ARIC Manuscript Proposal #4170

PC Reviewed: 12/13/222	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of Plasma Ferritin Levels with Total and Cause-Specific Mortality

b. Abbreviated Title (Length 26 characters): Ferritin and Mortality

2. Writing Group: Ethan Cannon, Iman Aboelsaad, Christie Ballantyne, Leo Buckley, John Leister, Jeffrey Misialek, Jim Pankow, Pamela Lutsey

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _EC__ **[please confirm with your initials electronically or in writing]**

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3. Timeline: Data analysis to being immediately, anticipated draft completion spring 2023

4. Rationale:

Iron deficiency in the elderly is an important public health problem, as it is a leading cause of anemia, which has a prevalence of over 10% in adults above age 70 [1]. Indeed, the primary function of iron is to form hemoglobin, a protein in erythrocytes that delivers oxygen to cells throughout the body [2]. However, beyond erythropoiesis, iron is also a component of numerous other proteins and enzymes, and therefore is important to many aspects of human physiology. Ferritin is an iron storage protein found throughout the body that protects cells from

oxidative damage caused by free iron [3]. Serum or plasma ferritin is found in trace amounts and is an indicator of overall iron status [4].

Common causes of iron deficiency in younger individuals, such as growth, pregnancy or breastfeeding, and menstruation do not apply to elderly persons. Among older individuals, inadequate dietary intake, impaired absorption, and blood loss (such as gastrointestinal bleeding) are primary causes [6]. There is a paucity of generalizable data about the prevalence of iron deficiency among U.S. elderly populations, for several reasons. For instance, diagnosis of iron deficiency using common biomarker tests is complicated, and iron deficiency is heterogeneous across levels of sex, age, region, and race, among other reasons [7, 8].

Because iron plays a critical role in all organ systems, there is biologic plausibility that low iron could contribute to morbidity and mortality, independent of anemia [9]. Iron is linked with DNA repair [10], and iron deficiency has been associated with poor immune function [11]. Iron deficiency, with or without anemia, is predictive of diminished aerobic work capacity [12] and quality of life [13] among adults. Additional research suggests it may have further deleterious effects. For individuals with comorbidities, iron deficiency is strongly correlated with hospital readmission and mortality, independent of anemia status [14, 15]. In a meta-analysis of clinical trials of patients with heart failure, correcting iron deficiency showed promise as a means of reducing mortality [16]. Among the general population, prospective research of the effects of iron deficiency on mortality has yielded mixed results [15, 17]. Hsu *et al* found a link between low iron and all-cause mortality among elderly individuals living in long-term care facilities [18]. Using data from the English Longitudinal Study of Ageing, Philip *et al* reported iron deficiency to be associated with greater risk of overall mortality, and particularly cancer and respiratory mortality, while the finding for CVD mortality was null [7]. Cross-sectional studies have also reported an elevated prevalence of iron deficiency among patients with inflammatory diseases such as heart failure, chronic kidney disease, and IBD [4, 9, 14].

Iron overload also contributes to disease, generally through the deposition of iron into organ tissues. In the elderly, this condition is typically caused by hereditary hemochromatosis or acquired in association with repeated blood transfusions or other conditions such as liver cirrhosis and viral hepatitis [19]. The prevalence of hereditary hemochromatosis is approximately 0.5% in the U.S., but iron overload is far more common [20]. Excess iron may cause oxidative stress [4] and be toxic to various organs [21]. Its negative effects on cardiovascular health are well documented [22, 23].

Iron deficiency is a relatively treatable medical condition. While intravenous repletion is often required (particularly in the presence of comorbidities), first steps include increasing dietary iron, decreasing consumption of substances that inhibit iron absorption, and iron supplementation [6]. Despite the relative simplicity of these solutions, iron deficiency in elderly populations may often go unrecognized and untreated due in part to the overlap of its symptoms with those of other diseases. This is particularly true in non-anemic iron deficiency, which is more prevalent than iron deficiency with anemia, yet where symptoms are less well-defined. If low ferritin levels independently predict cause-specific mortality, then it follows that the prioritization of treatment for iron deficiency could lead to improved health outcomes.

5. Main Hypothesis:

- Both low and high levels of plasma ferritin will be associated with greater all-cause mortality, as well as mortality due to cardiovascular disease, cancer, and respiratory disease.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study Design

Prospective cohort from Visit 5 to most recent follow-up.

Inclusion/Exclusion

Participants with SomaLogic plasma ferritin data from visit 5 will be included. Individuals that are neither African American nor white, as well as African Americans from the MN and MD centers, will be excluded due to low numbers. Additionally, we will exclude participants with anemia (Hg<13 g/dL in men or 12 g/dL in women) from our primary analysis.

Variables

Exposures: The primary exposure will be plasma ferritin light chain levels, measured by modified aptamer on the SomaLogic SomaScan platform. Plasma ferritin heavy chain/ light chain complex levels will be a secondary exposure.

Outcome: All-cause mortality, CVD mortality, cancer mortality, and respiratory disease mortality based on hospital ICD codes for underlying cause of death.

Potential effect modifiers and/or mediators: Age, sex, and race

Other confounders:

- Demographics (e.g., age, sex, race*study site (5-level variable), educational attainment)
- Behaviors (e.g., smoking and alcohol consumption)
- Physiologic traits, inflammatory biomarkers and clinical conditions (e.g., obesity, eGFR, CRP, and hypertension)

Data analysis

Baseline characteristics of participants will be described using means and proportions stratified by quintiles of the exposure. Cox proportional hazards regression will be used to explore relationships between plasma ferritin and hazard of all-cause mortality as well as mortality due to CVD, cancer, and respiratory disease. Restricted cubic splines will be employed to visualize non-linear trends. The primary analysis will likely consider quintiles of serum ferritin. However, based on what is observed in the spline analysis, we may subclassify the tails (e.g., look specifically at participants in the bottom 5% or top 95% of the distribution). Additionally, we will also evaluate the association of 50% lower levels by taking the log of ferritin level and multiplying by -1.

Final covariate decisions will be made after seeing the association of covariates according to serum ferritin quintiles. We will use a series of nested models. Our first model with adjust for demographics (e.g., age, sex, race*study site (5-level variable), educational attainment). Our second model will further adjust for behaviors (e.g., smoking and alcohol consumption). Our

third model will adjust for physiologic traits, inflammatory biomarkers, and clinical conditions (e.g., obesity, eGFR, CRP, and hypertension). We will also the explore the effect of including eGFR earlier in the model building process.

Cross-product terms will be used to evaluate whether race, sex, and/or age modify the associations of plasma ferritin with risk of incident mortality (all-cause and cause-specific) on the multiplicative scale. Stratified results will be presented, as appropriate. Sex-specific findings will be presented regardless of whether the interaction is statistically significant given inherent interest.

We will perform a sensitivity analysis to see how results change when participants with anemia are included.

Because we are interested in the length of the course of action for ferritin status on each cause of mortality, we will carefully inspect the proportional hazards assumption for each outcome.

Methodologic Limitations or Challenges

Data using the SomaScan do not scale to standard clinical metrics. Therefore, it is not possible to employ clinical cut points. This also means that we will not be able to address the prevalence of iron deficiency in the elderly. Tangentially, if more validation studies are being done with traditional assays vs. Soma, ferritin may be worthwhile to add.

The complex role of ferritin creates another limitation. Ferritin is known to be an acute-phase protein, and increased levels of ferritin are found in certain inflammatory or other diseases, even in the presence of iron deficiency [5]. Therefore, different criteria are utilized in diagnosing iron deficiency in individuals with these conditions. We will utilize model covariates to make appropriate comparisons, but there remains the possibility of confounding.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No
(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes __X__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.

ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php

___X___ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

PUBLISHED

#2481 Silvestre OM, Gonçalves A, Nadruz W Jr, Claggett B, Couper D, Eckfeldt JH, Pankow JS, Anker SD, Solomon SD. Ferritin levels and risk of heart failure-the Atherosclerosis Risk in Communities Study. Eur J Heart Fail. 2017 Mar;19(3):340-347. doi: 10.1002/ejhf.701. Epub 2016 Dec 14. PMID: 27976478; PMCID: PMC5334451.

#1661 Raynor LA, Pankow JS, Duncan BB, Schmidt MI, Hoogeveen RC, Pereira MA, Young JH, Ballantyne CM. Novel risk factors and the prediction of type 2 diabetes in the Atherosclerosis Risk in Communities (ARIC) study. Diabetes Care. 2013 Jan;36(1):70-6. doi: 10.2337/dc12-0609. Epub 2012 Aug 28. PMID: 22933437; PMCID: PMC3526210.

#1038 Jehn ML, Guallar E, Clark JM, Couper D, Duncan BB, Ballantyne CM, Hoogeveen RC, Harris ZL, Pankow JS. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 2007 May 1;165(9):1047-54. doi: 10.1093/aje/kwk093. Epub 2007 Feb 6. PMID: 17284722.

#194A Iribarren C, Sempos CT, Eckfeldt JH, Folsom AR. Lack of association between ferritin level and measures of LDL oxidation: the ARIC study. Atherosclerosis Risk in Communities. Atherosclerosis. 1998 Jul;139(1):189-95. doi: 10.1016/s0021-9150(98)00070-7. PMID: 9699907.

#231 Moore M, Folsom AR, Barnes RW, Eckfeldt JH. No association between serum ferritin and asymptomatic carotid atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 1995 Apr 15;141(8):719-23. doi: 10.1093/oxfordjournals.aje.a117493. PMID: 7709914.

NOT YET PUBLISHED

#4013 Iman Aboelsaad…Victoria Arthur. Iron metabolism, incident heart failure and adverse cardiac remodeling in community-dwelling adults: the Atherosclerosis Risk in Communities Study

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __X__ No

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number* _____)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central. gjgjggj

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20. *StatPearls*. 2022.
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