Decreased Cancer Risk After Iron Reduction in Patients With Peripheral Arterial Disease: Results From a Randomized Trial

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Background Excess iron has been implicated in cancer risk through increased iron-catalyzed free radical–mediated oxidative stress.

Methods A multicenter randomized, controlled, single-blinded clinical trial (VA Cooperative Study #410) tested the hypothesis that reducing iron stores by phlebotomy would influence vascular outcomes in patients with peripheral arterial disease. Patients without a visceral malignancy in the last 5 years (n = 1277) were randomly assigned to control (n = 641) or iron reduction (n = 636). Occurrence of new visceral malignancy and cause-specific mortality data were collected prospectively. Cancer and mortality outcomes in the two arms were compared using intent-to-treat analysis with a Cox proportional hazards regression model. Statistical tests were two-sided.

Results Patients were followed up for an average of 4.5 years. Ferritin levels were similar in both groups at baseline but were lower in iron reduction patients than control patients across all 6-month visits (mean = 79.7 ng/mL, 95% confidence interval [CI] = 73.8 to 85.5 ng/mL vs 122.5 ng/mL, 95% CI = 115.5 to 129.5 ng/mL; $P < .001$). Risk of new visceral malignancy was lower in the iron reduction group than in the control group (38 vs 60, hazard ratio [HR] = 0.65, 95% CI = 0.43 to 0.97; $P = .036$), and, among patients with new cancers, those in the iron reduction group had lower cancer-specific and all-cause mortality (HR = 0.39, 95% CI = 0.21 to 0.72; $P = .003$; and HR = 0.49, 95% CI = 0.29 to 0.83; $P = .009$, respectively) than those in the control group. Mean ferritin levels across all 6-monthly visits were similar in patients in the iron reduction and control groups who developed cancer but were lower among all patients who did not develop cancer than among those who did (76.4 ng/mL, 95% CI = 71.4 to 81.4 ng/mL, vs 127.1 ng/mL, 95% CI = 71.2 to 183.0 ng/mL; $P = .017$).

Conclusions Iron reduction was associated with lower cancer risk and mortality. Further studies are needed to define the role of body iron in cancer risk.

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Body iron stores accumulate imperceptibly with aging because intake exceeds loss and no biologic mechanism exists for excretion of iron in excess of physiological requirements (1). Iron accumulation has been implicated in the risk of several chronic diseases, including cardiovascular disease and cancer, through increased iron-catalyzed free radical–mediated oxidative stress (1–9). Clinical cohort studies have found that measures of body iron stores or dietary iron intake may be associated with increased risk of cancer and cancer mortality (9–14). Colorectal cancer has received particular attention in this regard (6,11). Cancer risk rises after menopause in women (5,12) in association with rising iron stores (1). A cause-and-effect relationship between levels of iron stores and cancer risk is suggested by studies showing that blood donation (which reduces body iron) is associated with lower cancer risk (13,14) and that blood transfusion (delivery of an iron load) adversely affects cancer outcome (15,16). Kato et al. (17) reported a cohort of patients with hepatitis C who were treated with iron reduction and followed for 12 years. These patients had a statistically significantly lower risk of developing hepatocellular carcinoma compared with a demographi-

cally similar cohort not treated with iron reduction. Although studies in animal models suggest that limitation or removal of iron may

prevent cancer occurrence and growth (18–20), no such data exist for malignancy in humans.

A randomized trial of calibrated phlebotomy was conducted in patients with advanced peripheral arterial disease (21–24). Although this clinical trial was designed as a cardiovascular disease study, it provided a setting for controlled, prospective collection of data on risk of new malignancy. The occurrence of new visceral malignancy according to histological type and organ of origin and death according to cause was determined in this cohort of patients, who had no clinical evidence of visceral malignancy within the preceding 5 years.

Subjects and Methods

Patients

The methodology for this randomized, controlled, single-blinded trial has been reported (21–24). Patients with stable peripheral arterial disease as defined previously (22,23) were recruited from the inpatient and outpatient populations of participating Veterans Affairs (VA) hospitals. Entry criteria minimized accrual of patients with acute-phase ferritin elevation, and patients with visceral malignancy within the preceding 5 years were excluded (22). Patients were required to have a hematocrit of greater than 35% (in the absence of iron deficiency) and serum ferritin levels of less than 400 ng/mL, but there was no predefined floor ferritin-level requirement. The protocol was approved by the Institutional Review Boards (IRBs) at each participating institution and by a national IRB. All patients provided written informed consent. Patient entry began on May 1, 1999, and ended on October 31, 2002; follow-up ended on April 30, 2005. The 6-year study duration included 3.5 years of accrual and a 2.5-year minimum follow-up (22,23). ClinicalTrial.gov Identifier: NCT00032357.

Randomization, Intervention, and Outcome Measures

Patients were assigned to control or iron reduction groups by computer randomization that was stratified according to participating hospital, age (≤60 and >60 years), baseline ferritin level (based on the rolling mean among prior entrants), diagnosis of diabetes mellitus requiring treatment (yes or no), smoking status (ever used tobacco products regularly [yes or no]), and the ratio of high-density lipoprotein cholesterol to low-density lipoprotein cholesterol (also based on the rolling mean among prior entrants). A pilot study showed the accuracy of the formula used for calculating the amount of blood to be removed to achieve the desired ferritin-level reduction without causing iron deficiency (21). Phlebotomy was scheduled at 6-month intervals to maintain trough ferritin levels of about 25 ng/mL and peak ferritin levels measured before the next phlebotomy episode of about 60 ng/mL, a range presumed to be optimal (1). Compliance with phlebotomy was assessed as the cumulative percent of the amount of blood calculated for removal that was actually removed across all phlebotomy episodes and by analysis of the effect of phlebotomy on the separation of ferritin levels over time between control and iron reduction groups (23). Follow-up data on the occurrence of new visceral malignancy by histological type and organ of origin and death according to cause were recorded at 6-month intervals by an observer who was blinded to intervention. An external data and safety monitoring committee reviewed all

CONTEXT AND CAVEATS

Prior knowledge
Having excess iron in the blood is associated with an increased risk for some cancers.

Study design
Analysis of cancer risk among patients with peripheral arterial disease who participated in a multicenter randomized controlled single-blinded trial of iron reduction through blood collection for prevention of atherosclerotic complications of arterial disease.

Contribution
Patients in the iron reduction group had a lower risk of subsequent cancer, and those who developed cancer had lower cancer-specific deaths and deaths from any cause than patients in the control group who developed cancer. At baseline, iron levels were similar in the two study arms. However, during follow-up, patients in the iron reduction group had lower iron levels than patients in the control group. In both groups, iron levels of all patients who developed cancer were higher during follow-up than those of patients who did not develop cancer.

Implications
Iron reduction is associated with reduced risk of cancer and death in this population.

Limitations
The trial was not originally designed to compare risks of cancer in the two groups. The lower ranges of iron levels reported here among patients who did not develop cancer cannot be used as a guide to reduce cancer incidence in the general population.

From the Editors

data during the study. An external endpoints adjudicating committee that was blinded to intervention adjudicated primary (all-cause mortality) and secondary (death plus nonfatal myocardial infarction and stroke) study endpoints, including death due to cancer (23).

Statistical Analysis
Statistical methods used in this study have been reported (21–31). The main study was designed to have sufficient (85%) power to detect a 30% decrease in the effect on the primary outcome of all-cause mortality. For the secondary outcomes of new cancer incidence and cancer-specific mortality, confidence intervals (CIs) were used to measure the precision of achieved estimates of treatment effects. Analyses were based on intent to treat. All patients were either followed up to the end of the study or tracked through the Department of Veterans Affairs National Patient Care Database, which is maintained in Austin, TX. Baseline patient characteristics and certain other outcomes were compared using the χ^2 test, Wilcoxon rank sum test, or analysis of variance. Because of possible competing risks, the nonparametric maximum likelihood estimators of the cumulative incidence curves (29) in addition to event curves according to the method of Kaplan and Meier (30) were used to characterize the timing of new cancer diagnoses and cancer deaths during follow-up. The log-rank statistics of treatment vs control comparisons in these curves were calculated without adjustment or stratification for potential confounders. The Cox

Table 1. Organ site of histologically confirmed new cancer diagnoses during follow-up in control (n = 641) and iron reduction (n = 636) groups

Organ site	Total	Control	Iron reduction
Lung	38	23	15
Colorectal	13	9	4
Prostate	12	8	4
Upper aerodigestive	7	6	1
Bladder	8	1	7
Pancreas	1	0	1
Other*	19	13	6
Total	98	60	38

* The 19 cancers at other sites include melanoma (n=4); lymphoma, soft tissue sarcoma, adenocarcinoma of unknown primary, malignant carcinoid, and hepatocellular (n=2 each); and multiple myeloma, renal cell, metastatic pancreatic endocrine carcinoma, gastric, and cholangiocarcinoma (n=1 each).

proportional hazards regression model (31) according to the method of Fine and Gray (32) was used to compute hazard ratios (HRs) and 95% CIs with adjustment for covariates. Assumptions of proportionality of hazard were verified by the Schoenfeld residuals plot and the plot of $\log(-\log(1 - CI))$ vs $\log(\text{time})$ (33). To describe the nonlinear effect of compliance with phlebotomy on cancer outcomes, the percent compliance was fitted in the linear tail-restricted cubic spline function with three knots in the Cox proportional hazards model, and the log relative hazards were plotted using Frank Harrell's Design and Hmisc packages in R (34,35). All statistical tests were two-sided. *P* values less than .05 were considered to be statistically significant.

Results

The CONSORT trial flow diagram for this study and a table showing comparability between treatment groups at entry to the study
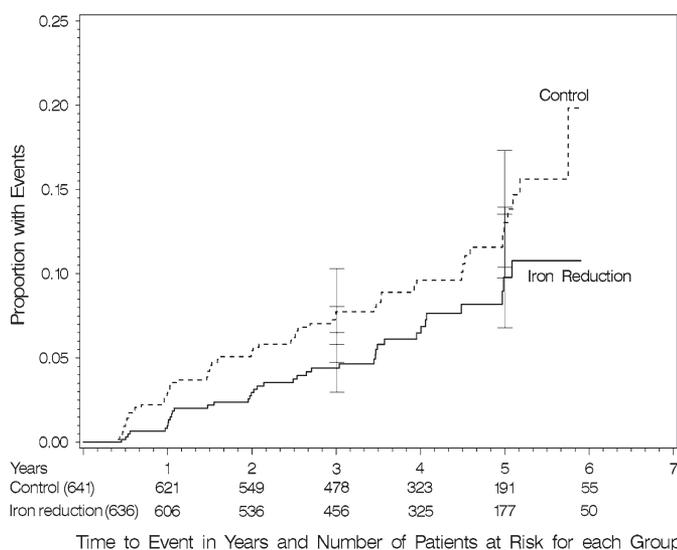

Figure 1. Kaplan–Meier event curves for new cancer diagnoses for the entire study cohort by intervention group. Hazard ratio = 0.65, 95% confidence interval = 0.43 to 0.97; *P* = .036 (two-sided log-rank test); Cox proportional hazards χ^2 = 4.4088. The **vertical axis** represents the Kaplan–Meier event estimates. Numbers below the **horizontal axis** represent the numbers of patients under observation at each follow-up time point. **Error bars** represent 95% confidence intervals.

have been published (23). Patients who were randomly assigned to iron reduction (n = 636) and control (n = 641) groups were demographically comparable at entry. The population was 98.8% male, with a mean age of 67 years. Mean ferritin levels were the same in control and iron reduction groups at baseline (mean = 122.4 ng/mL, 95% CI = 116.0 to 128.8 ng/mL, and 121.8 ng/mL, 95% CI = 115.3 to 128.2 ng/mL, respectively; *P* = .86), resembling levels in the general population of comparable age (1) and in the pilot study (21). The observed status of all 1277 patients entered was complete at the study conclusion based on clinical records at participating hospitals; the observed follow-up was approximately 4000 patient-years and the average observed follow-up 3.1 years. Because patient status could be tracked through the Austin database that captured outcome events that may have occurred elsewhere (usually at different VA hospitals), the average total follow-up was 1649 days or an average of 4.5 years per patient. Follow-up intervals for control and iron reduction patients were similar. A total of 3141 phlebotomy episodes were conducted among the 636 patients who were randomly assigned to iron reduction.

Details on overall compliance with phlebotomy in patients who were randomly assigned to iron reduction have been reported (23). Eighty-eight percent of patients who were assigned to the iron reduction group had the required amount of blood removed within the first year after randomization. Sixty-five percent of patients had 50% or more of the calculated amount of blood actually removed (ie, >50% compliance with intervention). The average patient had 72% of the calculated amount of blood removed over the course of the study. Compliance with phlebotomy was unaffected by the severity of vascular disease and comorbid conditions at entry (23).

A new diagnosis of histologically confirmed visceral malignancy was made in 98 patients (7.7% of the total study cohort) during follow-up. There is little basis for determining whether this incidence resembles that expected in the general population. Two patients had two new primary cancers diagnosed after entry: one patient in the control group had lung and colon cancer, and one patient in the iron reduction group had lung and upper aerodigestive cancer. These two patients were analyzed for the first occurrence of malignancy only (lung cancer in both cases). As reported previously (23), participants in the control and iron reduction groups had comparable demographic variables (age; race; sex; tobacco and alcohol use; diagnosis of diabetes mellitus and hypertension; body mass index; levels of cholesterol, ferritin, fibrinogen, and homocysteine; and comorbidities) at baseline. Similarly, no differences were observed in these demographic variables at entry to the study among patients who subsequently developed cancer during follow-up vs those who did not.

Of the 98 new malignancies, 60 occurred in control patients and 38 in iron reduction patients (Table 1) (χ^2_6 = 5.1644; *P* = .023). Risk of lung, colorectal, upper aerodigestive, prostate, and other cancers (Table 1) was lower with iron reduction. In a time-to-event analysis, the HR for a new cancer diagnosis was 0.65 (95% CI = 0.43 to 0.97; *P* = .036; Figure 1). Displaying the data as cumulative incidence curves also showed reduced cancer risk with iron reduction (HR = 0.61, 95% CI = 0.49 to 0.92; *P* = 018; Figure 2).

We considered the possibility that ferritin levels before cancer diagnosis might be associated with subsequent cancer occurrence. However, mean ferritin levels at baseline (when populations were
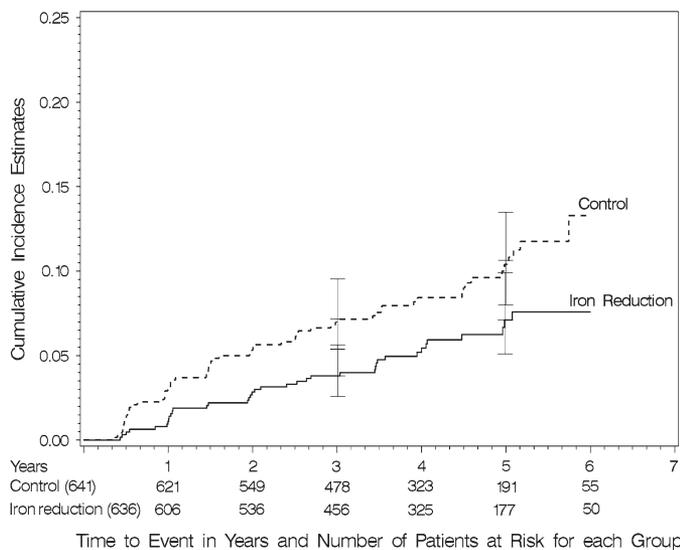
Figure 2. Cumulative incidence curves of new cancer diagnoses for the entire study cohort by intervention group. Hazard ratio = 0.61, 95% confidence interval = 0.49 to 0.92; *P* = .019 (two-sided log-rank test); Cox proportional hazards χ² = 5.4837. Numbers below the **horizontal axis** represent the numbers of patients under observation at each follow-up time point. **Error bars** represent 95% confidence intervals.

Figure 3. Kaplan–Meier event curves for cancer as the primary cause of death for the entire study cohort by intervention group. Hazard ratio = 0.39, 95% confidence interval = 0.21 to 0.72; *P* = .003 (two-sided log-rank test); Cox proportional hazards χ² = 8.9631. The **vertical axis** represents the Kaplan–Meier event estimates. Numbers below the **horizontal axis** represent the numbers of patients under observation at each follow-up time point. **Error bars** represent 95% confidence intervals.

otherwise apparently similar) were similar among all patients who subsequently developed cancer (n = 98) and among those who did not (n = 1179; 115.1 ng/mL, 95% CI = 98.4 to 131.8 ng/mL, vs 122.5 ng/mL, 95% CI = 117.7 to 127.2 ng/mL, respectively; *P* = .31). Mean baseline ferritin levels were also similar for subjects who were randomly assigned to control vs iron reduction who subsequently acquired cancer (114.5 ng/mL, 95% CI = 93.5 to 135.5 ng/mL vs 116.1 ng/mL, 95% CI = 87.4 to 144.8 ng/mL, respectively; *P* = .93). A non–statistically significant trend toward increasing ferritin levels over time of follow-up was observed in patients who subsequently developed cancer (see below). Cancer was the primary cause of death for 36 (5.6%) patients in the control group vs 14 (2.2%) in the iron reduction group (*P* = .003). Time-to-event analysis showed a lower rate of death due to cancer in patients who were randomly assigned to iron reduction vs control (HR = 0.39, 95% CI = 0.21 to 0.72; *P* = .003; Figure 3). Displaying the data as cumulative incidence curves also showed reduced death due to cancer (HR = 0.39, 95% CI = 0.21 to 0.72; *P* = .002; Figure 4). All-cause mortality in patients diagnosed with cancer was also reduced among patients who were randomly assigned to iron reduction (HR = 0.49, 95% CI = 0.29 to 0.83; *P* = .009).

Mean ferritin levels across all 6-monthly follow-up visits remained unchanged from baseline levels among control patients (122.5 ng/mL, 95% CI = 115.5 to 129.5 ng/mL) but declined statistically significantly among iron reduction patients (79.7 ng/mL, 95% CI = 73.8 to 85.5 ng/mL) (*P* < .001). The mean ferritin level during follow-up in patients who were randomly assigned to iron reduction having 50% or greater compliance with phlebotomy was 58.3 ng/mL, 95% CI = 55.2 to 61.4 ng/mL, which was consistent with levels that were targeted by the protocol (19,20). Overall mean ferritin levels during follow-up were non–statistically significantly higher in patients who developed cancer (n = 98, 115.3 ng/mL, 95% CI = 91.8 to 138.8 ng/mL) than in patients who did not (n = 1179,
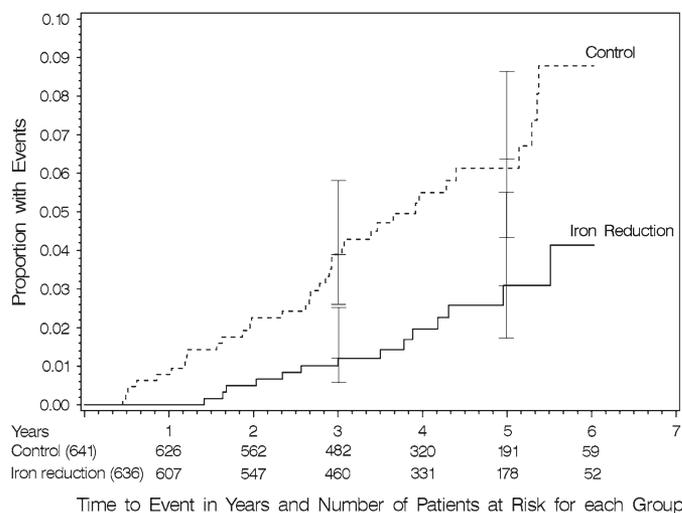
100.3 ng/mL, 95% CI = 95.6 to 105.0 ng/mL) (*P* = .098). Mean ferritin levels during follow-up remained unchanged from baseline levels in control patients who developed cancer (107.7 ng/mL, 95% CI = 91.0 to 124.5 ng/mL) and were comparable to baseline levels in iron reduction patients who developed cancer (127.1 ng/mL, 95% CI = 71.2 to 183.0 ng/mL) (*P* = .4).

Mean ferritin levels across all follow-up visits among patients who were randomly assigned to iron reduction were statistically significantly lower in patients who did not develop cancer (76.4 ng/mL, 95% CI = 71.4 to 81.4 ng/mL) than in those who did
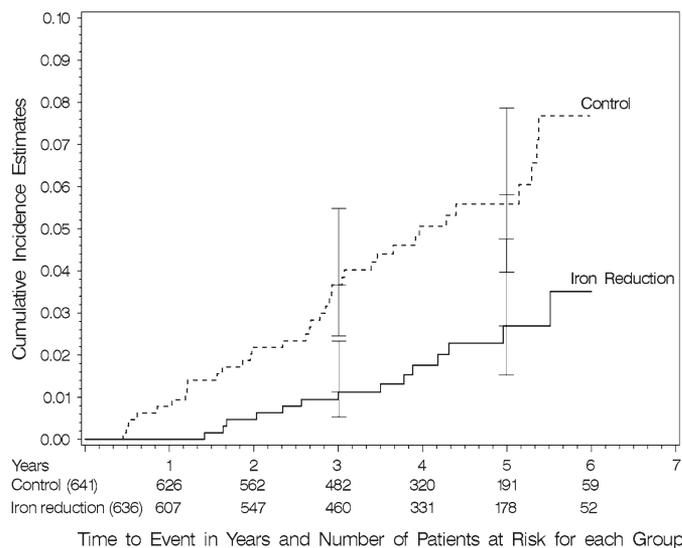

Figure 4. Cumulative incidence curves of cancer as the primary cause of death for the entire study cohort by intervention group. Hazard ratio = 0.39, 95% confidence interval = 0.21 to 0.72; *P* = .002 (two-sided log-rank test); Cox proportional hazards χ² = 9.6476. Numbers below the **horizontal axis** represent the numbers of patients under observation at each follow-up time point. **Error bars** represent 95% confidence intervals.
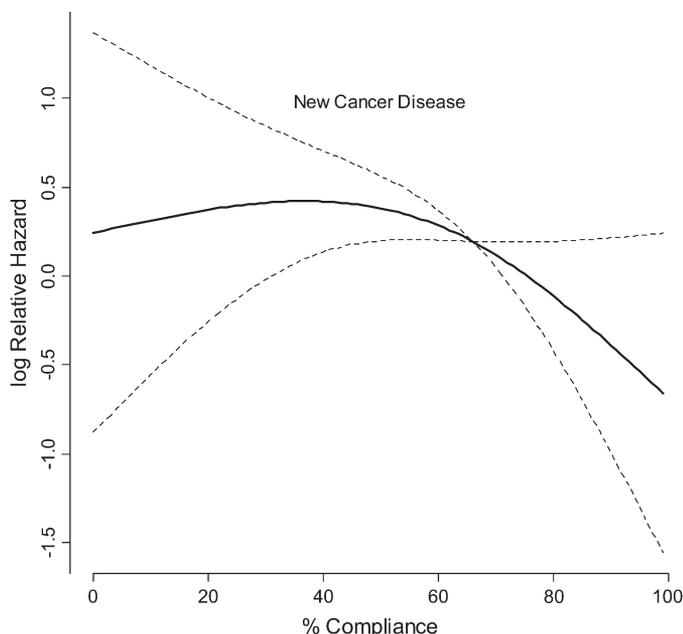
Figure 5. Association between percent compliance with the intervention (**horizontal axis**) and the log relative hazard for new cancer diagnoses (**vertical axis**) for patients who were randomly assigned to the iron reduction intervention. The **solid line** indicates percent compliance; **broken lines** delimit the 95% confidence intervals.

(127.1 ng/mL, 95% CI = 71.2 to 183.0 ng/mL) (P = .017). Thus, patients who were randomly assigned to iron reduction and did not develop cancer appeared to have better compliance with the intervention than patients who developed cancer. The effect of compliance with intervention was examined by plotting the log relative risk for new cancer diagnoses vs percent compliance with intervention. The results (Figure 5) appear to show reduced risk of new cancer in patients having greater than about 60% compliance with phlebotomy, corresponding to a ferritin level of less than 54.8 ng/mL, 95% CI = 51.6 to 57.9 ng/mL. Seventy-five percent of new cancers occurred among patients having mean ferritin levels during follow-up of greater than about 57 ng/mL. An attempt was made to fit the Cox model with an effect for compliance more than vs less than 60%, but differences were not statistically significant because of insufficient statistical power.

Discussion

This randomized trial of phlebotomy for reduction of stored iron was conducted in older, mostly male patients with advanced peripheral arterial disease having no history of clinically apparent malignancy for 5 years preceding entry. Analysis showed a 37% reduction in overall cancer incidence with iron reduction and reduced cancer-specific and all-cause mortality among patients who developed cancer in the iron reduction arm compared with those in the control arm. Reduced cancer risk and cancer-specific mortality were intent-to-treat findings based on the original randomization, whereas the difference in all-cause mortality in patients who developed cancer was defined by the occurrence of cancer after randomization. Risk of new malignancy was lower with iron reduction for several common tumor types. One patient with pancreatic cancer was observed,

and this patient was randomly assigned to iron reduction. We have no explanation for this observation or for the observation that of the eight patients with bladder cancer observed, one was randomly assigned to control and seven to iron reduction. As stated earlier, patients who were randomly assigned to iron reduction who developed cancer were less compliant with the intervention than those who did not develop cancer. Cancer risk after randomization was associated with the ferritin level during follow-up but not with other demographic or randomization variables at baseline, including baseline ferritin level.

Findings from this study support the hypothesis that ambient levels of body iron stores represented by the serum ferritin level are associated with cancer risk and that lowering iron levels reduces cancer risk. These data do not address possible effects of reducing stored iron on risk of malignancy in females, in other age groups, or in patients without established vascular disease. Both preclinical and epidemiological studies suggest that iron-induced free radical damage may play a role in early stages of tumor development (2,4,6,9,10,13,14,16,17), although such damage may also support the growth of established tumors (18–20). In this study, iron reduction appeared to begin reducing cancer risk relatively soon (within 6 months) after randomization. This timing presumably reflects an effect on incipient malignant disease and is consistent with the observation that increased risk of malignancy following blood transfusion is evident within 6 months (16). These data do not address possible effects of iron reduction on established malignancy or possible differences in threshold (at-risk) levels of body iron among individuals or according to tumor type. Yu et al. (36) reviewed the role of iron in neoplasia and aptly stated that "elucidation of the complex effects of Fe-reduction on the expression of cell cycle control molecules remains in its infancy."

These observations provide incentive for future studies and insight into optimal clinical trial design. The apparent dose–effect relationship between nadir ferritin levels and cancer risk in the lower range of ferritin values suggests that ferritin levels between about 12 ng/mL (the approximate lower limit of normal) and about 50 ng/mL should be targeted experimentally to rigorously test the hypothesis. Among patients in the iron reduction group, mean ferritin levels during follow-up were statistically significantly higher among patients who developed cancer than among patients who did not. The finding that patients who were randomly assigned to iron reduction and developed cancer were less compliant with the intervention than those who did not develop cancer emphasizes the need to achieve compliance in future studies. The apparent lack of an acute-phase effect before diagnosis of malignancy suggests that the ferritin level may be a reliable guide to calibrated iron reduction that avoids iron deficiency (21,23). A 6-month phlebotomy schedule appears adequate for maintaining low-risk ferritin levels.

These data suggest that ambient levels of iron stores may be noxious and constitute a "public" problem that affects large segments of the population. Iron ingested in excess cannot be recognized by our senses as potentially noxious. Iron stores rise slowly with aging to levels that are not obviously related temporally to disease that appears capriciously, for no obvious reason (1). The term "ferrotoxic disease" is useful to distinguish these diseases

from acute iron toxicity and the classic iron overload disorder, hereditary hemochromatosis (1). There may be a need to redefine the normal range for the serum ferritin level based on associated disease risk rather than on population statistics that are performed on apparently healthy individuals. Thus, iron deficiency may exist when ferritin levels decline to less than about 12 ng/mL, whereas ferrotoxic disease may occur with levels greater than about 50 ng/mL (1,23). However, threshold levels of body iron capable of contributing to disease risk may vary according to the disease in question, the antioxidant status of the individual, and other factors. Patterns of iron increase over time may account for differences in disease risk according to age and sex and also in blacks, whose levels of body iron exceed those of whites (1). The results of past randomized cancer clinical trials in which blood collection was used in one group to monitor chemotherapy toxicity but not in the control group having no chemotherapy (37) may warrant reinterpretation. The administration of iron to iron-replete cancer patients with anemia (38) should be evaluated for possible adverse effects on cancer outcomes.

This study has potential limitations. Data reported here must be considered preliminary because they were collected (albeit prospectively) during a study that was designed for vascular outcomes. It cannot be concluded from this study that maintenance of serum ferritin levels in the range of 12–50 ng/mL will reduce cancer incidence in unselected healthy individuals or in women (few women were included in this study). It is conceivable that phlebotomy may reduce cancer risk by a mechanism independent of iron reduction. Nonetheless, our data exhibit statistical strength and mechanistic plausibility and coincide with previous data on reduced cancer risk with lower body iron stores in premenopausal women (5,12), blood donors (13,14), and phlebotomized patients with hepatitis C (17). Further studies of the relationship between levels of body iron and cancer outcomes are needed.

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