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Serum ferritin is a biomarker for liver mortality in the Hemochromatosis and Iron Overload Screening Study

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ABSTRACT

Background. We identified no reports of long-term follow-up of participants in hemochromatosis screening programs. We evaluated causes of death and survival in non-C282Y homozygous Canadian participants in the primary care-based hemochromatosis and iron overload screening (HEIRS) study. **Material and methods.** Initial screening (IS) included transferrin saturation (TS), serum ferritin (SF), *HFE* genotyping (C282Y, H63D), and health questionnaire responses. By definition, participants without C282Y or H63D had *HFE* wt/wt. We linked 20,306 Canadian participants to the Ontario Death Registry for dates and causes of death 9 y after IS. We computed Cox proportional hazards to identify factors with increased death risks and Kaplan-Meier curves to estimate survival of non-C282Y homozygous participants with SF ≤ 1,000 µg/L and > 1,000 µg/dL. **Results.** There were 19,052 evaluable participants (IS mean age 49 y; 60% women; 93 C282Y homozygotes). There were 988 deaths. Significantly increased hazard ratios for all-cause mortality were positively associated with TS, SF, men, and C282Y homozygosity, and liver disease, diabetes, and heart failure reports. Non-C282Y homozygous participants with SF > 1,000 µg/L had lower survival than those with SF ≤ 1,000 µg/L ($p < 0.0001$). **Conclusions.** Nine years after initial screening, non-C282Y homozygous participants and SF > 1,000 µg/L was associated with decreased survival.

Key words. Survival analysis. Population screening. Hyperferritinemia.

INTRODUCTION

Serum ferritin (SF) measurements are indicators of iron deficiency or iron overload. Because SF is also an acute-phase reactant, SF levels are also elevated in persons who have acute and chronic inflammation, tissue injury, or malignancy. Severe SF elevation occurs in some persons with marked iron overload due to *HFE* C282Y homozygosity, deleterious mutations in non-*HFE* iron-related genes (e.g., *HAMP, HJV, TFR2*, and *SLC40A1)*, ineffective erythropoiesis (e.g., X-linked sideroblastic anemia, congenital dyserythropoietic anemias, beta-thalassemia major), and chronic erythrocyte transfusion (e.g., beta-thalassemia major, sickle cell disease).[1] Other common causes of severe hyperferritinemia include chronic ethanol ingestion, obesity, steatohepatitis, chronic viral hepatitis, anemia of chronic disease, renal failure, recent surgical operations, and uncontrolled malignancy (especially hematologic neoplasms).[1] Uncommon causes of severe hyperferritinemia include other chronic liver disorders, rheumatoid arthritis and other autoimmune disorders, and hereditary hyperferritinemia-cataract syndrome.[1] In 25 index patients with "benign" familial hyperferritinemia unassociated with iron overload or inflammation, 12 index patients (48%) were heterozygous for the p.Thr30Ile mutation in the NH2 terminus of L ferritin subunit *(FTL)* on chromosome 19q13.33.[2] All 20 relatives of the *FTL* p.Thr30Ile-positive index patients who had hyperferritinemia were also positive for this allele, whereas 10 relatives without hyperferritinemia did not have p.Thr30Ile.[2]

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We hypothesized that survival of persons with markedly elevated SF levels without *HFE* mutations is lower than that of persons without markedly elevated SF levels because severe hyperferritinemia is typically associated with serious medical conditions. The hemochromatosis and iron overload screening (HEIRS) study performed screening on 101,168 adults participants from primary care and laboratory sites in North America and Canada using SF and transferrin saturation (TS) measurements and DNA analyses to identify the C282Y and H63D polymorphisms of the *HFE* gene.[3] In the present substudy, we evaluated survival, causes of death, and their relations to *HFE* genotype and SF levels of 19,052 Canadian HEIRS Study participants without C282Y homozygosity over a 9-year interval after HEIRS Study initial screening. We discuss the present results in the context of other evaluations of causes of death and survival analyses of persons with hyperferritinemia without *HFE* mutations.

MATERIAL AND METHODS

Study participants

The multi-center HEIRS Study was sponsored by the National Institutes of Health to determine the prevalence of primary iron overload in adult primary care patients of various races/ethnicities in the US and Canada. Screening of 101,168 participants was performed by measuring SF and TS levels and testing for *HFE* C282Y and H63D mutations as previously described.[4]

Screening was performed during a two-year interval (February 2001 to March 2003) at five Field Centers (Washington, DC, Birmingham, AL, Irvine, CA, Portland, OR-Honolulu, HI, and London, Ontario, Canada). The target population was primary care patients identified through primary care clinics and medical laboratories where blood was drawn for diagnostic testing. Persons accompanying patients were also potential participants for screening. No advertising was used to recruit participants. Eligibility criteria included age ≥ 25 years and the ability to provide informed consent. Participants were asked how they learned about the study and whether they had been previously diagnosed to have iron overload or hemochromatosis. All participants were asked to respond to questions about whether or not they had a history of arthritis, liver disease, diabetes, congestive heart failure, impotence and infertility as part of initial screening, before results of *HFE* mutation analyses were available.

The present investigation is a secondary analysis of the 19,052 participants without *HFE* C282Y homozygosity recruited from the HEIRS Study Field Center in London, Ontario. This ancillary study was approved by the HEIRS Study Steering Committee and by the local institutional review board at the University of London.

Initial screening laboratory methods

Blood specimens were obtained without regard to fasting. Initial screening SF levels were measured by a turbidometric immunoassay (Hitachi 9/11 Analyzer, Roche Applied Science, Madison, WI, USA). Serum iron concentration and unsaturated iron-binding capacity were measured using a spectrophotometric method (Hitachi 9/11 Analyzer, Roche Applied Science, Madison, WI, USA). TS was calculated using corresponding serum iron and total iron-binding measures. By definition, elevated TS and SF values were those that exceeded Study thresholds (TS > 50% and SF > 300 μg/L for men; TS > 45% and SF > 200 μg/L for women), regardless of *HFE* genotype.

HFE C282Y and H63D alleles were detected in DNA in blood spots using a modification of the Invader assay (Third Wave Technologies, Madison, WI, USA). This method increases the allele-specific fluorescent signal by including 12 cycles of locus-specific polymerase chain reaction before the cleavase reaction. Participants in whom C282Y and H63D mutations were not detected were defined as having *HFE* genotype wt/wt (wild-type).

Treatment of participants with iron overload

Treatment of iron overload was beyond the scope of the HEIRS Study. Participants in the study discovered to have evidence of iron overload, regardless of *HFE* genotype, during screening typically presented to physicians of their choice and underwent weekly phlebotomy of 500 mL until SF was approximately 20 μg/L.[5] This established that they had achieved iron depletion. Many Canadian participants who had elevated SF were evaluated and treated at the Canadian HEIRS Study Field Center site.

Post-screening follow-up of Canadian HEIRS Study participants

The HEIRS Study collected the name, address, and date of birth of each participant. Using probabilistic

linkage, the Institute for Clinical Evaluative Sciences (ICES) linked the HEIRS Study data to the Ontario Death Registry database. Privacy legislation did not permit the present authors to ascertain the exact linkage between personal medical data and outcomes. Survival data were analyzed an average of 9 years after each participant was enrolled in the HEIRS Study. Causes of death were determined from analysis of the death certificates in the Ontario Death Registry.

Mortality and survival analyses

Cox proportional hazards models were used to identify the initial screening attributes associated with mortality. Survival data were evaluated by Kaplan-Meier analyses based on *HFE* genotype and by initial screening SF ($> 1{,}000$ μg/L or $\leq 1{,}000$ μg/L). Differences in survival were analyzed by the log-rank test. We defined values of $p < 0.05$ to be significant. Ferritin was also studied as a continuous variable in the Cox proportional hazards model.

RESULTS

General characteristics of Canadian participants

ICES evaluated observations on 19,159 Canadian HEIRS Study participants during a mean interval of 9 years after initial screening. Mean age at baseline was 49 years; 60% of participants were women. There were 12,902 *HFE* wild-type participants, 312 compound heterozygotes (C282Y/H63D), 370 H63D homozygotes, and 5,375 simple heterozygotes (C282Y or H63D). There were 988 deaths during the 9-year follow-up interval. There were 107 participants lost to follow-up (0.6%).

There were 34 wild-type participants with SF $>$ 1,000 μg/L. Six participants without *HFE* C282Y homozygosity underwent phlebotomy therapy. None had significant iron overload (> 5 g of iron removed by phlebotomy).

All-cause mortality

A Cox proportional hazards model for all-cause mortality demonstrated that initial screening SF and TS, male gender, and self-reported liver disease, diabetes, and congestive heart failure were associated with significantly increased hazard ratios (Table 1).

Survival

The survival of participants with *HFE* wt/wt (n = 12,902) and those with *HFE* genotypes C282Y/H63D (n = 312), H63D/H63D (n = 370), H63D/wt and C282Y/wt combined (n = 5,375) did not differ significantly.

A comparison of survival of participants with SF $>$ 1,000 μg/L (n = 34) and participants with SF $\leq$ 1,000 μg/L (after excluding C282Y homozygotes, n = 18,925) revealed that survival of participants with SF $>$ 1,000 μg/L was lower ($p < 0.0001$, log rank test) (Figure 1). The genotypes of the 34 participants with ferritin $>$ 1,000 μg/L were (0 compound homozygotes, 2 H63D/H63D, 15 H63/wt and C282Y/wt, and, 17 wt/wt). Other causes of elevated SF in non-C282Y homozygotes included alcohol abuse (3), arthritis (1), hepatitis C (2), and hepatitis B (1). Three participants underwent liver biopsy and iron overload was absent in each of the three.[6]

Table 1. Parameters of a Cox model for all-cause mortality.

Parameter	Hazard ratio	Lower 95% CI	Upper 95% CI	Value of p
Serum ferritin, µg/L	1.001	1.0	1.002	< 0.0001
Transferrin saturation, percent	0.984	0.978	0.99	< 0.0001
Age, y	1.093	1.087	1.099	< 0.0001
Male *vs.* female	1.382	1.207	1.582	< 0.0001
Arthritis reports	1.002	0.876	1.147	0.9743
Diabetes reports	1.294	1.099	1.523	0.002
Liver disease reports	1.925	1.427	2.597	< 0.0001
Congestive heart failure reports	1.418	1.188	1.692	< 0.0001
Impotence reports	1.149	0.909	1.45	0.2449

CI: confidence interval. Participants provided dichotomous (yes/no) responses to initial screening questions about arthritis, diabetes, liver disease, congestive heart failure, and impotence.

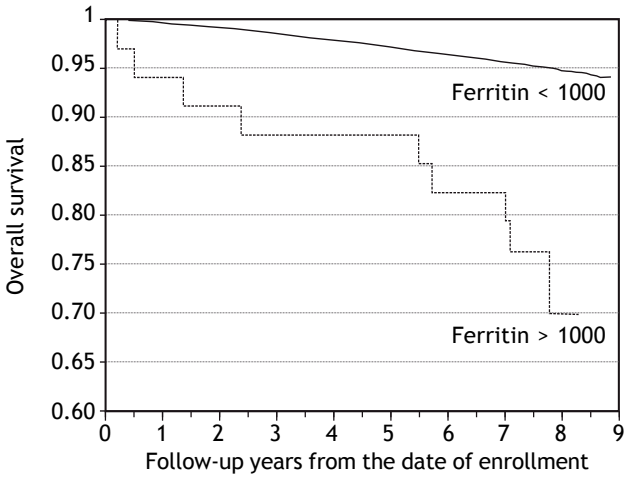

Figure 1. *Kaplan-Meier survival curves of Canadian HEIRS Study participants grouped by serum ferritin > 1,000 μg/L (n = 34) and ≤ 1,000 μg/L (n = 18,925) (p < 0.0001; log-rank test). C282Y homozygotes were excluded in this analysis.*

DISCUSSION

The ICES database of the province of Ontario provided a unique opportunity to study the natural history of HEIRS Study participants with elevated SF at initial screening. Canadian participants in the HEIRS study were followed for long-term mortality in the Ontario Death Registry.

Survival of Canadian HEIRS Study participants with *HFE* genotypes with C282Y or H63D mutations other than *HFE* C282Y homozygosity did not differ significantly from that of *HFE* wt/wt participants. A previous study with a smaller sample size but a longer follow-up interval detected no differences in survival of untreated participants across all six *HFE* genotypes.[7]

Survival was decreased in the present non-C282Y homozygous participants with SF > 1,000 μg/L. SF levels are increased in many patients with serious medical conditions including diverse non-iron liver disorders and the metabolic syndrome, chronic inflammation/infection, or malignancy. The privacy restrictions of the ICES database do not permit revelation of the linkage of individual outcomes with SF. Because participants were not randomized to phlebotomy or no treatment, it is not possible to discern the beneficial or adverse effects of phlebotomy in the present study. In other studies, iron depletion induced decreased liver fibrosis, although reversal of cirrhosis after iron depletion is rare.[8,9] The effects of therapeutic phlebotomy on fatty liver disease or viral hepatitis have been inconsistent and therefore phlebotomy is not widely advocated for management of these conditions. It has been proposed that phlebotomy would reduce mortality from vascular diseases[10] and decrease insulin resistance in persons with non-alcoholic fatty liver disease and hyperferritinemia[11,12] and metabolic syndrome[13] and type 2 diabetes mellitus, with or without *HFE* mutations.[14,15]

In the present study, SF > 1,000 μg/L was associated with an increased risk of death. Thus, SF, like C-reactive protein (CRP),[16] may be a biomarker of increased mortality risk. Both SF and CRP probably represent inflammation and are not direct causes of death. SF is a predictor of liver mortality in patients with decompensated cirrhosis [17] and in patients awaiting liver transplantation,[18,19] Explant siderosis is an adverse prognostic sign in such patients.[19]

In the HEIRS Study, the frequency of the synonymous *FTL* allele p.L55L was greater in whites with high TS/SF than controls.[20] This suggests that p.L55L is linked to another locus on chromosome 19q13.33 that causes higher TS/SF levels. *FTL* p.Thr30Ile[2] was discovered after the HEIRS Study. SF levels of 37 subjects with the *FTL* p.Thr30Ile mutation ranged from 400 μg/L to 6,000 μg/L. There were significant fluctuations of the SF levels, either with time for the same individual or between different individuals within the same family.[2] One individual underwent six phlebotomies of 400 mL each over a nine-month period and SF decreased from 3,900 μg/L to 676 μg/L. No characteristic clinical symptoms could be identified in the 37 individuals with p.Thr30Ile, although four complained of joint pains, three complained of asthenia, and one had bilateral cataracts. The brother of one p.Thr30Ile-positive proband was also a *HFE* C282Y/H63D compound heterozygote and had hepatic iron overload.[2] Taken together, these observations indicate that some persons with SF > 1,000 μg/L have *FTL* p.Thr30Ile or other alleles on 19q13.33 that may be associated with morbidity, although we identified no survival observations for such subjects.

It is plausible that managing the underlying causes of SF > 1,000 μg/L, if possible, could improve long-term patient outcomes including survival. Population-based studies demonstrate that SF values increase slowly with age in adults.[21] It has been postulated that increasing SF levels are related in part to ethanol consumption, dietary iron content, increasing trends in prevalence of obesity and metabolic syndrome, and age-related changes in iron homeostasis. Iron overload disorders, "benign"

hyperferritinemia,[1] and the higher SF levels typical of presumably healthy African-Americans, Asians, and Pacific Islanders[22,23] also contribute to age-related increments in SF in general populations. Population-based studies have also demonstrated increased mortality with increasing SF[18,24] and TS,[25] but not all studies have found these associations.[26] The present results suggest that clinicians should consider investigating the cause of severe hyperferritinemia in all patients to determine whether effective clinical intervention is possible.

ABBREVIATIONS

- **HEIRS:** hemochromatosis and iron overload screening.

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DISCLOSURES

The authors have no disclosures relative to this manuscript.

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