts prognosis in these patients after diagnosis or whether iron overload after diagnosis is an independent risk factor compared with *HFE* genotype.

Therefore, in this study, we investigated total and cause-specific mortality according to increased transferrin saturation in patients with late-onset type 1 diabetes and in patients with any type of diabetes. Second, we investigated whether mortality is driven by *HFE* genotypes. Finally, we investigated whether early measurement of transferrin saturation in patients with diabetes and subsequent early intervention helps to alter mortality outcome.

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## RESEARCH DESIGN AND METHODS

### Cohorts

**Cohort 1.** Between April and November 1999, at the Steno Diabetes Centre in Denmark, we cross-sectionally recruited all patients with late-onset type 1 diabetes mellitus developed after age 30 years (12): 792 patients were asked to participate and 716 (90%) accepted. At study entry in 1999, both clinicians and patients were unaware of the patients' iron and genotype status, and patients had a blood sample taken to measure transferrin saturation and genotyping for hereditary HFE. This study is retrospective in the sense that in 1999 we examined the relation between HFE genotype C282Y/C282Y and late-onset type 1 diabetes. All patients carrying this genotype also had increased transferrin saturation (12). **Cohort 2.** Based on results from cohort 1 (12), a decision was made at the Steno Diabetes Centre to introduce routine measurement of transferrin saturation during the first meeting with an endocrinologist for all patients attending the center; increased transferrin saturation above 50% was followed by genotyping for HFE. Thus, we compared these two cohorts, allowing us to examine the effect of this measurement on mortality.

Danish patients with any diabetes ( $n = 6,129$ ) were recruited from 2001–2007 (13); 9 patients were lost to follow-up, leaving 6,120 patients for this study: 2,656 with type 1 diabetes (ICD-10 code E10), 3,176 with type 2 diabetes (ICD-10 code E11), and 288 with other diabetes (ICD-10 codes E13 or E14). All individuals were from Copenhagen County and were referred to the Steno Diabetes Centre, which is a first-line referral clinic for type 1 diabetes in Copenhagen County; thus, this cohort is representative of a general population of about 600,000 inhabitants living in the larger area of Copenhagen suburbs. Patients with type 2 diabetes typically are referred because of failure of oral hypoglycemic agents with a consequent need to start insulin therapy. A minority of patients were referred from departments of nephrology and endocrinology because of late diabetes complications. All patients had transferrin saturation measured during the first meeting with the endocrinologist.

There were no duplicate individuals between cohorts 1 and 2. Both cohorts were treated appropriately on the basis of the results of transferrin saturation and

received antidiabetic treatment according to existing guidelines; however, because of earlier measurement of transferrin saturation, patients in cohort 2 received earlier treatment for iron overload than those in cohort 1.

Thus, we compared these two cohorts, allowing us to examine the effect of introducing this early measurement and intervention on mortality.

### Baseline covariates

Baseline covariates were obtained when transferrin saturation was measured (beginning of follow-up). Information on weight, height, smoking status, alcohol intake, and antidiabetic treatment were obtained from electronic patient records at the Steno Diabetes Centre. BMI was calculated as weight (kg)/height ( $m^2$ ) and was available for 91.6% of cohort 1 and 96.5% of cohort 2. For information on smoking and alcohol, the registered categorical values were, unfortunately, not exclusive (within the cohorts) and differed between cohorts and therefore were not included in this study. Information about antidiabetic treatment was available for 93.3% of cohort 1 and 73% of cohort 2 (some patients were enrolled and subsequently died before the introduction of electronic patient records).

Baseline diabetes diagnosis and complications were obtained from the Danish National Patient Registry (ICD-10 supplemental codes for complications: renal, 0.2; ophthalmological, 0.3; neurological 0.4; circulatory, 0.5; and other, 0.0, 0.1, 0.6, 0.7, 0.8).

### Transferrin saturation

Transferrin saturation (as a percentage) was calculated as  $(\text{iron}[\mu\text{mol/L}]/[2 \times \text{transferrin}(\mu\text{mol/L})]) \times 100$ . Transferrin was measured by turbidimetry, and iron levels were measured by colorimetry using a Hitachi 912 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Transferrin saturation  $>50\%$  was considered suggestive of increased transferrin saturation, in accordance with accepted clinical practice (14–16).

### Genotyping

Genotyping for C282Y (single nucleotide polymorphism database rs1800562), a G/A nucleotide change at position 845 in the HFE gene (17), and H63D (single nucleotide polymorphism database rs1799945), a C/G nucleotide change at position 187 in the HFE gene (17), was done using allele-specific amplification

(18) and confirmatory restriction enzyme digestion (12,17). The amplification refractory mutation system simultaneously detected both HFE mutations, C282Y and H63D, including sense and antisense primers for C282Y and H63D, with human growth hormone as an internal amplification control (18).

### Hereditary hemochromatosis

Patients were diagnosed with HFE (ICD-10 code E83.1) if transferrin saturation was  $\geq 50\%$  and the HFE genotype was C282Y/C282Y or C282Y/H63D.

### End point definitions

Using the patients' civil registry numbers, which are unique to every Danish citizen, information on total and cause-specific mortality was obtained from the time of blood sampling (through linkage to the Danish Civil Registration System) until 7 June 2011, and from the National Register of Causes of Death (NRCD) until 31 December 2009. Total mortality data was available up until 2011; however, data in NRCD on the causes of death was only available up until 2009. This registry is running behind in data collection due to an enormous amount of data that has to be processed, filed, and registered. Total mortality data was taken from the Danish Civil Registry System, and cause-specific data was taken from the NRCD. The NRCD contains information on all underlying and contributing causes of death coded by the Danish National Board of Health and based on patients' death certificates completed by physicians in hospitals, general practice, or forensic medicine. The NRCD has used ICD codes consistently, using ICD-10 codes in particular since 1994. The following ICD-10 codes were used for cause-specific deaths in this study: neoplasms (C00–D48, liver cancer C220, C221, C223, C229); endocrine, nutritional, and metabolic diseases (E00–E90); and diseases of the circulatory system (I00–I99). Because of a significantly longer median follow-up time for late-onset type 1 diabetes in cohort 1 than in cohort 2, the follow-up time in cohort 1 was adjusted to a median follow-up of 8 years using the "right truncation" statistical method (described below) to balance the follow-up of patients with late-onset type 1 diabetes in cohort 2.

### Statistics

STATA/SE 11.0 statistical software (Stata Corp., College Station, TX) was used. Mann-Whitney  $U$  tests and Pearson  $\chi^2$

Table 1—Baseline characteristics of patients with diabetes

Characteristics	Cohort 1		Cohort 2			
	Late-onset type 1 diabetes (n = 716)	All (n = 6,120)	Type 1 diabetes (n = 2,656)	Late-onset type 1 diabetes (n = 1,115)	Type 2 diabetes (n = 3,176)	Other (n = 288)
Sex, n (%)						
Men,	401 (56.0)	3,450 (56.4)	1,415 (53.4)	632 (56.7)	1,868 (58.8)	167 (58.0)
Women	315 (44.0)	2,670 (43.6)	1,241 (46.7)	483 (43.3)	1,308 (41.2)	121 (42.0)
Year(s) recruited	1999	2001–2007	2001–2007	2001–2007	2001–2007	2001–2007
Age, years	58 (49–68)	56 (43–66)	45 (35–56)	58 (50–65)	63 (55–71)	57 (43–68)
BMI, n (%)						
≥25	313 (47.7)	3,590 (60.8)	991 (37.8)	429 (39.1)*	2,524 (81.6)	75 (38.7)
Missing data	60 (8.4)	213 (3.5)	36 (1.4)	18 (1.6)	83 (2.6)	94 (32.3)
Age at diabetes onset, years	46 (38–56)	47 (28–59)	27 (18–38)	41 (35–50)*	57 (49–66)	51 (38–62)
<30 years, n (%)	0	1,669 (27.3)	1,541 (58.0)	0	89 (2.8)	39 (13.5)
≥30 years, n (%)	716 (100)	4,451 (72.7)	1,115 (42.0)	1,115 (100)	3,087 (97.2)	249 (86.5)
Diabetes duration, years	20 (16–26)	15 (8–25)	26 (17–31)	22 (13–29)	10 (6–15)	8 (0–16)
Diabetes complications, n (%)†	342 (48)	2,829 (46)	1,411 (53)	567 (51)	1,321 (42)	97 (34)
Renal	10 (1.4)	331 (5.6)	31 (1.2)	29 (2.6)	286 (9.2)	14 (6.5)
Ophthalmological	29 (4.0)	430 (7.2)	72 (2.7)	65 (5.9)	347 (11.2)	11 (5.1)
Neurological	31 (4.3)	523 (8.8)	47 (1.8)	45 (4.1)	462 (14.9)	14 (6.5)
Circulatory	28 (3.9)	226 (3.8)	113 (4.3)	40 (3.6)	104 (3.4)	9 (4.2)
Other	294 (41.1)	2,116 (34.6)	1,298 (48.9)	495 (44.4)	736 (23.2)	82 (28.5)
Antidiabetic medication, n (%)‡						
Missing data§	48 (6.7)	1,652 (27.0)	567 (21.4)	266 (23.8)	913 (28.7)	172 (59.7)
Insulin only	664 (92.7)	2,828 (46.2)	2,060 (77.6)	840 (75.3)	673 (21.2)	95 (33.0)
Oral antidiabetics only	0	1,375 (22.5)	19 (0.7)	0	1,343 (42.3)	13 (4.5)
Insulin + oral	4 (0.6)	258 (4.2)	10 (0.4)	9 (0.8)	240 (7.6)	8 (2.8)
Diet only	0	7 (0.1)	0	0	7 (0.2)	0
Iron, umol/L	16 (13–20)	16 (12–20)	17 (13–21)	16 (13–20)	16 (12–20)	14 (10–20)
Transferrin, umol/L	28 (26–32)	31 (27–34)	29 (26–32)	28 (26–31)	32 (29–35)	31 (27–34)
Transferrin saturation, median (IQR), all (%)	28 (22–36)	26 (20–34)	29 (22–38)	28 (22–35)	24 (19–31)	23 (16–31)
Transferrin saturation <50%						
n (%)	670 (93.7)	5,847 (95.5)	2,463 (92.7)	1,049 (94.1)	3,108 (97.9)	276 (95.8)
Median (IQR)	27 (21–34)	26 (20–32)	28 (21–35)	28 (21–34)	24 (18–30)	23 (15–30)
Transferrin saturation ≥50%						
n (%)	45 (6.3)	273 (4.5)	193 (7.3)	66 (5.9)	68 (2.3)	12 (4.2)
Median (IQR)	56 (52–62)	56 (52–64)	56 (52–63)	56 (52–63)	55 (52–66)	57 (52–71)
Hemochromatosis genotype						
All						
Wild type/wild type	474 (66.2)	—	—	—	—	—
H63D/wild type	143 (20.0)	—	—	—	—	—
H63D/H63D	15 (2.1)	—	—	—	—	—
C282Y/wild type	67 (9.4)	—	—	—	—	—
C282Y/H63D	8 (1.1)	—	—	—	—	—
C282Y/C282Y	9 (1.3)	—	—	—	—	—
Transferrin saturation ≥50%¶						
Wild type/wild type	24 (53.3)	129 (51.8)	100 (54.6)	35 (56.5)	25 (43.1)	4 (50.0)
H63D/wild type	6 (13.3)	55 (22.1)	40 (21.7)	14 (22.6)	13 (22.4)	2 (25.0)
H63D/H63D	1 (2.2)	8 (3.2)	3 (1.6)	2 (3.2)	5 (8.6)	0
C282Y/wild type	4 (8.9)	33 (13.3)	27 (14.8)	6 (9.7)	5 (8.6)	1 (12.5)
C282Y/H63D	1 (2.2)	13 (5.2)	9 (4.9)	4 (6.5)	4 (6.9)	0
C282Y/C282Y	9 (20.0)	11 (4.4)	4 (2.2)	1 (1.6)	6 (10.3)	1 (12.5)
Total number of genotypes for transferrin saturation ≥50%*	45	249	183	62	58	8

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Table 1—Continued

Characteristics	Cohort 1			Cohort 2		
	Late-onset type 1 diabetes (n = 716)	All (n = 6,120)	Type 1 diabetes (n = 2,656)	Late-onset type 1 diabetes (n = 1,115)	Type 2 diabetes (n = 3,176)	Other (n = 288)
Total mortality						
Median follow-up, years	8 (7–8)	7 (5–8)	8 (7–9)	8 (7–8)	7 (5–8)	5 (2–7)
Alive, n (%)	538 (75.1)	4,827 (78.9)	2,314 (87.1)	865 (77.6)	2,340 (73.7)	173 (60.1)
Dead, n (%)	178 (24.9)	1,293 (21.1)	342 (12.9)	250 (22.4)	836 (26.3)	115 (39.9)

Data for continuous variables are presented as median and interquartile range; other variables are presented as indicated. Analysis used the Mann-Whitney U test for continuous variables and Pearson  $\chi^2$  test for categorical variables (for the comparison of late-onset type 1 diabetes in cohort 1 vs. cohort 2). Type 1 diabetes (WHO E10), type 2 diabetes (E11), other diabetes (E13, E14). \* $P < 0.001$ . †Because of the difference in missing values for late-onset type 1 diabetes in cohort 1 and cohort 2, no  $P$  value was calculated. ‡See text for explanation of antidiabetic medication. §ICD-10 codes for diabetes complications: renal 0.2, ophthalmological 0.3, neurological 0.4, circulatory 0.5, other 0.0, 0.1, 0.6, 0.7, 0.8. ¶In cohort 2, HFE genotypes were only available for patients with transferrin saturation above 50%. || $P < 0.01$ .

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 transferrin saturation  $\geq 50$  vs.  $< 50\%$  or hemochromatosis genotype (C282Y/C282Y, C282Y/H63D, C282Y/wild type, H63D/H63D, or H63D/wild type vs. wild type/wild type); sex (because penetrance of clinically manifest HFE differs markedly between the two sexes [19]); cohort; type of diabetes; and onset of diabetes. Cumulative survival was plotted using Kaplan-Meier curves, and differences between transferrin saturation levels were examined by log-rank tests. Cox proportional hazards regression was used to estimate hazard ratios with 95% CIs. We used left truncation (or delayed entry) with age as the time scale, thereby allowing automatic adjustment for age. We used right truncation for cohort 1 to adjust the exit time to a median follow-up that was similar for patients with late-onset type 1 diabetes in cohort 2. Adjustments were made for age, sex, onset of diabetes ( $< 30$  and  $\geq 30$  years), BMI ( $< 25$  and  $\geq 25$  kg/m<sup>2</sup>), and diabetes complications (no/yes). Because of missing values for BMI, imputations were made depending on age, sex, and cohort so power was not lost in the adjusted analyses. To test for bivariate multiplicative interactions between transferrin saturation ( $< 50$  or  $\geq 50\%$ ) or genotype and sex, BMI, onset diabetes, and complications on total mortality in Cox regression models, two-factor interaction terms were included individually in the different models and tested for significance with a likelihood ratio test. No statistically significant interactions were observed. The assumption of proportional hazards was

tested using Schoenfeld residuals, and no violations were observed. For each analysis, we calculated the hazard ratio that could be detected with 80% power assuming a two-sided  $P < 0.05$  using NCSS Pass software (NCSS, Kaysville, UT).

## RESULTS

### Baseline characteristics

By design, cohorts 1 and 2 were diverse. Cohort 1 was a population of late-onset type 1 diabetes, whereas cohort 2 was a population of any kind of diabetes (Table 1). In late-onset type 1 diabetes, cohorts 1 and 2 were similar with respect to sex distribution, age, diabetes duration, prevalence of complications, and levels of iron, transferrin, and transferrin saturation, but they were dissimilar with respect to BMI ( $P < 0.001$ ) and diabetes onset ( $P < 0.001$ ), and for patients with transferrin saturation  $\geq 50\%$  also dissimilar with respect to genotype distribution ( $P < 0.001$ ). The difference observed for BMI should be interpreted with caution because of the difference in missing values (cohort 1, 8.6%; cohort 2, 1.6%). In addition, because of the difference in missing values for antidiabetic treatment of late-onset type 1 diabetes in cohort 1 (6.7%) and cohort 2 (23.8%), no comparison was made.

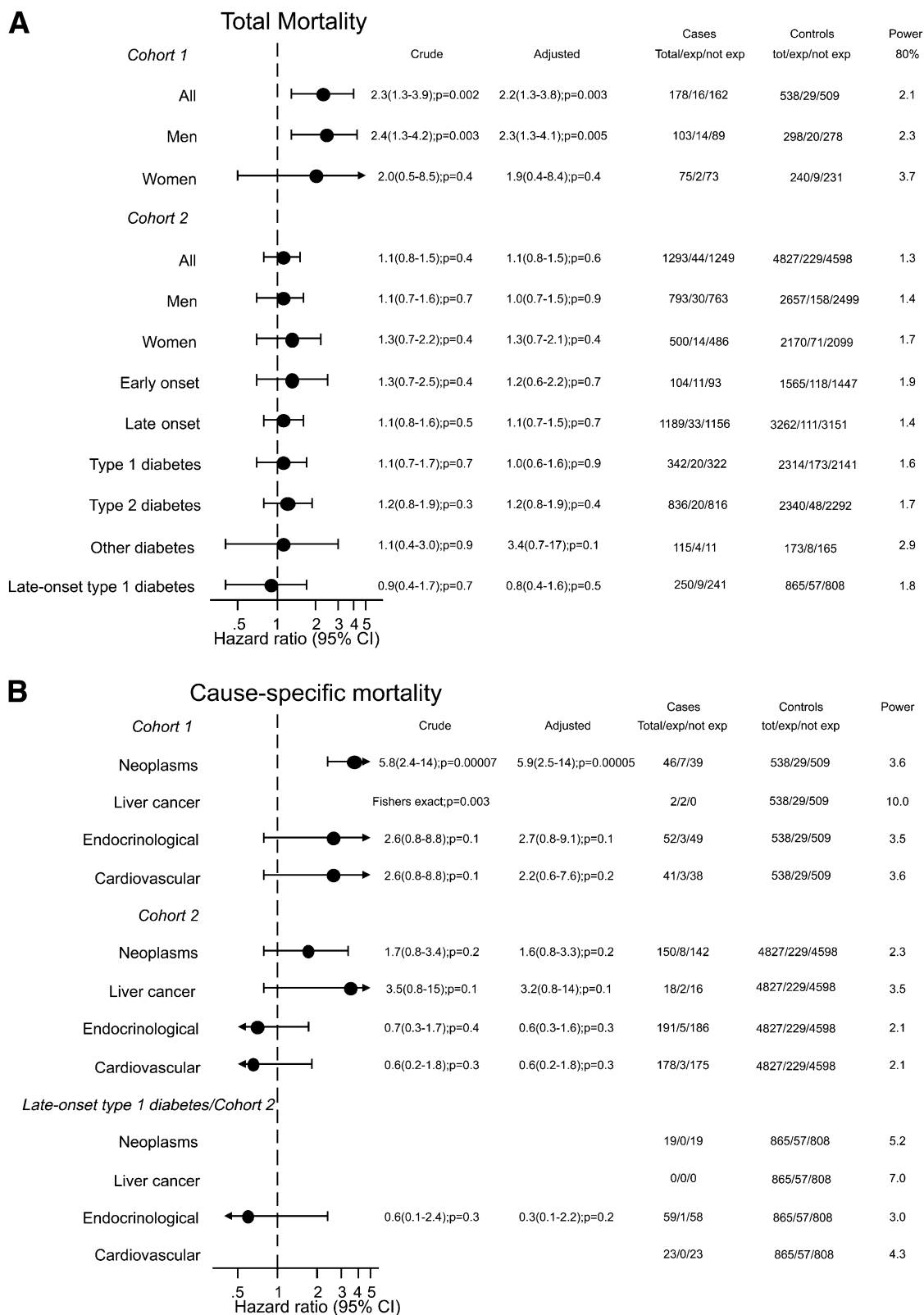
### Total and cause-specific mortality

#### Cohort 1: late-onset type 1 diabetes.

Total mortality was increased in patients with transferrin saturation  $\geq 50$  vs.  $< 50\%$ , with an age- and sex-adjusted hazard ratio of 2.3 (95% CI 1.3–3.9;  $P = 0.002$ ) overall and 2.4 (1.3–4.2;  $P = 0.003$ ) in men (Fig. 1A); the result

for women was not significant (Fig. 1A). Mortality due to neoplasms was increased in patients with transferrin saturation  $\geq 50$  vs.  $< 50\%$ , with an age- and sex-adjusted hazard ratio of 5.8 (2.4–14;  $P = 0.00007$ ) (Fig. 1B). Two of seven cases of neoplasms among patients with transferrin saturation  $\geq 50\%$  were liver cancers, whereas no liver cancers were observed among patients with transferrin saturation  $< 50\%$  (Fisher exact test,  $P = 0.003$ ). Mortality due to endocrinological or cardiovascular causes was not different. For total mortality for patients with transferrin saturation  $\geq 50\%$ , we had 80% power to detect a hazard ratio of 2.1 overall, 2.3 in men, and 3.7 in women. We had 80% power to detect a hazard ratio of 3.6 for neoplasms, 10.0 for liver cancer, 3.5 for endocrinological causes, and 3.6 for circulatory causes.

Total mortality was increased in patients with the C282Y/C282Y versus wild type/wild type genotype, with an age- and sex-adjusted hazard ratio of 4.0 (95% CI 1.2–13;  $P = 0.01$ ) (Table 2); mortality due to neoplasms was increased as well, with an age- and sex-adjusted hazard ratio of 13 (3.6–49,  $P = 0.0001$ ). Two of three cases of neoplasms among patients with the C282Y/C282Y genotype were liver cancers, whereas no liver cancers were observed among patients with the wild-type genotype (Fisher exact test,  $P < 0.0001$ ) (Table 2). There were no deaths registered as due to endocrinological diseases or diseases in the circulatory system among patients with the C282Y/C282Y genotype. For total mortality for patients with the C282Y/C282Y genotype, we had 80% power to detect a hazard ratio of 4.0 overall, 5.9 in men, and 6.8 in women. We had 80% power to detect a hazard



**Figure 1**—Total mortality (A) and cause-specific mortality (B) in cohort 1 and cohort 2. The “crude” hazard ratio is adjusted for age and sex, whereas the “adjusted” hazard ratio is multifactorially adjusted for age, sex, BMI, diabetes onset, and complications.

Table 2—Total and cause-specific mortality by genotype in cohort 1 (late-onset type 1 diabetes)

HFE genotype	No. of patients alive (n = 538)	No. of patients dead (n = 178)	Hazard ratio (95% CI), adjusted for age and sex	P value	Hazard ratio (95% CI), multifactorially adjusted*	P value	80% Power*
<b>Total mortality</b>							
<b>Men and women</b>							
Wild type/wild type	357	117	1.0		1.0		
H63D/wild type	107	36	1.0 (0.7–1.5)	0.8	1.0 (0.7–1.5)	0.9	1.6
H63D/H63D	12	3	0.8 (0.3–2.6)	0.6	0.9 (0.3–2.8)	0.8	3.1
C282Y/wild type	51	16	0.8 (0.5–1.3)	0.4	0.8 (0.5–1.4)	0.4	1.9
C282Y/H63D	5	3	1.1 (0.3–3.5)	0.9	1.0 (0.3–3.3)	0.9	4.3
C282Y/C282Y	6	3	4.0 (1.2–13)	0.01	4.0 (1.2–13)	0.02	4.0
<b>Men</b>							
Wild type/wild type	205	63	1.0		1.0		
C282Y/C282Y	2	3	9.3 (2.7–32)	0.0004	9.4 (2.7–33)	0.0005	5.9
<b>Women</b>							
Wild type/wild type	152	54	1.0	—	1.0	—	—
C282Y/C282Y	4	0	—	—	—	—	6.8
<b>Transferrin saturation &lt;50% among men</b>							
Wild type/wild type	278	89	1.0		1.0		
<b>Transferrin saturation ≥50% among men</b>							
C282Y/C282Y	2	3	10 (2.8–33)	0.0003	9.9 (2.9–35)	0.0003	5.9
<b>Cause-specific mortality</b>							
<b>Neoplasms</b>							
Wild type/wild type	357	29	1.0		1.0		
C282Y/C282Y	6	3	13 (3.6–49)	0.0001	14 (3.7–51)	0.00008	6.7
<b>Liver cancer</b>							
Wild type/wild type	357	0	1.0		—	—	—
C282Y/C282Y	6	2	Fisher's exact	<0.0001	—	—	7.2
<b>Endocrinological disease</b>							
Wild type/wild type	357	34	1.0		1.0		
C282Y/C282Y	6	0	—	—	—	—	8.0
<b>Cardiovascular disease</b>							
Wild type/wild type	357	28	1.0		1.0		
C282Y/C282Y	6	0	—	—	—	—	8.7

Data shown as hazard ratio (95% CI) unless otherwise indicated. \*Multifactorially adjusted: adjusted for age, sex, BMI, and diabetes complications.

ratio of 6.7 for neoplasms, 7.2 for liver cancer, 8.0 for endocrinological causes, and 8.7 for circulatory causes.

Eliminating individuals with C282Y/C282Y or C282Y/H63D genotypes in the analyses of transferrin saturation did not change the results (Supplementary Fig. 1), except for reducing the number of deaths due to liver cancer to zero in individuals with transferrin saturation  $\geq 50\%$ . Multifactorially adjusted analyses of transferrin saturation and genotypes gave similar results.

**Cohort 2: any diabetes, any onset.** Total mortality (overall or stratified by sex, onset, or type of diabetes) (Fig. 1A) or cause-specific mortality (Fig. 1B) were not increased in patients with transferrin saturation  $\geq 50$  vs.  $< 50\%$  (Fig. 1A). For total mortality for patients with

transferrin saturation  $\geq 50\%$ , we had 80% power to detect a hazard ratio of 1.3 overall, 1.4 in men, and 1.7 in women. We had 80% power to detect a hazard ratio of 5.2 for neoplasms, 7.0 for liver cancer, 3.0 for endocrinological causes, and 4.3 for circulatory causes.

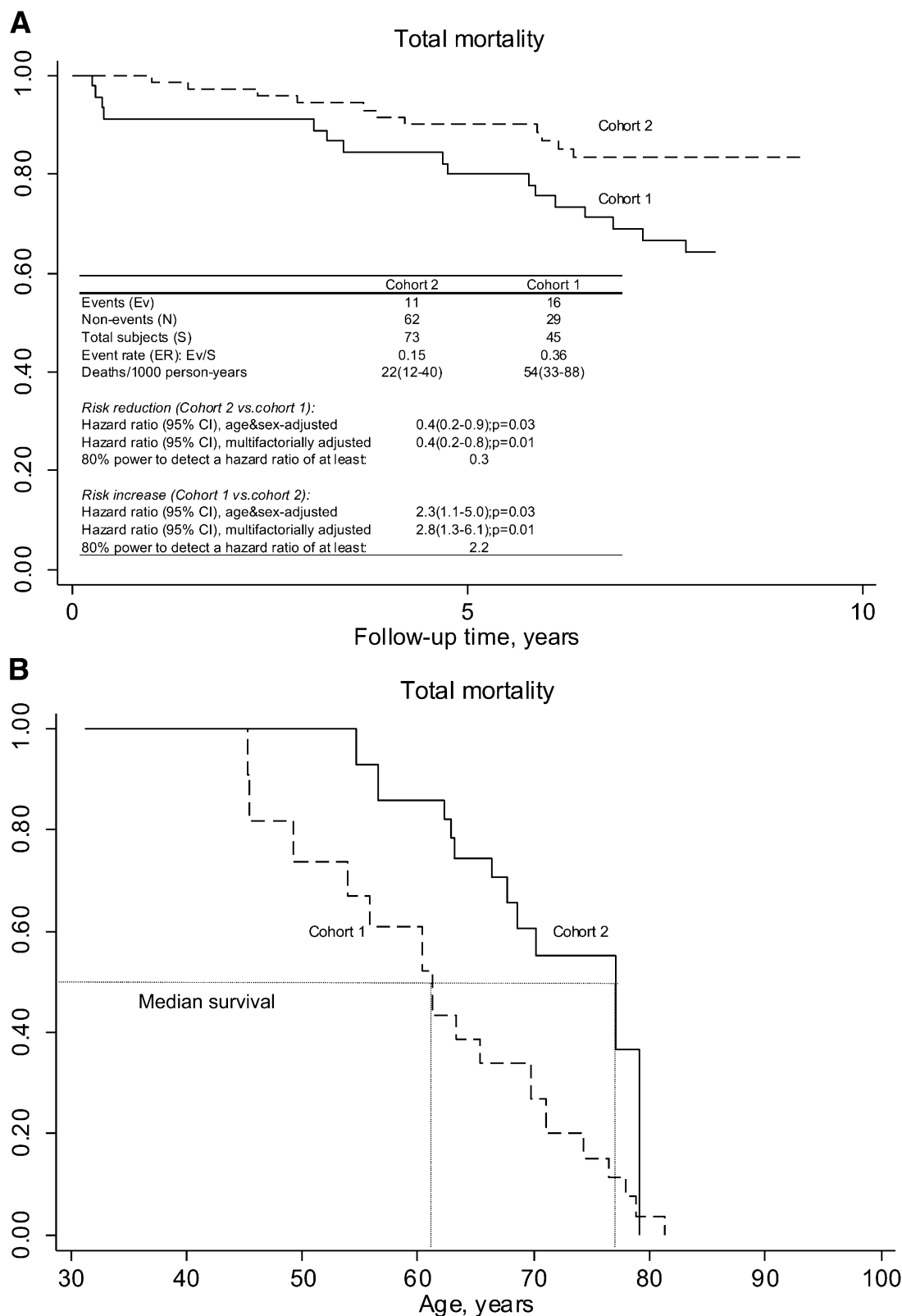
Eliminating individuals with the C282Y/C282Y or C282Y/H63D genotypes in the analyses of transferrin saturation did not change the results (Supplementary Fig. 1). Multifactorially adjusted analyses of transferrin saturation and genotypes gave similar results.

#### Patients with late-onset type 1 diabetes and transferrin saturation $\geq 50\%$

With a median follow-up of 8 years, 11 deaths (15%) occurred in cohort 2

compared with 16 (36%) in cohort 1, corresponding to 22 and 54 deaths per 1,000 person-years of follow-up, respectively (age- and sex-adjusted hazard ratio for cohort 2 vs. cohort 1, 0.4 (95% CI 0.2–0.9;  $P = 0.03$ ) (Fig. 2A). Median survival time was 77 years (cohort 2) and 61 years (cohort 1) (Fig. 2B). Multifactorially adjusted analyses gave similar results. Eliminating individuals with the C282Y/C282Y or C282Y/H63D genotypes from the analyses gave similar results (Supplementary Fig. 2). There were too few events to calculate cause-specific hazard ratios.

**CONCLUSIONS**—In this study we have shown that increased transferrin saturation and the C282Y/C282Y genotype predict total mortality in a population



**Figure 2**—Total mortality according to follow-up time (A) and age (B) in late-onset type 1 diabetes and elevated transferrin saturation ( $\geq 50\%$ ) in cohort 2 vs. cohort 1.

of patients with late-onset type 1 diabetes after diagnosis and that increased transferrin saturation after diagnosis is a risk

factor independent of *HFE* genotype. We also have shown that early measurement (at referral) of transferrin saturation in

patients with diabetes may improve the life expectancy of these patients. These are novel findings.

There is evidence for increased mortality in patients with clinically overt HFE (2,3,20–22) and among untreated patients, usually due to liver cirrhosis and diabetes mellitus (2,3). Previous general population studies have shown increased risk of premature death associated with increased transferrin saturation (1), and a recent study showed that diabetic patients with serum ferritin levels >700 ng/mL and who are receiving maintenance hemodialysis had increased 1-year mortality (5). This is in accordance with our results. Thus it may seem that iron overload is associated with increased total mortality in patients with late-onset type 1 diabetes, whereas death due to liver cancer may be specific for HFE.

A study of patients with HFE concluded that awareness of the diagnosis in general will translate into a greater life expectancy (11). Another study showed that health checkups and family screening allow detection of HFE at an earlier age and that survival was comparable to the normal population (23). Our study shows that unawareness of high iron stores (as in cohort 1) in patients with late-onset type 1 diabetes poses an unnecessary high risk of premature death in general and that these patients will benefit from early measurement of transferrin saturation and appropriate intervention, gaining 16 years of life. However, whether the test should be transferrin saturation, ferritin, or both needs to be resolved. We have not examined whether early measurement of transferrin saturation in patients with diabetes other than late-onset type 1 is beneficial.

Limitations of this study included lack of ferritin measurements, smoking history, and genotyping of patients with transferrin saturation <50% in cohort 2. Validity of antidiabetic treatment in cohort 2 was low (27% missing data compared with 6% in cohort 1). The study lacked information on well-known risk factors for liver disease (e.g., history of alcohol use and viral hepatitis), weakening the conclusions drawn on the basis of transferrin saturation as a risk factor for liver cancer. Missing BMI values were not equally distributed between the cohorts; however, adjusted analyses including BMI showed results similar to those of unadjusted analyses.

Oxidative stress as a result of the Fenton reaction is thought to be the biological mechanism between iron overload and associated complications (24) and to play a central role in the development of diabetes complications (25). Accordingly, the intracellular marker for

oxidative stress, 8-OxoGuo, a widely used marker of RNA oxidation, predicts outcome in patients with type 2 diabetes (26); this marker is also present in patients with HFE (27).

A series of considerations—some of which are known as “Bradford Hill Criteria”—are needed to assess causality in epidemiological association studies (28). Overall, our study fulfils these criteria. The association of transferrin saturation or C282Y/C282Y genotype and total mortality, neoplasms, and liver cancer in cohort 1 is supported by a high hazard ratio and low *P* value, as is the difference in total mortality between cohort 1 and cohort 2. Our findings on total mortality, cancer, and early measurement of iron overload are consistent with previous studies (1,23,29,30). The consistency is also underscored by women having a lower risk than men because of regular loss of menstrual blood (and iron). The effect (death) occurred after the cause (transferrin saturation, genotype). A sort of biological gradient exists: men generally have a greater exposure to iron (because of longer exposure time) than women (exposure begins at menopause). A plausible biological mechanism (described above) between iron overload and total mortality exists (2,5,19,26,27). Furthermore, given the many statistical tests performed, correction for multiple hypothesis testing could be considered. If the significance level is restricted to  $P < 0.01$ , most of the analyses that initially were significant in our study would still be significant. The reason for correction for multiple testing is to reduce false-positives; however, this is done at the expense of an increasing number of false-negatives. It has been argued that if the rationale for the hypothesis and the biological mechanism behind it are well defined (and not just a “fishing expedition”), then there is no reason for multiple comparison correction (31) since in such a situation there is no theoretical basis for a “universal null hypothesis,” and chance would not be the first explanation of the observed results (31). The power to detect total mortality in cohorts 1 and 2 was within the observed reference ranges; power was highest for results in cohort 1 overall, in men, and for neoplasms; however, there was not sufficient power to exclude a modestly increased risk of total mortality among patients with late-onset type 1 diabetes in cohort 2. In general, subgroup analyses of cause-specific mortality were not sufficiently powered.

In summary, we found that increased transferrin saturation and genotype C282Y/C282Y predict total mortality in patients with late-onset type 1 diabetes, and increased transferrin saturation after diagnosis is a risk factor independent of HFE genotype. Early measurement of transferrin saturation in these patients, leading to appropriate intervention, improves life expectancy.

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C.E., H.U.A., H.B., and T.M.-P. designed the study, interpreted the data, and created the figures and tables. C.E. and T.M.-P. analyzed the data and wrote the manuscript. H.U.A., A.T.-H., M.F., H.B., and B.G.N. collected data. All authors edited the manuscript. C.E. 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.

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