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Title

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Permalink

https://escholarship.org/uc/item/2185988j

Journal

Circulation Heart Failure, 9(1)

ISSN

1941-3289

Authors

Garimella, Pranav S
Katz, Ronit
Patel, Kushang V
et al.

Publication Date

2016

DOI

10.1161/circheartfailure.115.002124

Peer reviewed

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HHS Public Access

Author manuscript

Circ Heart Fail. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Circ Heart Fail. 2016 January ; 9(1): e002124. doi:10.1161/CIRCHEARTFAILURE.115.002124.

Association of Serum Erythropoietin with Cardiovascular Events, Kidney Function Decline and Mortality: The Health ABC Study

Pranav S. Garimella, MD, MPH[1], **Ronit Katz, DPhil**[2], **Kushang V. Patel, PhD**[2], **Stephen B. Kritchevsky, PhD**[3], **Chirag R. Parikh, MD, PhD**[4], **Joachim H. Ix, MD, MS**[5], **Linda F. Fried, MD, MPH**[6], **Anne B. Newman, MD, MPH**[6], **Michael G. Shlipak, MD, MPH**[7], **Tamara B. Harris, MD, MS**[8], and **Mark J. Sarnak, MD, MS**[1] **for the Health ABC Study**

[1]Tufts Medical Center, Boston, MA

[2]University of Washington, Seattle, WA

[3]Wake Forest School of Medicine, Winston-Salem, NC

[4]Yale University School of Medicine, New Haven, CT

[5]University of California, San Diego, CA

[6]University of Pittsburgh, Pittsburgh, PA

[7]University of California, San Francisco, CA

[8]National Institute on Aging, Bethesda, MA

Abstract

Background—Studies suggest that in patients with heart failure (HF), high serum erythropoietin is associated with risk of recurrent HF and mortality. Trials of erythropoietin stimulating agents in persons with kidney disease have also suggested an increased incidence of adverse clinical events. No studies have evaluated the association of endogenous erythropoietin levels with clinical outcomes in the community living older adults.

Methods and Results—Erythropoietin concentration was measured in 2,488 participants aged 70–79 years in the Health, Aging and Body Composition Study. Associations of erythropoietin with incident HF, coronary heart disease (CHD), stroke, mortality, and ≥30% decline in estimated glomerular filtration rate (eGFR) were examined using Cox proportional hazards and logistic regression over 10.7 years of follow up. Mean (SD) age was 75 (3) years and median (quartile 1, quartile 3) erythropoietin was 12.3 (9.0, 17.2) mIU/mL. There were 503 incident HF events and each doubling of serum erythropoietin was associated with a 25% increased risk of incident HF 1.25 (95% CI 1.13, 1.48) after adjusting for demographics, prevalent cardiovascular disease (CVD), CVD risk factors, kidney function and serum hemoglobin. There was no interaction of serum erythropoietin with chronic kidney disease or anemia (p>0.50). There were 330 incident

Correspondence to: Mark J. Sarnak, MD, MS, Division of Nephrology, Tufts Medical Center, 800 Washington St, Box 391, Boston, MA 02111, msarnak@tuftsmedicalcenter.org; Fax: 617-636-8329.

Disclosures: None.

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CHD events, 161 strokes, 1,112 deaths and 698 outcomes of ≥ 30% decline in eGFR. Serum erythropoietin was not significantly associated with these outcomes.

Conclusions—Higher levels of endogenous erythropoietin are associated with incident HF in older adults. Studies need to elucidate the mechanisms through which endogenous erythropoietin levels associate with specific outcomes.

Keywords

erythropoietin; heart failure; chronic kidney disease; cardiovascular outcomes; death

Anemia is common in persons with chronic kidney disease (CKD) and heart failure (HF) and is associated with increased risk of mortality and adverse cardiovascular (CVD) events.[1, 2] The etiology of anemia in both conditions is multifactorial and includes chronic inflammation, iron deficiency, erythropoietin deficiency and erythropoietin resistance.[3, 4] Although initial studies of anemia correction in CKD suggested a clinical benefit with use of erythropoietin stimulating agents (ESA's),[5] several recent large trials have shown an increased risk of adverse events including HF, myocardial infarction (MI), stroke, mortality and worsening kidney function with ESA therapy.[6–8] Similarly, the use of ESA therapy in HF has also been limited due to a higher risk of adverse events after early reports of success.[9, 10] However, it remains unclear whether it is the dose of ESA administered, the level of hemoglobin achieved or a lack of response to therapy that explains these adverse outcomes.

Observational studies have noted that endogenous erythropoietin levels are frequently elevated in persons with prevalent HF and associate with an increased risk of recurrent HF hospitalizations and mortality.[11, 12] Outside of the HF population, limited data also suggest an increased risk of mortality with elevated serum erythropoietin levels in persons with CKD and in the very old.[13, 14] To our knowledge there have been no large studies however in healthy community-dwelling adults that have evaluated the association of endogenous erythropoietin levels with the spectrum of clinical outcomes seen in trials. In this study we evaluate the association of serum erythropoietin with incident HF, coronary heart disease (CHD), stroke, mortality and kidney function decline in a large population of community-dwelling older adults.

METHODS

Participants

The Health Aging and Body Composition (Health ABC) is a longitudinal study of the impact of changes in weight and body composition on age-related physiologic and functional changes. Community-dwelling adults (N=3,075) aged 70 to 79 years were recruited from Medicare eligibility lists from March 1997 through July 1998 at two field centers in Pittsburgh, PA, and Memphis, TN. White participants were recruited from a random sample of the lists; black participants were recruited from all age-eligible individuals residing in the respective communities. Subjects were eligible if they reported no difficulty walking one-fourth of a mile, climbing up 10 steps, or performing basic activities of daily living; were free of life-threatening illness; planned to remain in the geographic area

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for ≥3 years; and were not enrolled in lifestyle intervention trials. Exclusion criteria included difficulties with activities of daily living, cognitive impairment, inability to communicate with the interviewer, intention of moving within the next 3 years, and active treatment for cancer in the preceding 3 years. All participants gave informed consent and the study was approved by the institutional review boards at the University of Tennessee Health Science Center and the University of Pittsburgh. Of the 3,075 participants at baseline, 2,921 participants were alive and returned for a follow up visit at year 3. Serum erythropoietin was measured in 2,499 persons of these participants and we excluded 11 who were missing cystatin C or ACR measures, for a final study sample of 2,488 (Figure 1).

Exposure Variable

The levels of erythropoietin were measured from frozen stored serum collected at the year 3 visit. Samples were obtained in the morning after an overnight fast, and after processing, the specimens were frozen at −70°C and shipped to the Core Laboratory at the University of Vermont. Erythropoietin was measured using a monoclonal antibody and polyclonal antibody conjugate in a sandwich ELISA format (Quantikine IVD Human Epo, R&D Systems, Minneapolis, MN) as per the manufacturer's instructions. The sensitivity of the ELISA (minimum detectable level) is < 0.6mIU/mL. The intra-assay coefficient of variation was 7.8% at a mean concentration of 6.45mIU/mL and 2.4% at a mean concentration of 84.12mIU/mL.

Outcomes

Surveillance for outcomes was conducted via in-person and alternating phone interviews every 6 months regarding any hospitalizations; when a hospitalization was reported, all records pertaining that were available were collected. Medical records for all overnight hospitalizations were reviewed at each site by local adjudicators. The Health ABC Study Diagnosis and Disease Ascertainment Committee reviewed all hospital records, death certificates, informant interviews, and autopsy data to adjudicate immediate and underlying causes of death. Complete details regarding ascertainment and adjudication of CVD outcomes are provided online (http://keeptrack.ucsf.edu/). Adjudication criteria for HF required, in addition to a physician diagnosis of HF: 1) medical record documentation of symptoms (e.g., shortness of breath, fatigue, orthopnea, paroxysmal nocturnal dyspnea) and physical signs (e.g., edema, pulmonary rales, gallop rhythm); 2) evidence of pulmonary edema on chest radiography or evidence supporting HF on echocardiography; and 3) medical therapy for HF, including at least a diuretic and a vasodilator and/or digitalis. These criteria are similar to those used in the Cardiovascular Health Study. Incident CHD was defined as hospitalization for MI, angina pectoris, or elective coronary revascularization, either surgical or percutaneous. Incident stroke included both fatal and nonfatal stroke events. We divided the cardiac outcomes into HF and CHD because of previous literature showing associations of erythropoietin levels with recurrent HF.[11] While evaluating associations with HF, CHD and stroke, we excluded persons with these conditions at baseline. Date and causes of death were taken from the death certificate. Progression decline in kidney function was defined as ≥ 30% decline in estimated glomerular filtration rate (eGFR) from year 3 visit to year 10. A 30% decline in eGFR has recently been associated with adverse outcomes and therefore recommended as an alternative endpoint for CKD

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progression.[15] Cystatin C was measured using a BNII nephelometer (Dade Behring Inc., Deerfield, IL) that used a particle-enhanced immunonepholometric assay (N Latex Cystatin C).[16] The eGFR was estimated using an equation that included serum cystatin C concentration, age, sex, and race derived in the CKD-EPI study,[17] and the use of this equation has been validated for change in eGFR over time.[18]

Covariates

Covariates included socio-demographic factors such as age, gender, race (self-defined), clinical site; prevalent CVD (history of coronary artery disease and stroke); CVD risk factors including smoking status defined by current *versus* former (>100 lifetime cigarettes) or never, diabetes (defined by use of hypoglycemic agents, self-report, fasting plasma glucose >126 mg/dL or an oral glucose tolerance test >200 mg/dl); use of individual anti-hypertensives including angiotensin converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARB), calcium channel blockers (CCB), diuretics, systolic blood pressure; left ventricular hypertrophy (LVH) and heart rate. The Minnesota code criteria were applied to diagnose left ventricular hypertrophy (LVH) from the baseline electrocardiogram: R >26mm in either V5 or V6, or R >20mm in any of leads I, II, III, aVF, or R >12mm in lead aVL, or R in V5 or V6 plus S in V1 >35mm.[19] Laboratory values including serum albumin, total cholesterol, C-reactive protein (CRP), interlukin-6 (IL-6), tumor necrosis factors-alpha (TNF-alpha), eGFR, urine albumin to creatinine ratio (ACR) and serum hemoglobin levels. Serum chemistries were measured by a colorimetric technique on a Johnson & Johnson Vitros 950 analyzer). Measures of IL-6, TNF-alpha, and CRP were performed using ELISA kits from R&D Systems (Minneapolis, MN). Detectable limits were 0.10 pg/ml for IL-6, 0.18 pg/ml for TNF-alpha, and 0.007 mg/L for CRP. Interassay coefficients of variation were determined by duplicate analyses of 150 specimens; 10.3, 8.0, and 15.8% for IL-6, CRP, and TNF-alpha, respectively.[20]

Statistical analysis

The starting time for this analysis was year 3 when serum erythropoietin was measured. While most covariates were available from year 3 a few were only collected at baseline and were therefore carried forward for these analyses. These included serum albumin, CRP, IL-6, TNF-alpha, urine ACR, LVH and heart rate. Participants with prevalent HF, CHD and stroke at year 3 were excluded in the analysis of the respective outcomes. We described the individual distribution of serum erythropoietin using summary statistics and frequency histograms. We then compared baseline participant characteristics (demographics, CVD risk factors and kidney function measures) across quartiles of serum erythropoietin. Because erythropoietin values were not normally distributed, erythropoietin was log transformed to the base 2 and analyses were reported per doubling of serum erythropoietin. We evaluated the association of serum erythropoietin with each outcome for the presence of non-linearity using restricted cubic splines. We performed a time to event analysis to estimate association of serum erythropoietin with HF, CHD events, stroke and mortality using four separate Cox proportional hazards regression models after adjustment for potential confounders. To evaluate the association of erythropoietin with progressive kidney disease, multivariate logistic regression was performed, as the outcome can only be reached at one discrete time-point when kidney function was measured. Participants with prevalent HF were excluded in

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analyses evaluating incident HF, and likewise, those with history of CHD and stroke were excluded in the CHD analysis and stroke analyses respectively. Finally we constructed Kaplan-Meier curves to assess the probabilities of survival free from HF, CHD, stroke and death stratified by quartiles of serum erythropoietin.

We used a series of models with sequential adjustment for potential confounding variables. Initial models were adjusted for demographic variables including age, gender, race and site. We adjusted for prevalent CVD in all outcomes except CHD and stroke. Models were adjusted for CVD risk factors including diabetes, use of anti-hypertensive medications systolic blood pressure, smoking status, serum albumin and CRP. Models for HF also included adjustment for heart rate, left ventricular hypertrophy, IL-6, TNF-alpha based on previous literature from the Health ABC showing their ability to improve prediction of incident HF.[21, 22] Next, given the association of serum erythropoietin levels with level of kidney function, we adjusted further for eGFR and UACR. Final models were adjusted for hemoglobin values given that erythropoietin levels are associated with serum hemoglobin.

We then assessed for an interactions between erythropoietin levels and CKD status (eGFR <60ml/min/1.73m^2 or ACR >30mg/g) for each of the clinical outcomes.[2] We also assessed the interaction between erythropoietin levels with presence of anemia (defined as serum hemoglobin <13gm/L in men and <12gm/L in women) and race for HF outcomes given that both anemia and race have been differentially associated with incident HF and mortality in blacks and whites.[23–26]

In sensitivity analysis, we evaluated the competing risk of mortality using the Lunn & McNeill method for heart failure, incident CVD and stroke and also evaluated whether our HF results were consistent when patients without prevalent CHD or without incident MI prior to the HF event were excluded. All analyses were performed using S-Plus (release 8.0, Insightful Inc, Seattle, WA) and SPSS statistical software (release 15.0, SPSS Inc, Chicago, IL). A two sided p-value <0.05 was considered statistically significant for all analyses including interaction terms.

RESULTS

The mean age of participants was 75.4 ± (2.8), 51% were female, and 39% were Black. The mean eGFR was 79 ±20 ml/min/1.73m^2. The median erythropoietin level (quartile1, quartile 3) was 12.3 (9.0, 17.2) mIU/mL. The distribution of participant characteristics across quartiles of erythropoietin levels is shown in Table 1. The percentage of blacks increased from 32% in the first quartile to 46% in the highest quartile (p<0.001). Prevalent CAD, HF and risk factors for CVD, including diabetes and hypertension and inflammatory markers TNF-alpha (p=0.006) and IL-6 (p<0.001) were highest in the fourth quartile of erythropoietin. Mean eGFR (p=0.002), serum albumin (p=0.044) and serum hemoglobin ($p \ll 0.001$) values were lower with higher erythropoietin quartile.

The mean follow-up time was 10.7 (SD=2.9) years. There were 503 incident HF events (incidence rate 2.5% per year), 330 incident CHD events (2.1%/year), 161 incident strokes (0.8%/year), 1,112 deaths (4.2%/year) and 698 events of kidney function decline (1.2%/

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year) Unadjusted incidence rates of the all clinical outcomes were highest in the fourth quartile of serum erythropoietin levels. Higher serum erythropoietin levels were associated with increased risk of HF (Figure 2). In continuous models, each two-fold increase in serum erythropoietin was associated with 30% higher risk of HF after adjusting for prevalent CVD, CVD risk factors and kidney function. This association remained significant after additional adjustment for hemoglobin levels (Table 2). After similar adjustments, the highest quartile (≥ 17.7 mIU/mL) of serum erythropoietin had 1.37 (95% CI 1.03–1.81) times increased risk of HF compared to the lowest quartile (<9.04 mIU/mL). There was no association between higher erythropoietin levels with CHD, stroke, all-cause mortality, or progressive decline in kidney function. Figure 3 shows the Kaplan-Meir curves for each of the outcomes by quartiles of serum erythropoietin.

The presence of CKD did not significantly modify the effect of erythropoietin levels on HF (p=0.82), MI (p=0.15), stoke (p=0.81), mortality (p=0.49) or eGFR decline (p=0.15). There was also no significant interaction between serum erythropoietin with either race (p= 0.50) or presence of anemia (p=0.81) with respect to the outcome of heart failure.

In a sensitivity analysis evaluating the competing risk of mortality with the other clinical outcomes, our results were unchanged (Supplemental Table). In another sensitivity analysis, we found that of the 503 participants who developed incident HF, 152 had prevalent CHD and 19 developed incident MI prior to development of HF. In the remaining 332 participants we evaluated the association between endogenous erythropoietin level and risk of HF. Each SD higher erythropoietin level was associated with a 27% increased risk of HF (HR 1.27, 95% CI 1.07, 1.50) and compared to the lowest quartile, persons in the highest quartile of serum erythropoietin had 41% increased risk of HF (HR 1.41, 95% 1.01, 2.02).

DISCUSSION

In this large cohort of community-dwelling older adults, we found that higher levels of endogenous erythropoietin were associated with risk of incident HF after adjusting for demographic variables, prevalent CVD, CVD risk factors, inflammatory markers, kidney function and hemoglobin level. A number of studies have demonstrated that levels of endogenous erythropoietin are higher in persons with prevalent HF and that they correlate with severity of disease.[11, 27–29] Elevated levels of erythropoietin are also associated with mortality [11, 12, 27, 28] and may be able to discriminate those who are likely to be hospitalized for recurrent HF or die during follow up.[11] Our results add to this literature by demonstrating an association of endogenous erythropoietin levels with incident HF. We did not find any significant associations between serum erythropoietin levels with CHD, stroke, mortality or progressive kidney function decline.

Large clinical trials of ESAs have noted a significantly greater risk of adverse outcomes in persons randomized to ESA treatment relative to placebo. The Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) noted an elevated risk of stroke but not HF in those receiving ESA therapy as compared to placebo,[30] and the Reduction of Events by Darbepoetin Alfa in Heart Failure (RED-HF) trial noted a trend towards increased risk of stroke and thrombotic events with ESA therapy compared with placebo.[9] Among

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patients with prevalent HF and CHD undergoing dialysis, the Normal Hematocrit Study found an increased risk of death and non-fatal myocardial infarction in those randomized to the normal hematocrit arm,[31] and in persons with CKD, the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial showed a 41% increased risk of HF related hospitalizations (p=0.07) in addition to higher risk for MI, stroke and mortality [6] in those randomized to higher hemoglobin targets with ESA therapy. Studies have attempted to elucidate whether the relationship between ESA and adverse CVD outcomes are directly through ESA or mediated by the hemoglobin levels achieved. *Post-hoc* analyses from both the CHOIR and TREAT studies have suggested that participants who were unable to achieve target hemoglobin values despite incremental doses of ESA (suggesting a hypo-responsive bone marrow) were at particularly higher risk of adverse outcomes compared to those who achieved their target hemoglobin values.[32, 33] However, in a more recent analysis of the CHOIR results, it was noted that irrespective of the hemoglobin response, participants who were maintained on a higher dose of ESA had an elevated risk of adverse clinical outcomes.[34] These findings may suggest that the dose of ESA used rather than the hemoglobin level achieved may be associated with adverse outcomes. Our results extend this discussion to endogenous erythropoietin levels and risk for clinical events.

There are several potential mechanisms through which erythropoietin may be associated with cardiovascular outcomes. First, the elevation of hemoglobin with ESA therapy may result in increased blood volume and viscosity, leading to strokes, hypertension and left ventricular remodeling. More recent analyses indicate that elevated hemoglobin levels do not independently confer increased risk independent of ESA dosing, and we noted higher risk for HF even after adjustment for levels of hemoglobin.[34] Second, erythropoietin has a number of non-hematopoietic roles including anti-apoptotic and antioxidant properties, and has been shown to promote angiogenesis in the heart.[35, 36] It has been hypothesized that due to its pleotrophic properties on angiogenesis, platelet aggregation and tumorigenesis, chronic exposure to elevated levels erythropoietin may have a causal role in the development of adverse cardiovascular events.[11, 13, 34, 37] Through numerous receptors in the heart, erythropoietin plays a cardioprotective role during ischemia-reperfusion injury by preventing apoptosis and promoting ventricular remodeling.[3839] However, it has been hypothesized that chronic stimulation of these receptors due to elevated or supra therapeutic levels of circulating erythropoietin may lead to disordered remodeling and adverse outcomes.[40] Third, elevations in endogenous erythropoietin and the need for high dose ESA in trials may reflect a hypo-responsive bone marrow, which in turn may reflect overall poorer health status.[28, 32, 41] Hypo-responsiveness to erythropoietin may also reflect underlying levels of chronic inflammation (clinical or subclinical).[42, 43] Our findings of increased HF risk persisted however despite adjusting for inflammatory markers such as CRP, TNF-alpha and IL-6 as well as comorbid conditions.

We did not note an increased risk of CHD events, stroke, mortality, or decline in kidney function in our study, in contrast to the trends seen in trials of ESA therapy. It is possible this difference is related to the absolute erythropoietin levels achieved with exogenous ESA where intravenous administration of as little as 3000 U can cause a peak serum erythropoietin level of nearly 1000 mIU/L, which is over 50 times the value of the highest

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quartile in our study.[44] One could also hypothesize that the chronic but relatively low grade elevation of serum erythropoietin in our study is sufficient to exert adverse effects an on cardiac myocytes and increase risk of HF, but not influence other outcomes. We found that excluding persons with prevalent CHD and MI prior to the development of HF did not alter our primary results which suggest that, the association between erythropoietin and HF may be mediated through non-atherosclerotic mechanisms. Lastly, it is possible that the rapid rise and fall of serum erythropoietin following exogenous ESA administration, which is distinctly different from the low grade sustained elevation seen in our study, differentially affects clinical outcomes.

Our study has a number of limitations. First, the observational nature of the study precludes our ability to ascribe cause–effect relationships. Although we adjusted for a number of potential confounders and inflammatory markers, residual or unmeasured confounding cannot be ruled out. Second, our study was not designed to evaluate the predictive value of serum erythropoietin for clinical events and/or whether measurement leads to a change in outcomes; therefore, we would not currently recommend measurement of serum erythropoietin in this population. Third, our population only included elderly blacks and whites and only a relatively small proportion had advanced CKD, therefore it remains unknown whether the results are generalizable to younger populations, those of different ethnicities and with more advanced CKD. Fourth, because some patients may have developed HF without reporting the event, our rates of HF may have been underestimated. Finally, due to the study design and timing of data collection, a number of covariates (CRP, IL-6, TNF-alpha, urine ACR, LVH and heart rate) which were included in our regression models were collected only at baseline and needed to be carried forward to year 3. This is important given that some of these independently predict incident HF. Our study also has a number of strengths. It is the largest study of endogenous erythropoietin levels in a community dwelling general population. The Health ABC cohort has detailed ascertainment of risk factors, covariates, and outcomes and the ability to adjust for measures of kidney function and inflammation. We also used a laboratory that has extensive experience with these measures.

CONCLUSIONS

Well-functioning community-living older persons with elevated levels of serum erythropoietin are at increased risk of incident HF. This association is independent of cardiovascular risk factors, inflammatory markers, kidney function and anemia status. This finding is consistent with those from randomized trials suggesting that high erythropoietin may lead to adverse clinical outcomes. Further studies are needed to evaluate the mechanisms explaining the results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Acknowledgments

Sources of Funding: This research was supported in part by the Intramural Research Program of the NIH, and the National Institute on Aging (NIA) Contracts N01-AG-6-2101; N01-AG-6-2103; N01-AG-6-2106; NIA grant R01-AG028050, and NINR grant R01-NR012459. Drs. Shlipak, Sarnak, Ix, and Katz were supported by NIA grant 5R01AG027002-07 and Dr. Patel was supported by National Center on Minority Health and Health Disparities, NIH grant MD-07-080. The study sponsors had no role in study design; collection, analysis, and interpretation of the data; writing the report; and the decision to submit the report for publication.

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Clinical Perspective

Therapy with high doses of erythropoietin stimulating agents is associated with higher risk of adverse events in dialysis patients and those with heart failure. While endogenous erythropoietin levels are elevated in the setting of prevalent heart failure and increase the risk of re-hospitalization, the association of erythropoietin with clinical outcomes in the general population is relatively unknown. We found that elevated levels of endogenous erythropoietin were associated with increased risk of incident heart failure over 10 years of follow-up in community dwelling adults aged 70–79 years enrolled in the Health ABC study. This novel association was independent of baseline anemia status, kidney function and other risk factors for heart failure. It is unknown however whether elevated levels of erythropoietin reflect a state of inflammation, thus serving as a marker of increased HF risk or whether erythropoietin is causally associated with incident heart failure. If causal, it may be that the untoward effects of elevated erythropoietin are mediated by non-atherosclerotic mechanisms given the lack of increased risk of coronary events or stroke associated with higher levels. Further studies are needed to evaluate if serum erythropoietin levels can be used to predict incident heart failure before routine measurement in clinical practice can be recommended.

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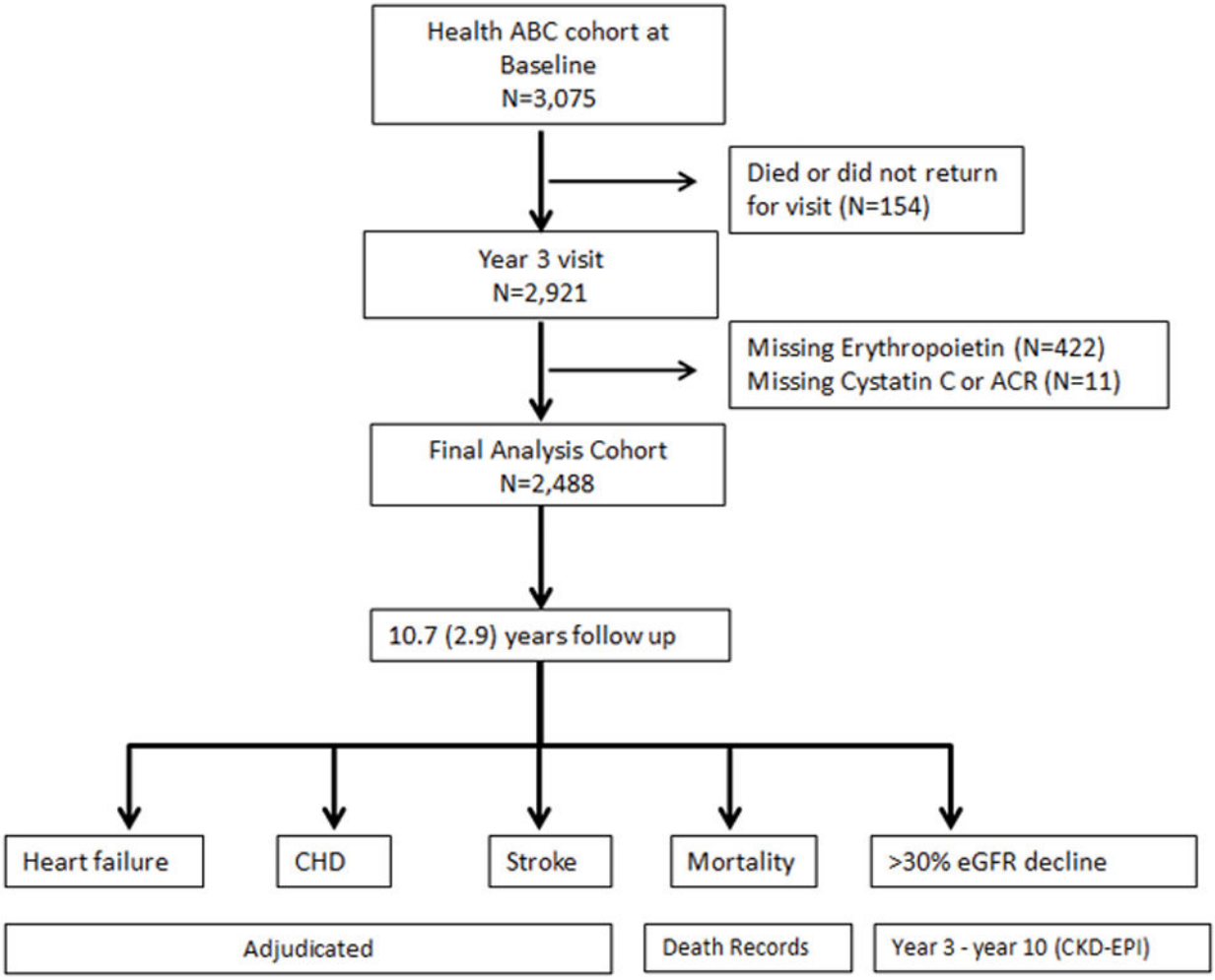

Figure 1.
Flow diagram of Health ABC analysis cohort

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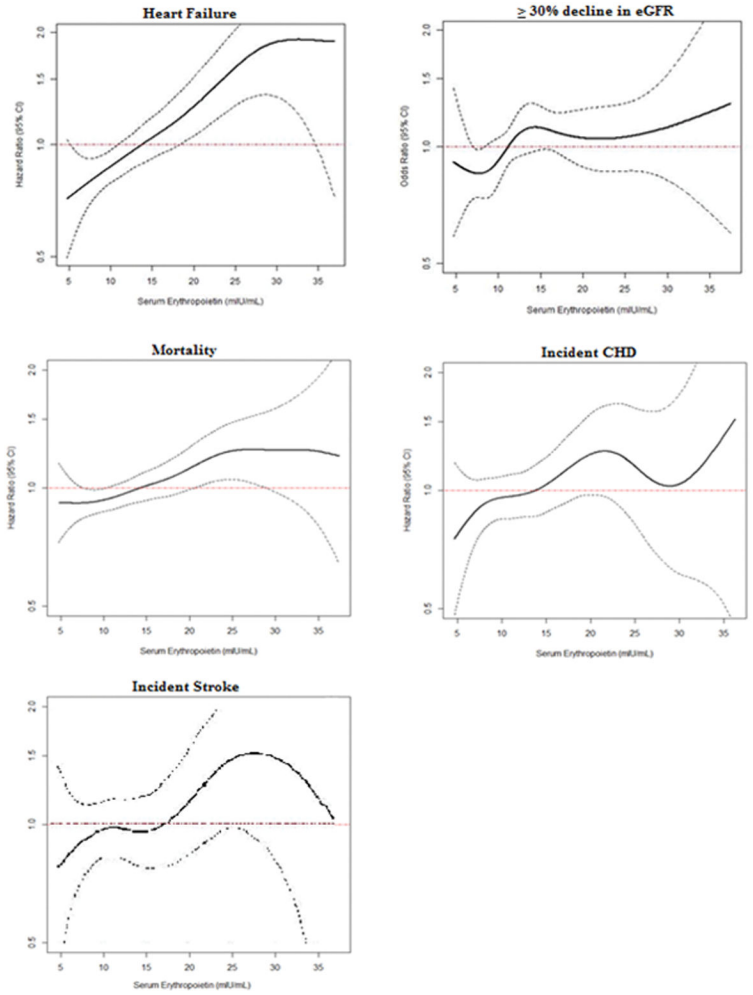

Figure 2. Spline of serum erythropoietin and clinical outcomes

Unadjusted splines show the hazard ratios for the associations between serum erythropoietin and clinical outcomes except >30% decline in kidney function (odds ratio). The solid line represents the point estimate and the dotted lines represent 95% confidence

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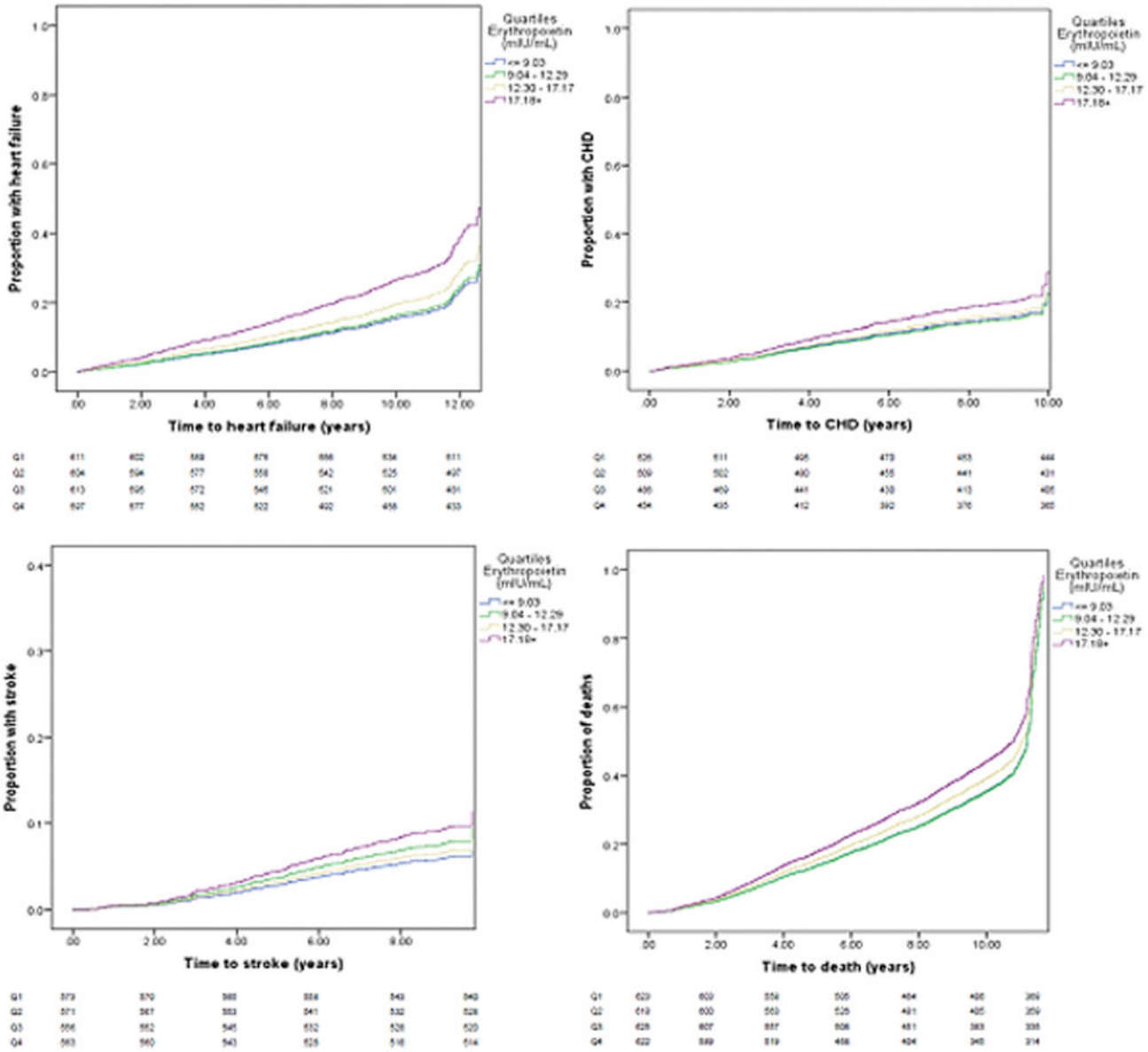

Figure 3. Kaplan Meir curves of clinical outcomes by serum erythropoietin levels

Unadjusted Kaplan Meir curves for incident heart failure, CHD, stroke and death by quartile of serum erythropoietin at year 3. CHD-coronary heart disease

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Table 1

Baseline participant characteristics by quartiles of serum erythropoietin

	Serum erythropoietin (mIU/mL)					
	Q1	**Q2**	**Q3**	**Q4**	**p for trend**	**All**
Range	≤ 9.0	9.1 – 12.3	12.4 – 17.2	> 17.2		
N	623	618	625	622		2488
Age (years)	75.4 (2.8)	75.5 (2.8)	75.9 (3.0)	75.6 (2.8)	0.018	75.6 (2.9)
Male	48	47	47	52	0.184	49
Blacks	32	39	37	46	<0.001	39
Site					0.099	
Memphis	54	49	49	47		50
Pittsburgh	47	51	51	54		50
Diabetes	18	18	21	26	<0.001	21
Hypertension	68	76	76	81	<0.001	75
SBP (mm Hg)	133 (20)	137 (20)	135 (20)	137 (21)	0.004	135 (20)
Smoking					0.659	
Never	43	47	46	43		45
Former	49	45	49	49		48
Current	8	8	5	8		8
CAD	16	18	23	28	<0.001	21
HF	2	2	2	4	0.035	3
CKD	25	24	27	26	<0.001	28
Stroke	7	6	10	8	0.107	8
LVH	9	14	11	12	0.201	12
Anemia[†]	7	13	12	30	<0.001	15
Heart rate	64 (11)	71 (12)	71 (11)	72 (12)	0.120	71 (12)
CRP[*] (mg/L)	1.6 (0.9, 2.9)	1.7 (1.0, 3.1)	1.6 (0.9, 2.9)	1.7 (0.9, 3.2)	0.04	1.7 (0.9, 3.0)
IL-6[*] (pg/mL)	1.7 (1.1, 2.5)	1.7 (1.1, 2.5)	1.7 (1.2, 2.6)	2.0 (1.3, 3.1)	<0.001	1.7 (1.2, 2.7)
TNF-alpha[*] (pg/mL)	2.9 (2.2, 3.7)	3.1 (2.4, 4.0)	3.1 (2.4, 3.9)	3.4 (2.6, 4.4)	0.006	3.1 (2.4, 4.0)
Albumin (g/dL)	4.00 (0.31)	3.99 (0.30)	3.99 (0.30)	3.96 (0.33)	0.044	3.99 (0.31)
Hemoglobin (g/dL)	14.0 (1.2)	13.8 (1.3)	13.7 (1.2)	13.1 (1.5)	<0.001	13.6 (1.3)

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	Serum erythropoietin (mIU/mL)					
	Q1	**Q2**	**Q3**	**Q4**	**p for trend**	**All**
eGFR (ml/min/1.73m^2)	81 (19)	80 (20)	79 (20)	77 (22)	0.002	79 (20)
ACR* mg/g	6.7 (3.6,15.3)	7.2 (4.2,14.5)	7.9 (4.6,16.9)	10.8 (5.8,29.3)	<0.001	7.9 (4.5, 18.7)
BP meds						
ACE-I	14	18	17	24	<0.001	451 (18)
ARBs	5	5	5	5	0.937	130 (5)
Beta blockers	13	17	18	21	<0.001	422 (17)
CCB	19	25	26	30	<0.001	618 (25)
Diuretics	8	10	9	9	0.639	221 (9)

All values expressed as % or mean (SD) except

*median (quartile 1, quartile 3),

†defined as serum hemoglobin <13gm/L in men and <12gm/L in women.

ACR- albumin to creatinine ratio, CAD- coronary artery disease, CKD-chronic kidney disease, CRP- C-reactive protein, eGFR-estimated glomerular filtration rate, HF- heart failure, IL-6- interleukin 6, SBP-systolic blood pressure, TNF-alpha- tumor necrosis factor alpha, BP med- blood pressure medication, ACE-I- angiotensin converting enzyme inhibitors, ARBs- angiotensin receptor blockers, CCB-calcium channel blockers

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Table 2

Association of serum erythropoietin with clinical outcomes

Erythropoietin	Number with events	Unadjusted HR (95% CI)	Risk factor adjusted* HR (95% CI)	Kidney function and hemoglobin adjusted† HR (95% CI)
Incident heart failure				
Continuous (per doubling)	503/2425	1.47 (1.29, 1.66) p<0.001	1.29 (1.13, 1.47) p<0.001	1.24 (1.08, 1.42) p=0.002
Quartiles				
< 9.04	100/611	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	107/604	1.05 (0.79, 1.41)	0.89 (0.66, 1.19)	0.87 (0.66, 1.18)
12.30 – 17.17	132/613	1.36 (1.03, 1.80)	1.10 (0.83, 1.46)	1.03 (0.78, 1.37)
≥ 17.18	164/597	1.83 (1.40, 2.39)	1.34 (1.02, 1.76)	1.25 (1.01, 1.69)
P for trend		<0.001	0.021	0.043
Incident CHD				
Continuous (per doubling)	330/1975	1.16 (1.00, 1.35) p=0.057	1.08 (0.93, 1.26) p=0.335	1.07 (0.91, 1.26) p=0.396
Quartiles				
< 9.04	82/526	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	78/509	0.93 (0.68, 1.38)	0.88 (0.64, 1.21)	0.86 (0.63, 1.19)
12.30 – 17.17	81/486	1.10 (0.80, 1.50)	1.02 (0.75, 1.40)	1.00 (0.73, 1.37)
≥ 17.18	89/454	1.29 (0.95, 1.76)	1.10 (0.80, 1.50)	1.09 (0.79, 1.50)
P for trend		0.182	0.569	0.570
Incident Stroke				
Continuous (per doubling)	161/2263	1.20 (0.97, 1.48) p=0.088	1.08 (0.87, 1.34) p=0.498	1.05 (0.84, 1.32) p=0.655
Quartiles				
< 9.04	33/573	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	43/571	1.24 (0.79, 1.97)	1.14 (0.72, 1.80)	1.14 (0.72, 1.81)
12.30 – 17.17	36/556	1.07 (0.66, 1.72)	0.94 (0.58, 1.52)	0.90 (0.56, 1.46)
≥ 17.18	49/563	1.50 (0.96, 2.34)	1.20 (0.76, 1.90)	1.17 (0.73, 1.87)
P for trend		0.269	0.710	0.661
Total Mortality				
Continuous (per doubling)	1112/2488	1.21 (1.11, 1.31) p<0.001	1.15 (1.06, 1.25) p=0.001	1.09 (1.00, 1.19) p=0.051
Quartiles				
< 9.04	255/623	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	259/618	1.00 (0.84, 1.19)	0.96 (0.81, 1.15)	0.94 (0.79, 1.13)
12.30 – 17.17	290/622	1.13 (0.95, 1.34)	1.07 (0.90, 1.27)	1.01 (0.85, 1.20)
≥ 17.18	308/622	1.35 (1.14, 1.60)	1.22 (1.03, 1.45)	1.13 (0.94, 1.34)
P for trend		0.001	0.030	0.229
≥30% decline in eGFR				
Continuous (per doubling)		1.30 (1.11, 1.52) p=0.001	1.20 (1.02, 1.41) p=0.026	1.02 (0.86, 1.21) p=0.811
Quartiles				

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Erythropoietin	Number with events	Unadjusted HR (95% CI)	Risk factor adjusted* HR (95% CI)	Kidney function and hemoglobin adjusted† HR (95% CI)
< 9.04	66/618	1.00 (ref)	1.00 (ref)	1.00 (ref)
9.04 – 12.29	80/617	1.28 (0.90, 1.82)	1.23 (0.86, 1.76)	1.16 (0.81, 1.67)
12.30 – 17.17	82/620	1.31 (0.92, 1.86)	1.19 (0.83, 1.71)	1.06 (0.74, 1.53)
≥ 17.18	102/621	1.65 (1.18, 2.32)	1.42 (1.01, 2.01)	1.09 (0.76, 1.58)
P for trend		0.035	0.272	0.883

*Adjusted for age, gender, race and site, diabetes, BP medication systolic blood pressure, smoking status, serum albumin, CRP. All models included adjustment for prevalent cardiovascular disease except for the outcome of incident CHD and incident stroke. Models for the HF also included adjustment for heart rate, left ventricular hypertrophy, IL-6, TNF-alpha, and individually for angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers and diuretics,

†further adjusted eGFR, UACR and serum hemoglobin