The association of serum ferritin and transferrin receptor concentrations with mortality in women with human immunodeficiency virus infection

Victor R. Gordeuk
Gladys Onojobi
Michael F. Schneider
Fitzroy W. Dawkins
Robert Delapenha
Yaroslav Voloshin
Victor von Wyl
Melanie Bacon
Howard Minkoff
Alexandra Levine
Mardge Cohen
Ruth M. Greenblatt

Background and Objectives. Whether degree of iron stores influences progression of human immunodeficiency virus (HIV) disease is controversial. We studied the relationship of indirect measures of iron stores with mortality in highly active antiretroviral therapy (HAART)-naïve participants from the Women's Interagency HIV Study.

Design and Methods. One hundred and fifty-eight HIV-infected women who died before July 1996 were individually matched by $CD4^+$ cell count (within ± 50 cells/μL) and HIV RNA level (within ± 0.50 $\log_{10}$ copies/mL) to 154 controls. Serum ferritin and transferrin receptor concentrations were measured in 151 pairs of women.

Results. Using multivariable conditional logistic regression models that were adjusted for self-reported antiretroviral therapy use, age, smoking status, ethnicity, hemoglobin concentration, C-reactive protein and aspartate amino transferase, a $\log_{10}$ increase in baseline serum ferritin concentration was associated with a 1.67-fold increase in the odds of death (95% CI: 0.98, 2.86) and a one-unit decrease in transferrin receptor to $\log_{10}$ ferritin ratio was associated with a 1.12-fold (95% CI: 1.01, 1.23) increase in the odds of death.

Interpretations and Conclusions. In this study, higher indirect measures of iron status were associated with reduced survival among HAART-naïve HIV-infected women. Additional prospective studies with data on direct measures of iron status along with randomized trials are needed to elucidate the current equipoise over whether iron supplementation is beneficial by preventing anemia or harmful by increasing iron stores in HIV-infected women.

Key words: HIV, ferritin, transferrin receptor, iron status, mortality, women.

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From the Center for Sickle Cell Disease and Dept. of Medicine, Howard University, Washington, DC, USA (VG, GO, FWD, RD, YV); Dept. of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA (MFS); Georgetown University, Washington, DC, USA (VvW, MB); Dept. of Obstetrics and Gynecology, Maimonides Medical Center and SUNY Health Sciences Center at Brooklyn, NY, USA (HM); University of Southern California School of Medicine, Los Angeles, CA, USA (AL); Cook County Hospital, Chicago, IL, USA (MC); Depts. of Medicine, Epidemiology and Biostatistics, University of California, San Francisco, CA, USA (RMG).

*Correspondence:
Victor R. Gordeuk, M.D.
Center for Sickle Cell Disease,
Howard University, 520 W St. NW,
Washington, DC 20059 USA.
E-mail: vgordeuk@howard.edu*

Human imunodeficiency (HIV) infection is associated with profound impairment of the body's immune system and progressive iron deposition in the bone marrow, liver, and other organs.[1] Pathways of developing increased iron stores include blood transfusions and sequestration of iron in macrophages because of chronic inflammation. In addition, any anemia that is not due to blood loss or hemolysis is associated with increases in macrophage stores because iron formerly present in hemoglobin enters storage during the development of anemia. Basal iron levels in neutrophils and macrophages are necessary for generating free radical reactions that attack pathogens.[2] On the other hand, increased iron stores might directly favor HIV progression by impairing key mediators in the host response[3,4] or enhancing growth of pathogens.[5] While several clinical studies have provided evidence that iron excess has adverse effects in patients with HIV disease,[6] only a few have examined the effect of iron status on HIV progression in women, who in their reproductive years are at increased risk of iron deficiency related to menstruation and childbearing, and the results are conflicting.[7,8] Our study was designed to quantify the relationship that indirect measures of iron stores (serum ferritin concentration, serum transferrin receptor (TfR) concentration, and the ratio of serum TfR to $\log_{10}$ serum ferritin concentration) have with mortality in a group of HIV-infected, highly active antiretroviral therapy (HAART)-naïve participants from the Women's Interagency HIV Study (WIHS).

Design and Methods

Study population

The WIHS is a multi-site prospective cohort study of HIV infection in women.[9] Briefly, 2628 women (2059 HIV-infected and 569 HIV-uninfected) were enrolled between October 1994 and November 1995. Participants attended semi-annual study visits where they were interviewed, examined and laboratory specimens were obtained. The study was approved by the Institutional Review Board at each participating institution.

Selection of cases and controls

We selected as cases all 158 HIV-infected women who died prior to July 1, 1996, had data on baseline CD4+ cell counts and HIV RNA levels, and did not report the use of HAART. We individually matched these cases to HIV-infected controls who survived beyond July 1, 1996 and did not report use of HAART as of July 1, 1996 by baseline CD4+ cell count (within ±50 cells/μL) and HIV RNA level (within ±0.50 $\log_{10}$ copies/mL). One hundred and fifty-four controls were available as matches for the 158 cases and complete data on indirect measures of iron status (serum ferritin and transferrin receptor concentrations) were available in 151 of these pairs, resulting in a study population consisting of 302 HIV-infected women. Self-reported antiretroviral therapy (ART) use in the absence of HAART was not an exclusion criterion.

The definition of HAART was guided by the DHHS/Kaiser Panel guidelines and defined as: (a) two or more nucleoside reverse transcriptase inhibitors (NRTI) in combination with at least one protease inhibitor (PI) or one non-nucleoside reverse transcriptase inhibitor (NNRTI); (b) one NRTI in combination with at least one PI and at least one NNRTI; (c) a regimen containing ritonavir and saquinavir in combination with one NRTI and no NNRTI; and (d) an abacavir-containing regimen of three or more NRTI in the absence of both PI and NNRTI. Combinations of zidovudine and stavudine with either a PI or a NNRTI were not considered HAART. Therapy regimens not classified as HAART were categorized as either monotherapy or combination ART.

Laboratory methods

All laboratory tests were done on samples obtained at the baseline study visit. CD4+ cell counts were determined on fresh blood in laboratories that participate in the NIAID flow cytometry quality control program. Plasma HIV RNA levels were determined using the Nuclisens (bioMérieux, Marcy l'Etoile, France) method in laboratories participating in the AIDS Clinical Trials Group quality assurance program. Complete blood counts and aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were determined by automated methods in the laboratories of participating institutions. Concentrations of ferritin, TfR and C-reactive protein (CRP) were determined by enzyme-linked immunosorbant assay (ELISA) from serum samples that had been reposited at -80°C. Kits from Ramco Laboratories, Inc. (Stafford, TX, USA) were used for ferritin and TfR and kits from ALPCO Diagnostic (Windham, NH, USA) for CRP. Expected plasma ranges provided by the manufacturers are 20-300 μg/L for ferritin, 2.9-8.3 mg/L for TfR, and 0.068-8.2 mg/L for CRP. Because serum levels of AST and ALT were determined in several laboratories with differing normal ranges, these variables were categorized as being normal or elevated for the analyses, based on the normal range provided by the source laboratory (upper limits of normal of 30, 40 or 45 U/L for AST and 40 or 50 U/L for ALT).

Statistical analysis

Categorical baseline characteristics which included ethnicity, level of education, smoking, alcohol consumption, intravenous drug use, ART use, AST, ALT, and hepatitis B and C status were compared between cases and controls using the Pearson χ^2 test of overall association. Continuous baseline characteristics which included age, hemoglobin, mean corpuscular volume, white blood cell count, platelet count, CRP, serum ferritin concentration, TfR concentration, and the ratio of TfR to $\log_{10}$ ferritin were compared between cases and controls using the Wilcoxon rank-sum test. Conditional logistic regression, which accounted for cases and controls being matched for CD4+ cell count and HIV RNA levels, was used to estimate the odds ratio (OR) and 95% confidence interval (CI) of death according to serum ferritin concentration, TfR concentration, and the ratio of TfR to $\log_{10}$ ferritin. Each of the three conditional logistic regression models were adjusted for ethnicity and baseline values of self-reported ART use, age, smoking status, hemoglobin concentration, CRP and AST.

Results

Baseline characteristics

Many participants had indicators of HIV progression at study enrollment: 85% (n=257) had been or were being treated with ART; the median CD4+ cell count was 35 cells/μL; and the median HIV RNA was 5.32 $\log_{10}$ copies/mL. All ART reported at study enrollment consisted of NRTI; PI and NNRTI were not reported by any of the 302 participants at study enrollment. Women who died before July 1, 1996 were older, had lower median hemoglobin concentrations, had higher median serum levels of CRP and were more likely to have elevated AST than controls (Table 1). ART use before and at baseline was similar between the two groups.

Relationship of indirect measures of iron status with mortality

Table 2 shows a comparison of unadjusted values for serum ferritin concentration, serum TfR concentration, and the ratio of serum TfR concentration to $\log_{10}$ serum ferritin concentration among cases and controls. Cases had higher median serum ferritin concentrations (392 μg/L versus 330 μg/L; p=0.027) and the interquartile range (IQR) of the serum ferritin concentrations among cases was slighter larger (161 μg/L, 736 μg/L) among the cases than among controls (84 μg/L, 625 μg/L). The cases also had higher median serum TfR concentrations than had the controls (6.5 mg/L versus 5.5 mg/L; p=0.048), but the variability shown in the middle 50% of serum TfR concentrations among cases (IQR=4.8 mg/L, 9.4 mg/L) was nearly identical to that in the controls (IQR=4.4 mg/L, 8.3 mg/L). The distribution of the ratio of TfR to $\log_{10}$ ferritin for both cases and controls was heavily skewed to the right with outliers in the upper tail. However, there were no differences in the median ratios among cases and controls (2.6 [IQR=1.9, 3.8, versus 2.7 [IQR=1.8, 3.9]; p=0.639). To more appropriately quantify the association that these indirect measures of iron status have with mortality more appropriately we used three separate multivariable conditional logistic regression analyses that were adjusted

Table 1. Baseline characteristics of 151 cases who died before July 1, 1996 and 151 matched controls who were alive as of July 1, 1996.*

Characteristic	*Cases*	*Controls*	*p value°*
Demographic and Behavioral			
Age (years)	38 (33, 43)	36 (31, 41)	0.023
Ethnicity			
African American	87 (58%)	98 (65%)	0.593
Latina	29 (19%)	22 (15%)	
White	30 (20%)	26 (17%)	
Other	5 (3%)	5 (3%)	
Completed college	8 (5%)	10 (7%)	0.627
Currently smoke	79 (52%)	70 (47%)	0.385
≥ 3 alcoholic drinks/week	29 (19%)	29 (20%)	0.931
History of intravenous drug use	64 (42%)	56 (37%)	0.347
HIV Treatment			
Used ART prior to enrollment	134 (89%)	123 (82%)	0.098
Zidovudine	130 (86%)	118 (79%)	0.091
Stavudine	28 (19%)	18 (12%)	0.109
Didanosine	71 (47%)	62 (42%)	0.345
Zalcitabine	41 (28%)	38 (26%)	0.694
Used ART at enrollment	69 (46%)	63 (42%)	0.518
Zidovudine	41 (27%)	33 (22%)	0.315
Stavudine	17 (11%)	12 (8%)	0.329
Didanosine	13 (9%)	18 (12%)	0.323
Zalcitabine	11 (7%)	17 (11%)	0.234
Laboratory test results			
Hemoglobin (g/dL)	10.8 (9.8, 11.8)	11.7 (10.7, 12.6)	<0.001
Mean corpuscular volume (fL)	91 (85, 98)	92 (85, 98)	0.649
White blood cells (cells/μL)	2800 (2100, 4300)	3200 (2400, 3900)	0.764
Platelets (no/μL)	194000 (135000, 249000)	201000 (152000, 249500)	0.627
Elevated AST	90 (60%)	73 (48%)	0.050
Elevated ALT	55 (36%)	49 (32%)	0.467
Hepatitis B surface antigen positive	9 (6%)	8 (5%)	0.825
Hepatitis C antibody positive	65 (45%)	63 (43%)	0.814
C-reactive protein (mg/L)	2.6 (0.6, 6.8)	0.9 (0.3, 3.4)	<0.001

*ART: antiretroviral therapy; AST: aspartate aminotransferase; ALT: alanine aminotransferase. *All continuous characteristics are median (quartile 1, quartile 3), all categorical characteristics are N (%). °Wilcoxon rank-sum test for continuous characteristics and Pearson χ^2 test of overall association for categorical characteristics.*

Table 2. Comparison of indirect measures of iron status between cases who died before July 1, 1996 and 151 matched controls who were alive as of July 1, 1996.*

	Cases	*Controls*	*p value°*
Ferritin (μg/L)	392 (161, 736)	330 (84, 625)	0.027
Transferrin receptor (mg/L)	6.5 (4.8, 9.4)	5.5 (4.4, 8.3)	0.048
Transferrin receptor/ $\log_{10}$ ferritin	2.6 (1.9, 3.8)	2.7 (1.8, 3.9)	0.639

**All values are median (quartile 1, quartile 3). °Wilcoxon rank-sum test.*

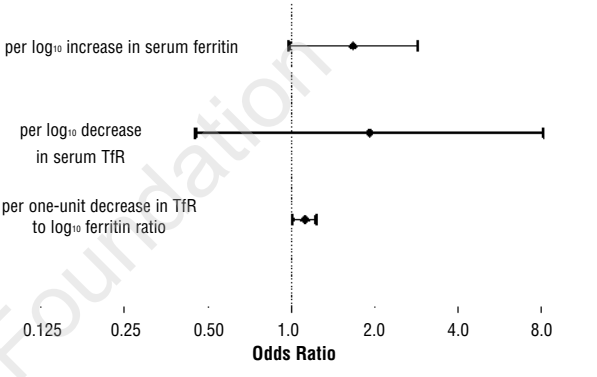

Figure 1. Odds ratios of dying obtained from three separate multivariable conditional logistic regression models adjusted for ethnicity, and baseline values of antiretroviral therapy use, age, smoking status, hemoglobin concentration, C-reactive protein and aspartate aminotransferase. The odds ratios denoted with a diamond in the figure are in the natural logarithmic scale and the horizontal bars are their corresponding 95% confidence intervals. In each case, the odds of death are higher for an indirect measure that indicates higher iron status (higher serum ferririn concentration and either lower serum transferrin receptor concentration or a smaller ratio of transferrin receptor to $\log_{10}$ ferritin).

for ethnicity, baseline ART use and smoking status, in addition to all baseline characteristics associated with mortality (variables with $p<0.05$ from Table 1). A $\log_{10}$ increase in serum ferritin concentration was associated with a 1.67-fold increase in the odds of death (95% CI: 0.98, 2.86), a $\log_{10}$ decrease in serum TfR concentration with a non-significant 1.92-fold increase (0.45, 8.13), and a one-unit decrease in the TfR to $\log_{10}$ ferritin ratio with a 1.12-fold increase (1.01, 1.23) (Figure 1).

Discussion

In this analysis, we sought to examine whether iron status may have an association with mortality among HIV-infected women by analyzing serum ferritin concentration, serum TfR concentration, and ratio of TfR to $\log_{10}$ ferritin, all indirect measures of iron status. The investigation was prompted by a number of previous studies suggesting that increased iron stores may adversely affect the course of HIV disease.[6] We decided to approach this question by studying participants who were enrolled in WIHS before the widespread adoption of HAART, reasoning that this type of therapy may obscure any potential adverse effect of increased iron stores on the natural history of HIV disease. Despite a study duration of only 1.5 years, we observed increased odds of death with higher iron stores as reflected by higher serum $\log_{10}$ ferritin concentrations (OR=1.67, p=0.06) and lower ratios of TfR to $\log_{10}$ ferritin (OR=1.12, p=0.03). A major limitation of this study is that we used indirect measures of iron status that can also be affected by inflammation, hepatic dysfunction and/or anemia, all frequent complications of HIV infection. A low serum ferritin concentration is highly specific for low iron stores, but the serum ferritin concentration may be in the normal range in patients with low iron stores in the setting of inflammation or hepatocellular dysfunction.[10-13] An elevated serum ferritin concentration may reflect increased iron stores, but it may also

reflect inflammation or hepatocellular dysfunction in the setting of normal iron stores. Conversely, the serum TfR concentration is increased with iron deficiency[14] and reduced with increased iron stores.[15] A study in which iron status was confirmed by bone marrow aspirate indicated that the ratio of serum TfR to $\log_{10}$ ferritin is a more accurate indicator of iron status in the setting of inflammation than either the serum ferritin concentration or the serum TfR concentration alone.[14] However, the TfR concentration is also increased in the setting of increased erythropoiesis due to hemolysis, ineffective erythropoiesis (destruction of erythroid precursors within the bone marrow), or hemoglobin regeneration after blood loss.[16] The gold standard for diagnosing iron deficiency is the absence of iron stores in the macrophages of a bone marrow aspirate, while the standard procedure for the diagnosis of increased iron stores is measurement of iron content in a liver biopsy specimen. Neither of these invasive procedures was possible within this study.

Given these considerations and our previous work indicating that elevated CRP concentration and anemia are predictors of mortality in HIV-infected women,[17,18] we adjusted for CRP, AST and hemoglobin in our conditional logistic regression models of the relationship of indirect measures of iron status with mortality. We also adjusted for age, ethnicity, smoking status and baseline ART use. The implication that both anemia and increased iron stores may predispose to mortality in the setting of HIV infection is not paradoxical because anemia of inflammation or bone marrow suppression associated with HIV infection tends to increase rather than decrease iron stores.[19-22] The associations observed in this study do not prove that increased iron stores promote HIV disease progression because we cannot infer a *cause and effect* relationship. Furthermore, it is conceivable that some underlying polymorphisms in genes such as *HFE*, ferroportin, or haptoglobin that influence iron status might underlie the observed effect on mortality rather than iron stores *per se*.[23,24]

Our study population had an advanced stage of HIV disease at baseline as evidenced by the low baseline CD4 cell counts and high HIV RNA levels. Although HIV does not usually present at such an advanced stage today in the USA and Europe, advanced disease at presentation remains the case too often in resource-limited settings. Furthermore, it is often not possible to provide HAART to immune-compromised individuals with high viral loads in such settings. Thus, the question raised by this study, whether iron status affects survival in HIV-infected women who do not receive HAART, and the related question, whether iron status influences the progression of early HIV infection to AIDS or a critical decline of CD4 count, are important considerations for many parts of the world. Our findings suggest that further studies of the relationship of iron status to HIV infection among women in resource-poor countries may be indicated. In particular, we believe that additional prospective studies with data on magnetic resonance imaging measurements of hepatic iron concentration[25] or direct measures of iron status and randomized trials are needed to elucidate the current equipoise over whether iron supplementation is beneficial by preventing anemia or harmful by increasing iron stores in HIV-infected women.

VRG: study conception, design, data analysis, writing the paper; GO: study conception, design, data analysis; MFS, FWD: study conception, design, writing the paper; RD: study conception and design; YV, VvW: study conception and design, data analysis; MB: study design, data analysis; HM, AL, MC, RMG: study design, data interpretation, writing manuscript.

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