THE ASSOCIATION BETWEEN HEMOGLOBIN LEVEL AND CANCER INCIDENCE, MORTALITY AND INFLAMMATORY BIOMARKERS IN POST-MENOPAUSAL WOMEN

by

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Andriene Grant, titled 'The Association between Hemoglobin Level and Cancer Incidence, Mortality and Inflammatory Biomarkers in Post-Menopausal Women' and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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DEDICATION

This work is dedicated to

Elsa Joyce and Abner Grant,

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and

to God

in whom all things are possible.

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ABBREVIATIONS

BDNF	Brain derived neurotrophic factor
CaD	Calcium and Vitamin D
CEA	Carcinoembryonic antigen
CK-MB	Creatine kinase-MB
CRP	C-reactive Protein
CT	Clinical Trial
DM	Dietary modification
DXA	Dual-energy X-ray absorptiometry
EPO	Erythropoietin
Fe	Iron
Hb	Hemoglobin
ICAM-1	Intercellular adhesion molecule 1
IFN-gamma	Interferon gamma
IL-1 alpha	Interleukin-1 alpha
IL-1 ra	Interleukin-1 receptor antagonist
IL-10	Interluekin-10
IL-6	Interluekin-6
LDD	Least detectable dose

ABBREVIATIONS – *Continued*

MDC	Macrophage derived chemokine
NHANES	National Health and Nutrition Examination Survey
OS	Observational Study
PAI-1	Plasminogen activator inhibitor-1
PHT	Post-menopausal hormone therapy
SHBG	Sex hormone binding globulin
SHR	Sub-hazard ratio
TNF-alpha	Tumor necrosis factor alpha
TNF-beta	Tumor necrosis factor beta/lymphotoxin alpha
TNFR1	Tumor necrosis factor receptor 1
TNFR2	Tumor necrosis factor receptor 2
VCAM-1	Vascular cell adhesion molecule 1
WHI	Women's Health Initiative
WHO	World Health Organization

ABSTRACT

Background: Knowledge regarding the associations of (i) hemoglobin level (Hb) prior to cancer diagnosis and cancer mortality (ii) the full range of Hb and cancer incidence and (iii) baseline inflammatory/other biomarkers and Hb in older populations is limited. The present study examined the associations of anemia status/Hb with cancer incidence and mortality, as well as the association with inflammatory biomarker levels in post-menopausal women.

Methods: Anemia was defined as Hb < 12 g/dl, while high Hb was defined as Hb >= 15 g/dl, or >= 16 g/dl. Associations were determined in three Women's Health Initiative Study sub-populations. The association between anemia/Hb with cancer mortality was determined in women without (N=21,021) or with (N=2,976) cancer history who had cancers on follow-up. The cross-sectional association of biomarkers and anemia/Hb was determined on 1,001 women with these available data. Finally, the association between anemia/Hb with cancer incidence was determined in women enrolled in the Observational Study/Clinical Trial who did not have a history of cancer/extreme energy intakes/missing follow-up time (N=140,269).

Results: Anemia was associated with a 21% higher hazard of total cancer death in participants with, and a 55% greater hazard in participants without cancer history. Anemic women with a history of cancer had twice the hazard of colorectal cancer death. C-reactive protein, TNF-alpha, TNF-beta and TNFR2 were significantly associated with anemia. IL-1 alpha and IL-10 were significantly associated with continuous Hb. Anemia was not associated with cancer incidence in the total population, but anemic African-American women had a reduced risk of any cancer incidence which was not observed in white women (p-interaction=0.03). Women with high Hb had an increased hazard of any (HR: 1.37; 95% CI: 1.17, 1.60) or breast cancer (HR: 1.42; 95% CI: 1.10, 1.84) incidence.

Conclusions: Anemia determined prior to cancer diagnosis was associated with total and colorectal cancer death. High Hb was associated with increased risk of total cancer and

breast cancer incidence. Anemia was associated with elevated levels of C-reactive protein, TNF-alpha, TNF-beta and TNFR2, while continuous Hb was associated with IL-1 alpha and IL-10. Further research is required to confirm associations and clarify causal mechanisms.

CHAPTER 1: INTRODUCTION

Anemia is a condition of global significance. It is estimated that 1.6 billion individuals (or 24.8% of the global population) are anemic[1]. Although children have the highest anemia prevalence worldwide[1], in the United States (U.S.) prevalence is greatest in adults aged 85 years and older[2]. The prevalence of anemia in the U.S. for the period 1988-1994 ranged from 1.5-26% depending on age and gender, and one in every 10 individuals (11%) over the age of 65 years in the U.S. was estimated to be anemic[2]. High anemia prevalence in the elderly is an important public health problem, as the condition is associated with a number of negative health outcomes including increased all-cause[3] and cancer mortality [4]. Approximately six hundred thousand (577,190) deaths from cancers occurred in the U.S. in 2012[5]. It is therefore important to study modifiable factors which could mitigate cancer mortality risk, given the magnitude of cancer deaths. Anemia is one such condition as it may be ameliorated, in some instances, by administration of oral or parenteral iron or other therapies[6].

Anemia is an independent prognostic factor of mortality in lung[4], head and neck[4], prostate[4], uterine[4], breast[7] and colorectal[8] cancer patients, but there is limited knowledge of when the association with cancer mortality begins. The condition may result from blood loss or inflammation which may be associated with cancer incidence, and hemoglobin levels may decrease prior to cancer diagnosis[9]. It is an important symptom which may be predictive of colorectal cancer diagnosis in clinical practice[10], but little is known of the association with cancer risk in a population-based setting. There also appears to be no literature on the association of anemia determined prior to cancer diagnosis with subsequent cancer mortality.

Although associations between anemia and various cancer mortalities have been demonstrated, risk of cancer incidence or mortality when hemoglobin is high has not

been fully elucidated. There is no established definition of a high hemoglobin level beyond the normal range of 12-16 g/dl in non-pregnant women[11]. Zakai et. al (2005) reported a 17% increased all-cause mortality risk in the highest hemoglobin quintile when compared with the reference, even after controlling for important confounders[3]. A corresponding association of high hemoglobin level with cancer mortality has not been extensively studied, though deaths from cancer may account for a modest proportion of total deaths attributed to high hemoglobin[12].

Hemoglobin level may be affected by dietary iron intake or inflammatory markers. Iron may play a role in carcinogenesis through the generation of reactive oxygen species[13], and dietary intake may be associated with breast[14] and colorectal[15] cancer incidence, though evidence is conflicting[16,17]. It is therefore important to determine whether the association of hemoglobin level with cancer incidence and mortality is independent of dietary iron intake.

Elevated levels of pro-and anti-inflammatory cytokines such as interleukin-6, interleukin-1, TNF-alpha and interleukin-10 may influence anemia pathogenesis by increasing ferritin expression and macrophage iron uptake, retarding erythropoietin expression and inhibiting erythroid precursor growth, making less iron available for erythropoiesis, and leading to diminished growth and survival of red blood cell precursors[18]. C-reactive protein, an acute phase reactant, is the biomarker most extensively studied with anemia level in observational studies. There is a dearth of literature on the association of inflammatory markers such as TNF-alpha and IL-10 with hemoglobin concentration in population-based observational studies of older individuals, and there is limited knowledge of whether these associations vary by race/ethnicity, age, or obesity status.

The study aims were therefore designed to use data from the Women's Health Initiative (WHI) Study to further clarify the association of anemia status and hemoglobin level with (i) cancer incidence and mortality, as well as (ii) biomarkers associated with

inflammation. The study explored associations with total, breast and colorectal cancer incidence and mortality. Breast and colorectal cancers accounted for the largest proportion of cancer deaths in the WHI, and were the first and third leading causes of cancers occurring during follow-up. The study also investigated the association between dietary iron intake and total cancer incidence.

The aims of the dissertation were:

(1) To investigate the association between anemia status and/or hemoglobin level with cancer mortality, controlling for potential confounding factors such as age and race/ethnicity;

We hypothesized that:

a. (i) anemia status and (ii) hemoglobin level are associated with cancer mortality, and that age and race/ethnicity modify these associations

(2) To investigate the association between anemia status and/or hemoglobin level with inflammatory biomarkers, controlling for potential confounding factors such as age, race/ethnicity and obesity status.

a. We hypothesized that anemia status and hemoglobin level are associated with inflammatory biomarkers, and that (i) age (ii) race/ethnicity and (iii) obesity modify these associations

(3) To investigate the association between anemia status, hemoglobin level, and dietary iron with cancer incidence;

We hypothesized that:

a. (i) anemia status and (ii) hemoglobin level are associated with cancer incidence, and that age and race/ethnicity modify these associations

As secondary aims we investigated the association between persistent anemia status and cancer mortality and incidence, as well as the associations of dietary iron and red meat intakes with cancer incidence.

Chapters 2 and 3 of the dissertation present a review of the epidemiology of anemia and high hemoglobin level, as well as the biology and epidemiology associated with colorectal and breast cancers. Chapter 4 will provide a brief background of the Women's Health Initiative Study, including aims, inclusion and exclusion criteria, outcomes and main findings. Chapters 5 to 7 present the background, methods, results and discussion for the studies addressing the three main aims outlined above. Finally, in Chapter 8 an overall conclusion will be provided which summarizes the main findings, study strengths and limitations, and provides suggestions for future research.

CHAPTER 2: ANEMIA AND HIGH HEMOGLOBIN LEVEL

Introduction

This chapter will cover the definition, diagnosis and epidemiology of anemia. It will briefly describe factors affecting iron homeostasis, including erythropoiesis, and the role of biomarkers in this process, It will also provide a discussion of the associations between anemia and chronic health conditions, including cancers. Finally, the chapter will include a brief description of the definition, causes of, and factors associated with high hemoglobin levels.

Anemia

Anemia Definition and Types

Anemia is defined as a hemoglobin level less than 12 g/dl in non-pregnant women, and less than 13 g/dl in men[19,20]. There are varying sub-types, but the most common forms in the elderly are nutrient deficiency anemia (including iron deficiency anemia), anemia of inflammation and anemia of unknown origin[2]. According to Handelman (2008) iron-deficiency anemia occurs when 'storage iron declines until iron delivery to the bone marrow is insufficient for erythropoiesis'[21]. This results when storage iron falls below 200 mg[21].

Megaloblastic anemia originates from abnormalities of red cell precursors in the marrow[22]. The two most common forms are vitamin B12 (cobalamin) and folate deficiency[22]. Pernicious anemia is one form of vitamin B12 deficiency which results from the autoimmune destruction of gastric mucosa[23]. Other causes of cobalamin deficiency are vegan diet, gastrectomy, malabsorption or ileal resection[23]. Causes of folate deficiency may include poor nutrient intake, low bioavailability, losses during food

preparation, alcoholism and use of medications such as methotraxate[24], phenytoin or trimethoprim/sulfamethoxazole[25].

Anemia of chronic disease is also called anemia of inflammation[21,2], and in this form there may be adequate iron stores, but they are not available for red blood cell formation[21]. Mechanisms associated with pathogenesis are discussed later in the Chapter. There are other less common forms, including anemia of renal disease and anemia of heart disease, which may occur due to disruption of erythropoietin production or action, or due to the effects of chronic inflammation[21].

Table 1 lists the causes of anemia in adults aged 65 years and older using data from the Third National Health and Nutrition Examination Phase III (NHANES III) Survey. Thirty-four percent (34%) of the anemia cases identified in the survey were attributable to nutrient deficiency, while approximately one third were due to each anemia of inflammation and unexplained anemia[2].

Table 1 Causes of Anemia in Adults Aged 65 Years and Older

Anemia Cause	Percent
Nutrient Deficiencies	**34.3**
Iron only	16.6
Folate only	6.4
B12 only	5.9
Folate & B12	2.0
Iron with Folate/B12	3.4
Inflammation/Chronic Disease	**32.2**
Renal insufficiency only	8.2
ACI only	19.7
Renal insufficiency and ACI	4.3
Unexplained Anemia	**33.6**

Adapted from. Guralnik et. al . *Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia*. Blood. 2004 104: 2263-2268

Key

ACI = Anemia of Chronic Inflammation

Diagnosis of Anemia

Anemia is diagnosed according to the WHO (1968) criterion of a hemoglobin level of less than 12 g/dl in women[19]. Further criteria are then required to diagnose sub-types. There have been challenges in distinguishing anemia of inflammation and iron-deficiency anemia, as ferritin (the main indicator of body iron stores) is also elevated in conditions associated with inflammation. Further tests are required to identify other nutrient deficiencies. Tests for vitamin B12 deficiency include (1) total cobalamin (2) methyl-alonic acid (MMA) and (3) holotranscobalamin [26] while tests for folate deficiency include serum and red cell folate, total homocysteine and methyl-malonic acid[22].

Epidemiology of Anemia

There are few published studies which have produced anemia incidence estimates[27]. Ania et. al (1997) reported an anemia incidence rate of 78 per 1000 person years in a

representative sample of Olmstead County residents aged 65 years and older[28]. Eight percent (8%) of participants in the InCHIANTI study who were free of anemia at baseline went on to develop anemia over a three year period of observation[29], while 7% of Korean adults aged 60 years and older developed anemia over a similar three-year period[30].

Anemia prevalence estimates are more readily available. McClean et. al (2008) utilized World Health Organization (WHO) Vitamin and Mineral Nutrition Information System data to estimate a global anemia prevalence of 24.8%[1]. In the United States the prevalence of anemia in the elderly has ranged from 8% to 52% depending on the population studied (Table 2). Guralnik et. al (2004) used data from NHANES III, a representative survey of the non-institutionalized U.S. population, to report an overall prevalence of 11%, in adults aged 65 years and older. However, prevalence proportions varied greatly by age, gender and race/ethnic group (Table 2)[2]. Women have higher prevalence proportions of anemia from ages 1 to 74 years, but men have a higher prevalence from age 75 onwards[2].

Risk factors Associated with Anemia

Nutrient deficiency anemia, anemia of inflammation and unexplained anemia all vary by race/ethnicity and age[2]. Alcohol consumption was found to be protective for iron deficiency anemia[31], while conditions associated with malabsorption, including celiac disease[23], may be associated with nutrient deficiency anemia and anemia of inflammation. The association between elevated cytokines in individuals diagnosed with nutrient deficiency anemia and anemia of inflammation has been reported in NHANES III[2] and InCHIANTI studies[32], while individuals with anemia of inflammation or unexplained anemia may have increased comorbidity status[2], or the presence of other inflammatory conditions[2]. Anemia may also be caused by medication use or cancer treatment. ACE inhibitors have been associated with anemia in heart failure patients[33], and anemia is a common complication of myelosuppressive chemotherapy[34] and radiotherapy[35].

Key Factors in Iron Metabolism and Erythropoiesis

The definitions of anemia or high hemoglobin are primarily based on blood hemoglobin concentration. Red blood cells (also called erythrocytes) are crucial to the delivery of oxygen to the lungs and tissues in the body[25], and iron is a critical component of hemoglobin[25]. Iron homeostasis and the recycling of hemoglobin are supported by the mononuclear phagocyte system (MPS, formerly called the reticuloendothelial system). The MPS is comprised of promonocytes, monocytes and macrophages[36]. Monocytes are formed in the marrow, and migrate in the bloodstream to tissues of target organs such as the spleen, liver, intestines, and kidney, where they differentiate into macrophages[37]. Macrophages of the MPS are also found in the bone marrow[37]. Lazar et. al (2005) have indicated that one of the main functions of the MPS involves phagocytosis of foreign particles and 'self-tissues' such as senescent red blood cells[36].

Iron metabolism is tightly regulated[21,37]; according to Andrews and Schmidt (2007), the main factors regulating iron homeostasis are (1) erythroid iron needs (2) hypoxia (3) iron deficiency and (4) iron overload and inflammation[38]. Most of the iron used to produce new blood cells is derived from the breakdown of red blood cells by macrophages of the MPS[37], which occurs primarily in the spleen[38]. In addition to senescent erythrocytes, a small proportion of iron is absorbed from the large intestine to supplement the body's needs. Dietary iron may be classified as *heme* or *non-hem*e, and is absorbed in the gut by different pathways[38]. Ferroportin is found on the surface of cells of the MPS, and is the main protein responsible for delivering iron from the intestinal mucosa to transferrin, which transports iron to the bone marrow[21,38]. Ferroportin is also the protein responsible for delivery of iron found in storage sites of the MPS to transferrin in the circulation. Ferroportin is degraded in instances of inflammation, resulting in less iron being released from storage cells of the MPS[21].

All blood cells, including erythrocytes, are formed from hematopoietic stem cells in the bone marrow[39]. Hematopoietic stems cells are differentiated into myeloid and lymphoid

lineages, with erythrocytes being derived from the myeloid line[39]. 'Erythropoiesis' is the process by which the bone marrow produces red blood cells (erythrocytes)[25], and this occurs in various stages. The first stage involves the growth of erythroid progenitor cells under the influence of specific growth factors. Burst forming erythroid units (BFU-E) are the first recognizable erythroid progenitors, and their growth is stimulated by stem cell factor (SCF), interleukin-3 (IL-3) and erythropoietin. BFU-E then matures into colony forming erythroid units (CFU-E) which are primarily stimulated by erythropoietin[39]. In the second stage these progenitors progress into erythroblast precursors which accumulate hemoglobin[39]. In the final stage the nuclei of mature erythrocytes are expelled, allowing new red blood cells to enter the bloodstream.

Excess iron in the MPS system that is not utilized is stored in ferritin[37]. As levels of unutilized iron increase, iron storage diverts to hemosiderin which is an insoluble protein that allows for greater iron storage per unit cell volume[37]. The main sites of iron stores are the liver, spleen and bone marrow[37].

The Role of Inflammatory Markers in Erythropoiesis

Pro and anti-inflammatory cytokines may also play a role in erythropoiesis. IL-6 (and to a lesser extent IL-1 alpha and IL-1 beta)[40] stimulate the production of hepcidin. Hepcidin is produced in the liver, and regulates iron homeostasis by promoting iron storage and reducing intestinal iron absorption[41]. Elevated levels of hepcidin lead to the degradation of ferroportin which prevents the release of iron stores from the MPS[41]. IL-1 alpha and TNF-alpha have been shown to inhibit erythroid progenitor growth[42,43], and erythropoietin expression[44] but to stimulate ferritin expression[45,46] in vitro. Interferon-γ also inhibits the growth of erythroid precursors[47], while IL-10 increases ferritin transcription[48,49] in vitro. CRP increases with inflammation, and is indicative of an inflammatory state which is associated with reduced storage iron[21]. Inhibition of erythroid precursors and erythropoietin expression would lead to diminished red blood cell production, while enhanced ferritin expression would lead to greater iron stores,

resulting in less iron being available for erythropoiesis. These factors play a role in the development of anemia of inflammation[18].

The Association with Adverse Health Outcomes, Including Cancers

Anemia is associated with a number of negative health outcomes. It is a risk factor for cardiovascular disease[50], is associated with poor cognition[51], depression[52], disability[53] and poor quality of life[54], and is associated with increased mortality in individuals with heart failure[55] and cancers[4]. The diagnosis of anemia in cancer patients is a common phenomenon, resulting from the action of chemotherapeutic agents, cytokines, nutrient deficiency and bleeding, among other causes[56].

Anemia is a prognostic indicator of survival in individuals with cancer [4]. The condition is associated with poor survival in breast cancer patients[7,57,58,59,60], though null associations have also been reported[61,62]. Anemia is also associated with poorer prognosis[8,10,63,64,65] in individuals with colorectal cancer. Studies exploring the association of anemia with breast and colorectal cancers have primarily been retrospective in nature, and have involved patients already diagnosed with cancer. In addition, different measures of hemoglobin have been used in analysis such as pre-treatment hemoglobin, or nadir hemoglobin level.

High Hemoglobin

Definition

There appears to be no standard definition for a high hemoglobin concentration. However, the hemoglobin normal range is 12-16 g/dl for women and 14-17 g/dl for men[11]. If a definition of 2 standard deviations outside of the mean reference population is used, then a high hemoglobin level would generally fall within the range of 15.5 to 16 g/dl for women[66]. Yip (2000) has proposed that a hemoglobin level of 16-17 g/dl could

be classified as a mildly high hemoglobin concentration, while a level greater than 17 g/dl could be classified as moderately high hemoglobin concentration[66].

Causes

High hemoglobin levels result from increased red blood cell production or a reduction in plasma volume[66]. Residence at high altitude, cigarette smoking and polycythemia vera are associated with increases in red blood cell mass or elevations in hemoglobin level[66]. The most common cause of reduction in plasma volume is severe dehydration[66].

Factors Associated with High Hemoglobin Levels

Factors associated with high hemoglobin level include residence at a high altitude, cigarette smoking, dehydration, use of or increased secretion of erythropoietin, polycythemia vera, lung disease, and congenital heart disease[66,67,68].

Association with Health Outcomes, Including Cancers

High hemoglobin levels may be associated with excess all-cause mortality[3,69,70], heart disease[70], diabetes[71] and Alzheimer's disease[72]. Studies have reported positive associations between hemoglobin level and metabolic syndrome[73,74] and hemoglobin levels and blood pressure[75], although mean hemoglobin levels in the study populations did not exceed the normal range. There is limited evidence of an association between high hemoglobin level and cancer risk or mortality in literature. However, randomized clinical trials of epoietin alpha and beta to increase hemoglobin levels in cancer patients indicated shorter survival[76,77] and increased disease progression[77].

Table 2 U.S. Prevalence Estimates of Anemia in Older Adults Stratified by Gender and Race/Ethnicity

Study	Cohort/ Study Name	Year(s) Samples Collected	Population	Study Design	Age Dist.	Prevalence (%)					
						Total	**Women**	**Men**	**NHW**	**African American**	**Mexican American**
Guralnik et. al 2004	NHANES III	1988-1994	National probability sample of civilian non-institutionalized population	Nationally representative survey. Children, older persons, African American and Mexican-Americans oversampled.	> 65 years	10.6	10.2	11.0	9.0	27.8	10.4
Zakai et. al 2005	Cardiovascular Health Study	1989-1990; 1992-1993	Residents of 4 counties: Forsyth County, NC; Sacramento County, CA; Washington County, MD Allegheny County, PA.	Prospective Cohort Study.	> 65 years	8.5	8.1	9.2	7.0	17.6	NM
Denny et. al 2006	The Duke Established Populations for Epidemiologic Studies of the Elderly.	1992	Residents living in and adjacent to Durham County, NC. African Americans were over-sampled	Prospective Cohort Study. Blood samples collected at the 6th annual contact.	71 – 102 years	24.0	23.9	24.7	13.8	33.6	NM
Patel et.	Health ABC	1997-1998	Community-	Prospective	71 –	NR	13.1	17.7	7.0,	20.9,	NM

Study	Cohort/ Study Name	Year(s) Samples Collected	Population	Study Design	Age Dist.		Prevalence (%)			
al 2007	Study		dwelling elderly living in Memphis, TN and Pittsburgh, PA	Cohort Study. Blood samples collected 2 years after baseline	82 years				women 13.7, men	women, 25.5 men
Haslam et. al 2011	Georgia Centenarian Study	2002-2005	Centenarians and octogenarians of 44 counties of GA	Population-based survey	83-109 years	26.2% octogenarian 52.1% centenarian	NR	NR	NR	NR

Adapted from Patel K. The Epidemiology of anemia in older adults. *Semin Hematol*. 2008 October ; 45(4): 210–217
Abbreviations: Dist. = Distribution. NM = Not Measured; NR=Not Reported; NHW = Non-Hispanic White

CHAPTER 3: OVERVIEW OF BREAST AND COLORECTAL CANCER

This chapter will cover breast and colorectal cancer biology and epidemiology, as well as mention factors which affect breast and colorectal cancer incidence and survival.

The Global and National Impact of Cancer

The International Agency for Research on Cancer (IARC) indicates that 12.7 million cancer cases and 7.6 million cancer deaths occurred worldwide in 2008[78]. Approximately 1.6 million new cases of cancer and 600,000 deaths were projected to have occurred in the United States (U.S.) in 2012. Breast cancer was the leading cause of incident cancer in women in the U.S. followed by cancers of the lung and bronchus, and colon and rectum[5]. The leading causes of cancer deaths in women were from cancers of the lung or bronchus, breast, and colon and rectum[5]. The lifetime probability of developing an invasive cancer in the U.S. is estimated to be 45% for men and 38% for women[5]. Approximately 1 in 8 women in the U.S. will have breast cancer during their lifetime[79], while the lifetime risk of developing colorectal cancer is approximately 5% in males and females[80].

Breast Cancer

Biology

The breast is comprised of glands, termed ducts and lobes, as well as fat and connective tissue[81]. A breast lobe begins with a single duct which opens at the nipple, which branches into a network of major and lesser ducts ending in terminal ductal lobular units[82]. Glands are formed from epithelium, of which there is a lower layer of myoepithelial cells and an upper layer of luminal cells, which are responsible for milk

secretion. Terminal ductal lobular units are the site of origin for all precancerous and cancer lesions[81].

The majority of breast abnormalities are benign[81]. When a breast cancer occurs it may be confined to the area of origin, and these cancers are termed *in situ*. However, most breast cancers are invasive[79]. Invasive ductal carcinoma is the most common type of invasive breast cancer[83]. The extent of invasion may be determined by cancer staging. The TNM system is based on the primary tumor (T), lymph node involvement (N) and distant metastases (M)[84]. SEER staging indicates whether tumors are local, regional or distant[84].

According to Rakha and Ellis (2009), breast cancer is no longer thought to be a homogenous group of cancers, but, rather, comprise different diseases that vary by morphology, biology and response to therapy[85]. The four main classes of breast cancer are (i) basal-like breast cancer (ii) luminal A cancers (ii) luminal B cancers and HER2 positive cancers[86]. These classes are useful in predicting how well a tumor will respond to treatment[87]. HER2 positive cancers show amplification of the ERBB2 (HER2) gene. HER2 positive tumors are more aggressive than HER2 negative tumors. Basal-like tumors closely correspond with triple negative tumors[85], though it may be argued that the two types of cancer are not synonymous[85]. Triple negative cancers are estrogen receptor negative, progesterone receptor negative and HER 2 receptor negative[86] which are generally more aggressive[85]. Luminal tumors express genetic markers typical of luminal epithelial cells of the normal breast[86].

Epidemiology

Global incidence of breast cancer in 2008 was estimated at 42 cases per 100,000 population[88]. Incidence in the U.S. for the period 2004-2008 was 121 per 100,000 person years[5]. The mortality rate was 23.5 per 100,000 person years[5]. Breast cancer incidence remained stable in the U.S. for the period 2005-2008[5]. However, incidence rates in other parts of the world such as in selected Asian countries (Japan, Hong Kong, Singapore,

Taiwan and Korea) increased over the period 1993 to 2002[88]. Incidence in non-Hispanic white women living in the U.S. for the period 2004-2008 was 125 per 100,000 population in comparison to 116 per 100,000 population in African-American women[79]. Incidence in women of other race/ethnic groups was much lower than rates for white or African-American women[79]. Breast cancer mortality rates have been on the decline since 1990[5].

Risk factors for breast cancer may be separated into those that are modifiable or non-modifiable (Table 3).

Table 3 Breast Cancer Risk Factors

Non-modifiable	**Modifiable**
Gender	Nulliparous
Age	Older age at first birth
Genetics	No breastfeeding
Family History	Hormone Replacement Therapy
Race/Ethnicity	Birth control pills
Breast density	Geography
Early menarche	Alcohol consumption
Late menopause	Obesity after menopause
Exposure to radiation	Physical inactivity
Height	
Increased Bone Density	

Source: Adapted from Thomas P. *Chapter 1 Overview.* In: Thomas P, editor. Breast Cancer and its Precursor Lesions. Making Sense and Making it EarlyNew York Dordrecht Heidelberg London: Springer; 2011. p. 1-6.

Breast cancer increases with age[89], and is more likely to occur in women[79]. Overall incidence is higher in non-Hispanic white women than women of other race/ethnic groups. However, African American women below 40 years of age have higher incidence rates than white women in the same age range[79]. Women with a first degree relative who had breast cancer, as well as women who have had mutations in BRCA1 and BRCA2

genes are at increased risk of developing breast cancer[79,89]. Factors related to increased hormone exposure over a woman's lifetime such as early menarche, nulliparity, late menopause and use of menopausal hormones (HRT) also increase risk[79]. The incidence of breast cancer is higher in developed countries compared with developing countries, and is likely to be due to lifestyle changes such as fewer births and older age at first birth[88]. Lifestyle-related factors such as obesity (post-menopausal), alcohol consumption and physical inactivity may also increase risk[89].

Factors influencing breast cancer survival include stage at diagnosis, age at diagnosis, race/ethnicity, socioeconomic status, lifestyle-related factors and tumor characteristics[79]. Women with advanced stage disease have lower survival. In addition, women diagnosed with breast cancer prior to the age of 40 years have lower survival than their older counterparts[79]. Although older African American women have lower incidence than white non-Hispanic women, they also have lower survival[79]. Socioeconomic factors such as poverty, lack of health insurance and lack of access to care also negatively influence survival[79]. Hormone receptor negative tumors, HER2 positive tumors and triple negative tumors are associated with worse prognosis[79].

Colon Cancer

Biology

The colon and rectum are components of the gastrointestinal tract. The colon is approximately five feet long and is responsible for absorption of water and minerals from waste[80]. The rectum is approximately six inches long, and is the passage from the colon to the anus for the expulsion of waste[80]. According to the American Cancer Society (2011), the colon comprises four sections; these are (i) the ascending colon, which is attached to the small intestine and ascends upwards on the right side (ii) the transverse colon which crosses from the right to left side (iii) the descending colon which is on the left side the abdomen and (iv) the sigmoid colon which joins the colon to the rectum[80].

Colon cancer originates from hyperplastic or adenomatous polyps in the mucosa of the colon[90]. The development of cancer is a slow process which may take as long as 10 to 15 years[80]. The majority of colon cancers originate from adenomas and the risk of cancer increases with increasing number of polyps[90]. The majority of colon cancers are adenocarcinomas[80] and approximately 50% of colon cancers are right-sided[90].

Epidemiology

The projected incidence of colorectal cancers over the period 2004 to 2008 in the U.S. was 55.7 per 100,000 population in males, and 41.4 per 100,000 population in females[5]. Colorectal cancer incidence has been on the decline in the U.S. since the mid-1980s[5,80], and this has mainly been attributed to increases in cancer screening and polyp removal[5,80]. Incidence rates vary by geographical region worldwide, with the highest rates occurring in the U.S., Europe, New Zealand and Australia, and the lowest rates occurring in Africa[78]. Incidence also varies by race/ethnicity, with rates being the highest in African-American males and females when compared with individuals in other race/ethnic categories[80]. Projected colorectal cancer mortality rates in the U.S. were 20.7 per 100,000 population in males, and 14.5 per 100,000 population in females[5]. Colorectal cancer mortality rates have been on the decline since 1980 for males and 1950 for females[80]. Mortality rates are higher in African American men and women[80].

Risk factors for colorectal cancer are given in Table 4.

Table 4 Colorectal Cancer Risk Factors

Unmodifiable	**Modifiable**
Age	High fat diet
Previous history of colorectal cancer	Low fruit/vegetable consumption
Personal history of polyps	Physical inactivity
Family history of CRC or adenomatous polyps	Obesity
Inherited genetic risk	Smoking
Personal history of inflammatory bowel disease (Crohn's disease or ulcerative colitis)	Alcohol consumption

Sources:

1. Benson AB,3rd. Epidemiology, disease progression, and economic burden of colorectal cancer. *J.Manag.Care.Pharm.* 2007 Aug;13(6 Suppl C):S5-18.
2. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. *Clin.Colon Rectal Surg.* 2009 Nov;22(4):191-197.

Risk factors include age (greater than 90% of colorectal cancers occur in persons over the age of 50), personal history of adenomatous polyps, inflammatory bowel disease, family history of colorectal cancer or adenomatous polyps, inherited genetic risk, diets high in fat or high in meat consumption, physical inactivity, excess body weight, cigarette smoking and alcohol consumption[92]. The two most common inherited conditions are familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome[92]. Individuals with Lynch Syndrome may develop other cancers, but the risk of developing colorectal cancer is highest[80]. FAP is a rare condition, accounting for less than one percent of colorectal cancers[92]. However, the majority of individuals with the condition will develop colorectal cancer. Individuals with FAP develop hundreds of polyps which will develop into malignancy if the colon is not removed[92]. In contrast, individuals with Lynch Syndrome develop few polyps, but cancers resulting from this condition account for 2-6% of all cancers, and the lifetime risk of colorectal cancer is 70-80% in individuals with the syndrome[92]. Inflammatory bowel disease is used to describe individuals with Crohn's disease or ulcerative colitis. Both conditions appear to result in increased colorectal cancer risk[92].

According to the American College of Pathologists (2000), prognostic factors which are supported by strong evidence or which have been extensively studied include the local extent of the tumor, lymph node metastasis, blood or lymphatic vessel invasion, residual tumor following surgery, tumor grade, radial margin status and residual tumor in the resection specimen[93]. Other factors associated with colorectal cancer mortality include smoking status[94], race/ethnicity[95] and socioeconomic status[96]. Current smokers, black/African American individuals and individuals of low socioeconomic status are at increased risk of colorectal cancer mortality. Post-menopausal hormone usage may reduce mortality risk[97,98] while diabetes mellitus is associated with greater mortality[99].

CHAPTER 4: THE WOMAN'S HEALTH INITIATIVE STUDY

Introduction

The Women's Health Initiative (WHI) was a large scale study involving 161,809 women aged 50 -79 years, designed to investigate factors which could lower the risk of cancer, cardiovascular disease and fractures in post-menopausal women. The study encompassed varying components with different hypotheses and inclusion and exclusion criteria, and these are discussed in the following paragraphs. The Chapter will also cover factors associated with enrollment into the Genetic Factors Related to Sarcopenia Study, which is an ancillary study of the WHI.

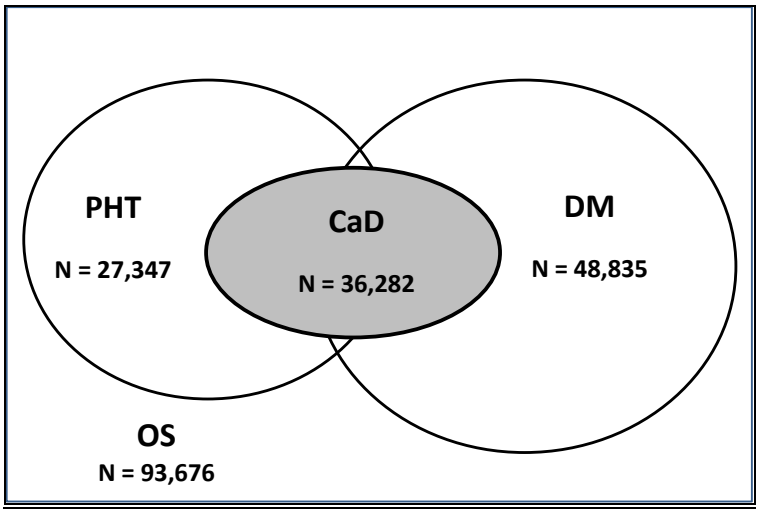
Study Components

The two main arms of the WHI were the clinical trial (CT) and the observational study (OS). The CT comprised three overlapping components. These included the dietary modification (DM) trial which was hypothesized to lower breast and colorectal cancer risk[100]. According to Anderson et. al (2003) the DM trial randomly assigned 48,836 women to a low fat diet or self-selected dietary behavior[101]. The postmenopausal hormone therapy (PHT) component comprised two trials designed to test the hypothesis that estrogen replacement therapy reduces the risk of coronary heart disease and fractures. The first trial randomized 10,739 women who had a hysterectomy to receive conjugated equine estrogen or placebo. The second trial randomized 16,608 women to estrogen plus medroxyprogesterone or placebo treatments[101]. A partial factorial design allowed women to enroll in the DM, PHT or both[102]. Approximately eight thousand women (8,050) were randomized to both the DM and PHT[101]. Women who joined the DM or PHT were invited at their first or second annual clinic visit[102] to join a Calcium and Vitamin D (CaD) trial which tested the hypothesis that administration of calcium and vitamin D would lower the

risk of hip fractures, other fractures and colorectal cancer[100]. The CaD trial randomized 36,282 women to receive 1,000 mg elemental calcium plus 400 international units of vitamin D3 daily, versus placebo[101].

The OS was designed to improve knowledge of risk factors for a number of diseases including cardiovascular disease, fractures and cancer[100]. Women were entered into the OS if they were interested in the dietary modification or hormone therapy trials, but were unwilling or ineligible to participate in the CT[103]. Figure 1 summarizes the number of women enrolled in each arm of the WHI.

Figure 1 Numbers Enrolled in WHI Study Components



Abbreviations:
CaD = Calcium and Vitamin D
DM=Dietary Modification
PHT = Post-menopausal Hormone Therapy

Source: Design and Protocol. Women's Health Initiative.
https://cleo.whi.org/about/SitePages/Design%20and%20Protocol.aspx

Recruitment and Enrollment

Women were recruited from 40 centers in twenty-four states[102] between 1993 and 1998[103]. Ten of the forty centers were selected as minority recruitment sites, and were

expected to have 60% minority enrollment[102]. Mass mailing was the most popular recruitment strategy used by clinics. Other recruitment strategies included newspaper articles and advertisements, public service announcements, brochures and health fairs[102]. Screening visits included viewing an introductory video, processing questionnaires, reviewing medical records, collecting anthropometric measures and drawing blood. The visit culminated with signing a consent form specific to the study component the participant was interested in joining[104].

Inclusion/Exclusion Criteria

Table 5 outlines the inclusion and exclusion criteria for the WHI study. Main eligibility criteria for both the CT and OS were post-menopausal status, willingness to provide informed consent, and intention to reside in the area of study for a minimum of three years (Table 5)[102]. Exclusion criteria for both trial arms included medical conditions which adversely affected three-year survival, as well as other conditions which were likely to affect adherence or safety (Table 5)[102]. Further exclusion criteria for the CT included any invasive cancer in the past 10 years, breast cancer at any time, a mammogram suspicious of breast cancer, myocardial infarction in the previous 6 months, stroke or transient ischemic attack (TIA) in the past 6 months, chronic hepatitis or severe cirrhosis (Table 5). In addition, the DM, PHT and CaD components of the CT had further exclusion criteria linked to adherence or safety (Table 5)[102].

Primary and Secondary Outcomes

Primary outcomes were those which were associated with the primary hypotheses of the clinical trials. These were coronary heart disease for the PHT, breast and colorectal cancer for the DM trial, and hip fracture for the CaD trials[104]. Secondary outcomes were those which were supportive of the primary hypothesis, or were being collected for data safety and monitoring reasons[104]. Secondary outcomes included hip and other fractures

for the PHT, coronary heart disease for the DM and other fractures and colorectal cancer for the CaD[104]. A full list of outcomes is given in Table 6.

WHI outcomes were identified by self-report at semi-annual or annual contact according to trial arms, and adjudication took place at the local and central level at the WHI clinical centers, the Clinical Coordinating Center or the NIH[104]. If the outcome was primary or secondary then relevant sections of the medical record were provided to a local adjudicator. All primary outcomes and safety endpoints which were locally adjudicated were also centrally reviewed. A sample of locally adjudicated secondary outcomes was also centrally adjudicated[104].

The five main cancer outcomes were cancers of the breast, colon, rectum, ovary and endometrium[104]. These cancers were coded for anatomic sub-site, date of diagnosis, extent of the disease and tumor morphology, while all invasive cancers were coded according to primary site. Breast cancers were also coded according to estrogen and progesterone receptor status. Recurrent cancers were not included[104]. Diagnosis of main cancer outcomes were substantiated by pathology report. Non-cancerous polyps, atypical benign breast disease and other premalignant benign conditions were not adjudicated as WHI outcomes[104]. Information on the primary cancer site was locally adjudicated according to 'ICD-O-2 codes, the date of diagnosis and tumor behavior'[104]. The main cancer outcomes were centrally adjudicated by trained cancer coders supervised by a cancer Epidemiologist, and a physician using National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) coding system for breast, colorectal, ovarian and endometrial cancers[105]. Difficult to code cases were sent to a reference cancer pathologist[104]. Agreement between local and central adjudication was high for breast (96%), colorectal (94%), endometrial (94%) and ovarian (89%) cancers. However, the percent agreement for in situ breast cancers was 78%[104].

Cause of death was classified based on data from medical records, autopsy reports or death certificates, with the first two sources being the preferred sources of information[104]. Records were matched with the National Death Index periodically to ascertain a cause of death for all participants[104]. All deaths were reviewed by two central adjudicators who were required to achieve consensus on each case[104]. In addition, a sample of the deaths was sent to the Cardiovascular Central Adjudication Committee on an annual basis for review[104]. Agreement between local and centrally adjudicated cancer deaths was 94%[104].

Length of Follow-up

Recruitment for the WHI took place between 1993 and 1998[103]. The hormone therapy trials of estrogen alone or estrogen plus progestin were stopped in 2004 and 2002 respectively, but participants were followed without intervention until the end of the study[106]. Women in the observational study were followed for 8-12 years[106]. The WHI study ended in March, 2005, with a closeout date for data collection of April 8, 2005[107].

In addition to the main WHI study, participants had the opportunity to enroll in the WHI Extension Study 1[107] to explore the long term effects of original interventions and document changes in hormone use[108] or the Extension Study 2 which focused on cardiovascular events and aging, particularly for African American or Hispanic participants, or women formerly enrolled in the PHT[109]. The Extension Study 1 ended in September 2010, while the Extension Study 2 is ongoing until 2015[107].

Primary Findings of the WHI

The postmenopausal hormone therapy trials (PHT) were stopped prematurely due to risks to participants outweighing benefits. Women who were randomized to estrogen plus progestin had increased risk of coronary heart disease (the primary outcome), stroke, venous thromboembolism and breast cancer (Table 7)[97]. However, they also had a lower

risk of colorectal cancer and fracture (Table 7)[97]. Women randomized to receive conjugated equine estrogen in the estrogen alone trial had increased risk of stroke, but a significantly reduced risk of hip fracture[110]. Although women who received calcium or vitamin D had reduced risk of hip fracture (Table 7), this risk was not significant when results were analyzed on an intention to treat basis[111]. The low fat diet did not confer a reduced risk of breast and colorectal cancer in women receiving the diet versus placebo (Table 7). Women had a non-significant increased risk of colorectal cancer[112] and a non-significant reduced risk of breast cancer[113] (Table 7).

Table 8 presents baseline characteristics for women enrolled in the OS. The mean BMI was 27 kg/m^2. The majority of women (83%) were white non-Hispanic; eight percent were black/African-American and four percent were Hispanic (Table 8). Approximately eight out of 10 (78.5%) women had post-high school education (Table 8). Sixteen percent of participants had an income <$20,000. Approximately half (50.9%) of women enrolled were never smokers and 4 out of 10 (38%) reported drinking greater than 1 drink per week. Greater than two-thirds of women (77.6%) had two or more live births (Table 8). Forty-eight percent reported the age at last menstrual bleeding as less than 50 years (Table 8).

Reliability measures were determined on 574 women who were enrolled in the Measurement Precision Study. In this sample questionnaires were administered at baseline and three months after enrollment, and the test-retest reliability was subsequently determined. Women completed four of the eight original baseline questionnaires. The majority of demographic values had weighted kappa or kappa statistics greater than 0.80, indicating good reliability. The reliability of a history of cancer was 0.77, while reliabilities for total expenditure/week from physical activity (METS) and history of hypertension, were 0.77 and 0.86 respectively (results not shown).

The Biomarkers and Genetic Factors Related to Sarcopenia Study

The Biomarkers and Genetic Factors Related to Sarcopenia Study is an ancillary study of the WHI. Women were selected exclusively from the OS arm at 3 centers which measured Dual-energy X-ray absorptiometry (DXA). These were the University of Arizona, the University of Pittsburgh and the University of Alabama, Birmingham. Participants for the WHI-OS DXA cohort were located in the communities where DXA scans were carried out. Two-thousand and eight hundred (2,800) Hispanic and non-Hispanic women were randomly selected from a possible pool of 5,428 eligible women in the WHI OS-DXA cohort to form a genetic study cohort. One thousand women (1,000) women were then randomly selected from the genetic cohort for determination of biomarker assessments. Eighty-nine biomarkers were analyzed for 1001 women[114].

Table 5 WHI Inclusion and Exclusion Criteria by Study Component

Component	Inclusion Criteria	Exclusion Criteria
CT + OS	50-79 years of age Postmenopausal Ability and willingness to provide written informed consent Intention to reside in area for at least 3 years	Competing risk: ▪ medical condition with predicted survival < 3 years Adherence or retention reasons: ▪ Alcohol or drug dependency ▪ Mental illness including severe depression ▪ Dementia ▪ Active participation in other randomized intervention trial
CT		Competing Risk: ▪ Any invasive cancer in previous 10 years ▪ Breast cancer at any time ▪ Mammogram or CBE findings suspicious of breast cancer ▪ MI in previous 6 months ▪ Stroke or TIA in past 6 months ▪ Chronic Hepatitis or severe cirrhosis Safety Reasons: ▪ Severe hypertension (SBP > 200 mm Hg/DBP > 105 mm Hg) ▪ Severely underweight (BMI < 18 kg/m2) ▪ Hematocrit < 32% ▪ Platelets < 75,000 cells/ml ▪ Current use of oral daily corticosteroids Adherence or retention reasons: ▪ Unwilling to participate in baseline/follow-up exam components
DM		Adherence or retention reasons: ▪ Dietary requirements incompatible with the intervention ▪ On a diabetic/low salt diet ▪ Gastrointestinal conditions contraindicating a high fiber diet ▪ Type 1 diabetes ▪ Colorectal cancer at any time ▪ Routinely eat >= 10 meals/week prepared out of the home

Component	Inclusion Criteria	Exclusion Criteria
		▪ Unable to keep a 4-day food record ▪ FFQ percent calories from fat < 32% ▪ FFQ energy intakes < 6000 or > 5000 kcal ▪ Previous bilateral prophylactic mastectomy
PHT		Safety reasons: ▪ Endometrial cancer at any time ▪ Endometrial hyperplasia ▪ Malignant melanoma at any time ▪ History of pulmonary embolism or deep vein thrombosis ▪ Previous osteoporosis-related fracture being treated with hormones ▪ History of bleeding disorder requiring transfusion ▪ History of hypertriglyceridemia ▪ Currently on anticoagulants ▪ Currently on tamoxifen ▪ Abnormalities in baseline pap smear/pelvic exam/pelvic ultrasound Adherence or retention reasons: ▪ Severe menopausal symptoms that would make placebo treatment intolerable ▪ Inadequate adherence to placebo run-in ▪ Unwilling or unable to discontinue use of PHT or testosterone ▪ Refusal to have baseline endometrial aspiration
CaD		Safety reasons: ▪ History of renal calculi or hypercalcemia ▪ Current use of oral corticosteroids or calcitrol ▪ Intention to continue taking >= 600 IUs of Vitamin D daily

Abbreviations: BMI = body mass index; CaD = Calcium and Vitamin D; CBE = clinical breast exam; CT = Clinical Trial; DBP = diastolic blood pressure; DM = Dietary Modification; MI = myocardial infarction; OS = Observational Study; PHT = Postmenopausal Hormone Therapy; TIA = transient ischemic attack.

Source: Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, et al. The Women's Health Initiative recruitment methods and results. *Ann.Epidemiol.* 2003 Oct;13(9 Suppl):S18-77.

Table 6 WHI Study Outcomes

Outcome	PHT	DM	CaD	OS
Cardiovascular	1°	2°	x	x
Coronary heart disease	2°	2°	x	x
Stroke	2°	2°	x	x
Congestive heart failure	2°	2°	x	x
Angina	2°	2°	x	x
Peripheral vascular disease	2°	2°	x	x
Coronary revascularization	2°	2°	x	x
Venous thromboembolic disease				
Pulmonary embolism	2°	x	x	x
Deep vein thrombosis	2°	x	x	x
Total cardiovascular	2°	2°	x	x
Cancer				
Breast	2°	1°	2°	x
Colorectal	x	1°	2°	x
Endometrial	2°	2°	x	x
Ovarian	2°	2°	x	x
Total cancers	2°	2°	2°	x
Fractures				
Hip	2°	x	1°	x
Other Fractures	2°	x	2°	x
Total Fractures	2°	x	2°	x
Other				
Diabetes mellitus requiring therapy	x	2°	x	x
Death from any cause	2°	2°	2°	x

Abbreviations:
CaD = Calcium and Vitamin D; CT = Clinical Trial; DM = Dietary Modification; OS = Observational Study; PHT = Postmenopausal Hormone Therapy; 1° = primary outcome; 2° = secondary or safety outcomes; x= ascertained

Source:
Curb JD, McTiernan A, Heckbert SR, Kooperberg C, Stanford J, Nevitt M, et al. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. *Ann.Epidemiol.* 2003 Oct;13(9 Suppl):S122-8.

Table 7 Risk of Primary Outcomes in WHI CT Trials

Study Outcomes	**HR**	**LCI**	**UCI**
Calcium Plus Vitamin D (CaD) Trial			
Hip Fracture [111]	0.88	0.72	1.08
Dietary Modification (DM) Trial			
Invasive Breast Cancer [113]	0.91	0.83	1.01
Colorectal Cancer [112]	1.08	0.90	1.29
Post-Menopausal Hormone Therapy Trial			
Estrogen Alone[1]			
Coronary Heart Disease (CHD) [110]	0.91	0.75	1.12
Estrogen Plus Progestin[2]			
Coronary Heart Disease (CHD) [97]	1.29	1.02	1.63

1 The Estrogen alone trial was halted by the NIH in 2004. Participants receiving conjugated equine estrogen had increased risk of stroke and reduced risk of hip fracture.

2 The Estrogen plus Progestin trial was halted in 2002 due to risk exceeding benefits. Participants receiving estrogen plus progestin had a higher risk of coronary heart disease, stroke, venous thromboembolism and breast cancer and lower risk of colorectal cancer and fracture.

Table 8 Selected Baseline Characteristics of Women Enrolled in the Observational Study

Characteristics	(N=93,676)		Reliability (κ), (N=564)
	Mean	SD	
Body Mass Index (BMI) kg/m^2	27.3	5.9	NR
Race/Ethnicity	N	%	
American Indian	422	0.5	0.99
Asian/Pacific Islander	2,671	2.9	
Black	7,639	8.2	
Hispanic	3,623	3.9	
White	78,013	83.3	
Unknown	1,308	1.4	
Education			
0-8 years	1,560	1.7	0.87
Some high school	3,288	3.5	
High school diploma (GED)	15,121	16.3	
School after high school	33,933	36.5	
College degree or higher	39,002	42.0	
Family Income			
< $10,000	3,916	4.5	0.81
$10,000-19,999	10,100	11.6	
$20,000-34,999	20,226	23.3	
$35,000-49,000	17,429	20.1	
$50,000-74,999	17,486	20.2	
$75,000+	17,608	20.3	
U.S. Region			
Northeast	21,273	22.7	
South	24,459	26.1	
Midwest	20,607	22.0	
West	27,337	29.2	
Smoking			
Never smoked	47,023	50.9	
Past smoker	39,514	42.8	
Current smoker	5,791	6.3	
Alcohol intake			
Never drinker	10,477	11.3	
Past drinker	17,555	18.9	
< 1 drink/month	10,733	11.5	
< 1 drink/week	18,728	20.1	
1-7 drinks/week	23,842	25.6	
7+ drinks/week	11,709	12.6	
Number of live births			
Never	9,357	10.1	
None	2,697	2.9	
1	8,779	9.4	
2-4	60,674	65.2	
5+	11,500	12.4	
Age last had any menstrual bleeding (y)			
< 40	10,836	12.6	0.83

Characteristics	(N=93,676)	Reliability (κ), (N=564)
40-44	11,644	13.6
45-49	18,416	21.5
50-54	30,134	35.1
55-59	10,709	12.5
60+	4,023	4.7

NR= Not Reported

Source: Adapted from Langer R, White E, Lewis C, Kotchen J, Hendrix S, et. al. The WHI OS: Baseline Characteristics of Participants and Reliability of Baseline Measures. *Ann Epidemiol* 2003;13:S107-S121.

CHAPTER 5: HEMOGLOBIN LEVEL, ANEMIA AND CANCER MORTALITY IN POST-MENOPAUSAL WOMEN

Introduction

Anemia is a common condition in the elderly, with prevalence ranging from 3 to 40% in elderly women[115]. It is estimated that approximately 4 million individuals over the age of 65 years are anemic[116]. The condition is not benign, as it is associated with increased risk of hospitalization and all-cause mortality[69,117]. It is also associated with a variety of chronic conditions including congestive heart failure[118,119] kidney disease[118] and cancers[2,4]. Anemia is considered a prognostic indicator of survival in individuals with cancer. A systematic review of the association of anemia with cancer mortality reported an increased risk of death in individuals with lung, cervicouterine, head and neck and prostate cancers, multiple myeloma and lymphoma[4]. Anemia or low hemoglobin level are associated with poor survival in patients with breast[7,57,58] and colorectal cancer[8,10,63].

Despite reported associations with cancer mortality, there is still a dearth of literature on risk of cancer death in anemic individuals in large prospective studies. Previous research has mainly involved retrospective analysis of patients already diagnosed with cancer. More importantly, although it is known that cancer treatment may lead to anemia, and cancer anemia may affect survival in cancer patients, what remains to be fully explored is whether having anemia before cancer diagnosis alters survival. Stapley et. al (2006) reported that mild anemia which was identified as a symptom prior to colorectal cancer diagnosis in the primary care setting was associated with advanced cancer staging and mortality[10]. The present study prospectively examined the cancer mortality risk in anemic individuals in a non-clinical setting.

Literature on the risk of *all-cause* mortality in anemic elderly individuals has been conflicting, with reports of increased mortality risk in white, but not black participants[120], countered by those demonstrating no difference in mortality risk by race/ethnic subgroups[121,3]. Few studies have examined racial variations in the anemia-cancer mortality association, which may be likely, since African-American women have higher mortality rates from total, breast and colorectal cancers[5] and different sizes or grades of tumors or precursors[122,123, 124]. These variations differ according to cancer type, and have been attributed to biological differences[125], differences in socioeconomic status[126,127], variations in access to care or treatment[128], or a combination of these factors. The association of elevated hemoglobin concentration with all-cause mortality[3,69] also suggests the possibility of an increased cancer mortality risk at higher hemoglobin levels, since cancer deaths account for one third of total deaths in women aged 45-64 years, and one fifth of total deaths in women greater than or equal to 65 years in the U.S.[12]. However, there appear to be few or no studies which have investigated the association of cancer mortality across the full range of hemoglobin concentration.

The Women's Health Initiative (WHI) cohort with its large sample size of adjudicated cancer cases and mortality information allowed evaluation of the association of anemia and hemoglobin level with total, as well as site-specific cancer mortality, controlling for a variety of potential confounders. Specifically, we sought to determine the association of anemia and/or hemoglobin level with mortality from total, breast and colorectal cancer. As a secondary objective, we sought to determine whether this association varies by cancer history and race/ethnicity.

Methods

Study Population

The study population initially comprised 24,309 women aged 50 to 79 years who were enrolled in the Women's Health Initiative Observational Study (OS) or Clinical Trial (CT) and who had cancer over the WHI follow-up period. Details regarding the study

design have been published elsewhere[100,102]. In brief, women were recruited from 40 centers in twenty-four states[102] between 1993 and 1998[103]. Ten of the forty sites were selected as minority recruitment sites [102]. The CT comprised three different components, including: (1) two hormone therapy versus placebo trials, which investigated reduction in cardiovascular risk (2) a dietary modification trial in which a low fat diet was predicted to reduce breast and colorectal cancer risk and (3) a calcium and Vitamin D trial to investigate fracture risk[100]. Women were entered into the OS if they were interested in the dietary modification or hormone therapy trials, but were unwilling or ineligible to participate in the CT[100]. Main eligibility criteria for both the CT and OS were post-menopausal status, willingness to provide informed consent, and intention to reside in the area of study for a minimum of three years[102]. Exclusion criteria for both trial arms included medical conditions which adversely affect three-year survival, as well as other conditions which were likely to affect adherence or safety[102]. The hormone therapy trials of estrogen alone or estrogen plus progestin were stopped in 2004 and 2002 respectively, but participants were followed without intervention until the end of the study time period. Informed consent was obtained from participants at each recruitment site[129].

Among the 24,309 eligible women there were 21,270 without cancer history at enrollment and 3039 women with cancer history. Women were stratified on cancer history due to limited data on cancer recurrence. Cancer history was determined from questionnaires at baseline. Analysis was restricted to women with baseline hemoglobin measures (N=24,025). Women who had hemoglobin values less than 5 and greater than 20 g/dl (N=28) were excluded from the analysis, leaving a final sample of 23,997.

Anemia Assessment

Hemoglobin levels were determined from a complete blood count (CBC) which was assessed from 12 hour fasting samples[129]. Anemia was defined using the World Health Organization definition of a hemoglobin level less than 12 g/dl[19,130]. Mild anemia was defined as a hemoglobin level of 11-11.9 g/dl[130]. Moderate anemia was defined as a

hemoglobin level of 8-10.9 g/dl and severe anemia was defined as a hemoglobin level < 8 g/dl[130]. High hemoglobin was defined as a hemoglobin level >= 15 g/dl. This threshold was based on elevated mortality risk, and the low number of breast and colorectal cancer deaths occurring in high hemoglobin thresholds beyond a cutpoint of 15 g/dl. Persistent anemia was defined as a hemoglobin level less than 12 g/dl at both baseline and year 3, while transient anemia was defined as anemia occurring at year 0 only.

Outcomes Ascertainment

Outcomes of interest were total, breast and colorectal cancer death. Deaths were reviewed by central physician adjudicators who were blinded to randomization allocation and by a Central Adjudication Committee[104]. WHI participants were also matched to National Health index data at regular 2 to 3 year intervals[104].

Other Covariates

Age, smoking status, race/ethnicity, education, supplement use, income and hospitalizations within the past 2 years were assessed at baseline using self-administered and interviewer-administered questionnaires. Dietary intake and alcohol intake were assessed using a semi-quantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Center[129]. Total intake of calcium, folate, iron, vitamin B12 and vitamin C were determined by summing dietary and supplemental intake. Total folate was derived from the sum of natural and synthetic folate accounting for the differential bioavailability of synthetic folic acid. Height and weight were measured by trained staff at a clinic visit[103].

Statistical Analysis

Hemoglobin values below 5 g/dl and greater than 20 g/dl were set to missing (N=20). Exploration of the proportion of deaths by hemoglobin level and perusal of Martingale residuals suggested that treatment of hemoglobin as a continuous variable would not be appropriate. Use of different hemoglobin levels were therefore explored, namely (i) the

WHO definition of a hemoglobin level < 12 g/dl (ii) high hemoglobin defined as a hemoglobin level >= 15 g/dl and (iii) hemoglobin g/dl increments The two cutpoints used in final modeling were < 12 g/dl, denoting anemia status, and high hemoglobin level, since exploratory models indicated that the risk of total cancer death increased below 12 and greater than 15 g/dl. The numbers of breast and colorectal cancer deaths in the high hemoglobin group were too small for stratification by age category and race/ethnicity in modeling.

Baseline characteristics were presented as medians (interquartile ranges) and numbers (%). Medians were presented due to the skewness of body mass index and total intake variables. Differences in baseline characteristics by anemia status and high hemoglobin level were tested using Wilcoxon rank sum tests and chi-squared analyses. Dietary intake variables were log transformed prior to consideration in survival models. The number of deaths and crude mortality rate were presented for total, breast and colorectal cancers using time on study as the time scale. Women who were lost to follow-up or did not die were right-censored at the date of last contact. Confounders were identified from variables which were significantly associated with anemia and hazard of cancer death. Since most of these variables changed the age-adjusted hazard ratio by less than 5%, final anemia models adjusted for smoking status (never, past, current), hospitalization overnight within the past 2 years (no, yes), log total calcium intake (mg), clinical trial assignment (no, yes), region of residence (northeast, south, midwest, west), race/ethnicity (white non-Hispanic, black/African-American, other race) and diabetes at baseline (no, yes). High hemoglobin models adjusted for body mass index (kg/m2), dietary fiber intake, race/ethnicity (white non-Hispanic, black/African-American, other race), education (less than college, college or above), income (< $20,000, $20,000-34,999,$35,000-49,999,>=$50,000), smoking status (never, past, current), clinical trial assignment (no, yes), cancer group (without cancer history, cancer history with cancer on follow-up), region of residence (northeast, south, midwest, west), diabetes at baseline (no, yes) and cardiovascular disease (no, yes).

Figure 2 Schematic of the Analyses Conducted for Chapter 5

Main Analysis	The association between anemia/hemoglobin level and cancer mortality
Secondary Analyses	Effect modification by age and race/ethnicity
	Effect modification by cancer group
	The association of persistent and transient anemia with cancer mortality
Sensitivity Analyses	Main analysis, excluding deaths occurring in first six months of enrollment
	Main analysis excluding deaths or censored observations occurring in first 2 years of follow-up

Competing risk models were used to determine the association between anemia/or high hemoglobin level and cancer mortality. The sub-hazard ratio was estimated according to the method of Fine and Gray[131]. Deaths from any other cause were coded as a competing event. Models used age as the time scale, and enrollment age as the entry time. Anemia models for total breast and colorectal cancer mortality were stratified on cancer history (with versus without cancer history). Differences between the two groups was formally tested by inclusion of interaction terms in final cancer models. High hemoglobin models were not stratified by cancer history, since the addition of cancer history interaction terms were not significant. Effect modification by race/ethnicity ("White non-Hispanic", "Black" and "Other Race") was tested by inclusion of interaction terms in models. Effect modification was only tested in models predicting total cancer deaths, due to the small numbers of anemic women in racial subgroups who died from breast or colorectal cancers.

As a secondary analysis the risk of total cancer death in women with persistent and transient anemia was determined for women enrolled in the OS, as year 3 hemoglobin results were unavailable for women enrolled in the CT, and the number of deaths in the

persistent anemia subgroup were too small for ascertainment of breast and colorectal cancer mortality.

Two sensitivity analyses were conducted. Firstly, cancer deaths occurring within the first six months of enrollment were excluded to account for women dying from causes other than anemia. Secondly, deaths or censored observations occurring within the first two years were excluded to determine whether women may have been experiencing subclinical changes affecting hemoglobin levels prior to a formal diagnosis of cancer. Figure 2 summarizes the analyses carried out for the study. Schoenfeld residuals and time varying covariates were used to test the proportional hazards assumption. Analyses were conducted using STATA 12.0 (Stata Corporation, College Station, TX).

Results

Study Population

The study population was comprised of 24,309 women enrolled in the Women's Health Initiative observational study and clinical trial who had cancer over the follow-up period. Thirteen percent of the population (3,039) had a history of cancer. Of these women, 23,997 (98.7%) had valid hemoglobin measures. Women who had missing hemoglobin measures had a lower body mass index (BMI), were more likely to be black/African American, college educated, have a history of cancer, be assigned to the observational study, and to reside in the south or midwest (data not shown).

Prevalence of Anemia and High Hemoglobin

Five percent (5.2%) of the participants were anemic at baseline (Table 10); 4.9% in women without cancer history and 7.6% in women who had a history of cancer. Approximately four percent of women (4.4%) had mild anemia. Less than one percent of the population had moderate (0.8%) or severe (0.03%) anemia (Table 10). The proportion of women with hemoglobin levels greater than or equal to 15 g/dl was 7%.

Less than one percent (0.3%) of women had hemoglobin levels greater than or equal to 16.5 g/dl (Table 10).

Factors Associated with Anemia Status and High Hemoglobin Concentration

Anemic women had a lower median BMI, dietary fiber intake and total calcium, vitamin B12 and zinc intake levels. A higher proportion of black/African American women (14.2%) were anemic in comparison with white non-Hispanic women (4.5%), and participants of other race/ethnic sub-groups (6.1%). The proportion of anemia was significantly higher in never-smokers (5.8%) in comparison with past (5.1%) and current (2.8%) smokers (Table 9). The proportion of women with anemia was also significantly higher in never drinkers (6.9%), those who were hospitalized overnight in the past two years (7.1%), who had a history of cancer (7.6%) or who were enrolled in the observational study (5.9%, Table 9). Women who resided in the south (6.8%) had higher proportions of anemia than women residing in the Northeast (5.5%), Midwest (4.6%) and West (4.1%).

Women with high hemoglobin levels were older, had a higher median body mass index and lower median dietary fiber, total calcium, folate and vitamin C intake (Table 9). Black/African American women had lower proportions of high hemoglobin (2.6%) than White non-Hispanic (7.4%) women, and women of other race/ethnic groups (7.6%). High hemoglobin proportions were greater in those who did not have college education (7.7%) and were in the lowest income category (9.3%, Table 9). Approximately one in five current smokers (20.5%) had high hemoglobin levels in comparison with approximately one in seventeen never (5.6%) and past (6.0%) smokers. The proportion of high hemoglobin was higher in women who consumed greater than or equal to 1 drink per day (8.2%), obese individuals (8.5%) and women enrolled in the clinical trial (7.7%). Women residing in the south had the lowest proportion of high hemoglobin (4.9%) when compared with women living in other regions, while those reporting diabetes (11.3%),

hypertension (9.1%) and cardiovascular disease at baseline (8.5%) had significantly higher proportions of high hemoglobin than those who did not (Table 9).

Mortality Risk by Anemia and High Hemoglobin Status

There were 7,348 cancer deaths occurring over 263,962 person-years of follow-up, and yielding an overall cancer mortality rate of 27.8 per 1000 person-years. There were 849 breast cancer deaths and 607 colorectal cancer deaths. Median time to cancer mortality was 16.3 years in non-anemic women and 15.5 years in women with anemia. Crude mortality rates were generally higher in individuals with anemia and high hemoglobin concentrations (Table 11). However, crude mortality rate ratios by high hemoglobin category were only significant for total cancer deaths in the combined population, and in women without a history of cancer (Table 11). Crude breast cancer mortality rate ratios for anemic versus non-anemic women were not significant in participants without cancer history (Table 11), but were significant in those with a history of cancer. Crude colorectal cancer mortality hazard ratios were significant in anemic versus non-anemic women in the combined population, as well as the cancer history subgroup (Table 11).

Cancer mortality by anemia results were stratified by cancer group, since there were significant cancer group by anemia interactions for total ($p=0.002$), breast ($p=0.084$) and colorectal cancer ($p=0.017$) deaths in Cox Models, and a significant interaction term for total cancer death in competing risk models (Table 14, $p=0.02$). In addition, colorectal cancer death sub-hazard ratios in competing risk models differed between cancer groups, with an increased hazard of colorectal cancer death only occurring in women with cancer history (Table 14). Anemia was a significant predictor of total cancer death in women without cancer history, (Table 12, SHR: 1.21; 95% CI: 1.09, 1.36). However, in women with cancer history, anemia was a significant predictor of total and colorectal cancer death (Table 12). Anemic women had a 55% higher risk of total cancer death (SHR: 1.55; 95% CI: 1.27, 1.89) when compared with women without anemia. Anemic women had

twice the hazard of colorectal cancer death than women who were not anemic (SHR: 2.01; 95% CI: 1.09, 3.72). Figure 5 shows the cumulative incidence of cancer deaths by anemia status or high hemoglobin level.

Women with hemoglobin levels greater than or equal to 15 g/dl were not at increased risk of total, breast or colorectal cancer death (Table 12). Although an increased risk of total cancer death was observed in age-adjusted models, smoking status attenuated the association. This observation also applied to higher hemoglobin thresholds (Figure 6). Participants with hemoglobin levels less than 10 g/dl were at increased risk of total cancer death in women without a history of cancer (Figure 7, SHR: 2.32; 95% CI: 1.32, 4.09). Women with moderate anemia were at increased risk of total cancer death in women without (Figure 7, SHR: 1.58; 95% CI: 1.20, 2.08) and with (SHR: 1.95; 95% CI: 1.20, 3.16) cancer history. Similarly, participants with mild anemia had significantly increased risk of total cancer death in both women without (SHR: 1.16; 95% CI: 1.02, 1.31) and with (Figure 7, SHR: 1.48; 95% CI: 1.20, 1.83) cancer history.

Table 13 gives the estimates for hazard of cancer death using hemoglobin categorized by g/dl increments. Women without cancer history who had hemoglobin levels less than 12 g/dl had a 24% increased hazard of total cancer death (SHR: 1.24; 95% CI: 1.10, 1.41) when compared with those who had hemoglobin levels in the reference range of 12 - 12.9 g/dl (Table 13). Women with cancer history who fell in the lowest hemoglobin category had a higher hazard of total (SHR: 1.28; 95% CI: 1.03, 1.59) and colorectal cancer death (SHR: 2.23; 95% CI: 1.08, 4.61) when compared with the reference. Women with a history of cancer who had hemoglobin levels of 13-13.9 g/dl and 14-14.9 g/dl had a decreased risk of total and breast cancer death (Table 13).

Effect Modification by Race/Ethnicity

Effect modification by race/ethnicity was tested in total cancer death models, but anemia by race/ethnicity interaction terms for differences in black versus white women were not

significant in cancer history groups. Anemia by high hemoglobin interaction terms were also not significant (Table 15, p=0.65). Both anemic white and black women with and without cancer history had an increased hazard of total cancer death, though the hazard ratio for black women was non-significant (Table 15). Women of other race/ethnic groups who were diagnosed with anemia were not at increased risk of cancer mortality. Anemia-race interaction terms were not significant (Table 15).

The Association of Persistent and Transient Anemia with Cancer Mortality

The effect of persistent anemia was explored in total cancer death models for women enrolled in the OS. Approximately three percent (2.6%) of the population had persistent anemia. The prevalence of persistent anemia by type of death was: no death (2.1%), breast cancer death (3.6%), colorectal cancer death (4.3%) and other cancer death (3.5%). The prevalence of persistent anemia was significantly higher in women with cancer history (3.5%) when compared with those without a history of cancer (2.4%, p for difference between groups=0.003). Although women with persistent anemia had a significantly higher hazard of total cancer death in women without cancer history (SHR: 1.38; 95% CI: 1.08, 1.75) when compared with non-anemic subjects, this association was not observed in those with transient anemia (SHR: 0.98; 95% CI: 0.76, 1.27). However, subjects with persistent (SHR: 1.39; 95% CI: 0.95, 2.02) and transient anemia (SHR: 1.46; 95% CI: 1.04, 2.04) had similar hazard ratios in women with a history of cancer, though the hazard ratio for persistent anemia was only marginally significant (p=0.091).

Sensitivity Analyses

A variety of sensitivity analyses were considered. Firstly, women who had cancers in the first two years of follow-up were excluded to account for women who may have had cancer prior to official diagnosis which would shorten the observed time between cancer diagnosis and death. Secondly, women with cancer deaths occurring within the first 6 months were excluded to account for women dying from causes other than anemia. Both approaches yielded results similar to the main analysis (results not shown) for total

cancer deaths. In women with no cancer history the hazard of total cancer death in analyses excluding incident cancers or deaths in the first 6 months were 1.21 (95% CI: 1.07, 1.37) and 1.20 (95% CI: 1.07, 1.34) respectively. Hazards of total cancer death using the same analytical approaches were 1.60 (95% CI: 1.30, 1.97) and 1.54 (95% CI: 1.26, 1.88) in those with a history of cancer. Anemia was not a significant predictor of colorectal cancer deaths when incident cancers or censored observations occurring in the first 2 years (SHR: 1.71; 95% CI: 0.82, 3.57) or deaths occurring within the first six months (SHR: 1.86; 95% CI: 0.99, 3.48) were excluded from analysis.

Discussion

Anemia was significantly associated with an increased hazard of total cancer death in women without cancer history and an increased hazard of total and colorectal cancer death in women with a history of cancer. Risk of total cancer death was higher at lower hemoglobin levels. However, women who had a history of cancer and mild anemia also had an increased risk of cancer mortality. Women with high hemoglobin levels were at increased risk of total cancer death in age adjusted, but not fully adjusted models.

The prevalence of anemia in women who had cancers on follow-up was 5.3%. Less than one percent of the population had moderate or severe anemia. The low anemia prevalence in the WHI in comparison to other population-based surveys[2,121,132] has been reported previously[129]. Reasons given for possible differences were the inclusion of younger women in the WHI sample, and the population being nutritionally replete or non-institutionalized[129]. While the age range of the Women's Health Initiative sample population was 50 to 79 years, the age of the NHANES population extended beyond 85 years[2]. NHANES prevalence estimates for women aged 50-64 years and 65-74 years were 6.8% and 8.5% respectively, which are closer to that observed in the WHI population[2].

Anemia has been identified as an independent predictor of all-cause[3,69,117] and cancer mortality[4]. Anemia or low hemoglobin level are associated with poor survival in individuals with a number of cancers[4] including breast[7,57,58] or colorectal cancers[8,10,63]. To our knowledge this is one of few prospective studies which have examined the association of anemia level prior to cancer diagnosis with cancer mortality. Stapley et. al (2006) reported on the association of symptoms at presentation to primary care (1 year prior to cancer diagnosis) and mortality from colorectal cancers[10]. The authors found that patients presenting with mild anemia had worse cancer staging and increased mortality. In the present study median time to cancer incidence was 5 years, and median time to cancer death in women with cancer history was 15 years. Therefore the probability distribution for cancer diagnosis at or greater than 5 years after baseline anemia measurements was 50%. The present study suggests that in addition to anemia being associated with an increased mortality risk in individuals with cancer, anemia prior to the diagnosis of cancer may also confer an increased hazard of cancer death.

The reason for the association of anemia prior to cancer incidence and subsequent mortality is unclear. It may be possible that individuals had already experienced subclinical changes or undetected cancer prior to formal cancer diagnosis, which may have resulted in lower hemoglobin levels and subsequent survival disadvantage. A study of blood donors found declining levels of hemoglobin up to 3 years prior to diagnosis in individuals with stomach cancer, multiple myeloma and lymphatic leukemia, and in small intestinal and colon cancers and Hodgkin lymphoma up to 2 years prior to diagnosis[9]. No decline in hemoglobin levels was found for breast cancers[9].

Another study of subjects eventually diagnosed with colorectal cancers reported hemoglobin decrements of 0.28 g/dl every 6 months up to four years prior to diagnosis[133]. Women diagnosed with cancer or were censored in the first two years of enrollment were excluded to account for pre-diagnostic hemoglobin changes, and this approach yielded similar conclusions to the main analysis for total cancer deaths, suggesting that the

increased mortality risk in anemic individuals remained after accounting for this pathway. In addition, 50% of the survival distribution had not been diagnosed with cancer up to 5 years after baseline anemia measurements. Exclusion of women diagnosed with cancer or censored in the first two years using a competing risks approach yielded a non-significant association between anemia and colorectal cancer mortality. The same analysis using a Cox proportional hazards rather than competing risks model yielded a hazard ratio of 2.29 (95% CI: 1.16, 4.53). The reason for the difference in the association with colorectal cancer mortality using the two methods is unclear. Examination of survival and cumulative incidence plots indicates that the majority of cancer deaths did not occur shortly after follow-up, and that the survival gap between women with and without anemia increased with time. This suggests that the difference between the main and sensitivity analysis is not due to sub-clinical changes affecting cancer mortality, but to methodological differences in accounting for deaths from other causes.

Another source of survival disadvantage in anemic women could be persistently low hemoglobin levels, which may increase the likelihood of anemia following cancer diagnosis and worsen survival. Increased risk of total cancer death in women without cancer history was observed in women with persistent, but not transient anemia. Women with cancer history also had a higher proportion of anemia at baseline and at year 3 when compared with women without a history of cancer. However, women with persistent or transient anemia were at elevated risk of cancer death in the cancer history group. These factors suggest that the worsened survival in the cancer history group may be due to a higher prevalence of the exposure at baseline and follow-up, resulting from previous disease and treatment, and/or subsequent anemia persistence leading to survival disadvantage. Despite this supposition, the present study is not able to fully elucidate the pathways by which persistent anemia may lead to increased cancer deaths. Pathways by which *tumor-associated* anemia may hasten cancer mortality may relate to tumor hypoxia[134], or anemia being indicative of other comorbidities which lower survival[135]. The latter concern was addressed by inclusion of cardiovascular disease, hypertension or

diabetes in the consideration of potential confounders, as these conditions may predict cancer mortality risk[99,136,137,138]. History of cardiovascular disease and hypertension status at baseline were not significantly associated with anemia, and were therefore not confounders of the anemia-cancer mortality association. Anemia was a significant predictor of cancer mortality, even after controlling for diabetes mellitus. The study is limited in the inability to control for renal disease, since end stage renal disease may be associated with increased cancer risk[139] and mortality[140].

The association of mild anemia with cancer mortality in women with a history of cancer is important, given that the majority of anemia diagnosed in the elderly is mild[2]. This may raise the question of anemia correction, which may occur in the form of iron therapy, blood transfusion, erythropoietin or administration of vitamin B12 or folic acid, depending on the type of anemia[6]. However, findings would have to be confirmed by other longitudinal studies before recommendations on anemia correction can be made. This will be a complex issue, since the condition may result from a number of causes[2], all of which may not be responsive to, or require iron supplementation[6,18] . Folate intake may yield a small reduction in cancer risk[141], but there is concern that folate may play a dual role of preventing cancers but also promoting the growth of established neoplasms[142]. In addition, meta-analyses of studies on the effect of erythropoietin stimulating agents in cancer patients indicate increased risk of thromboembolic events and possible decreased survival[143].

Previous studies have reported on the association between high hemoglobin level and *all-cause* mortality[3,69,121], but these findings have varied, with some estimates not attaining statistical significance[117,132]. Health consequences of high hemoglobin levels include increased blood viscosity and reduced oxygen delivery to tissues[66]. Cancer patients with high hemoglobin levels may also have diminished oxygen delivery to cancer cells[144,145], potentially leading to poor outcomes. In the present study there was an increased risk of total cancer death at higher hemoglobin levels in crude and age-adjusted models, but the

risk was attenuated after adjustment for smoking status. Smoking is associated with higher hemoglobin levels resulting from a compensatory mechanism to address diminished oxygen supply resulting from carboxyhemoglobin[146]. The association of high hemoglobin level with all-cause mortality after adjustment for smoking has been reported in few studies[3] and may be due to mechanisms hastening mortality from causes other than cancer, or to other factors associated with smoking that could not be controlled for in the analysis. Further research is needed to confirm the null association. Exploration of cancer mortality by hemoglobin g/dl increments suggests that higher hemoglobin concentration between 13-14.9 g/dl may actually be protective for total and breast cancer death in women with a history of cancer.

To our knowledge this is one of few studies to explore the modification of the anemia-cancer mortality association by race/ethnicity. Both white and black women without cancer history had an increased hazard of death in both cancer sub-groups, though the hazard ratio for black women was non-significant. Interaction term p values were not less than 0.05, indicating no difference in mortality risk by race/ethnic group. Racial differences in the association between anemia and all-cause mortality have been attributed to variations in the proportion of black/African-American individuals in the sample, the age range of participants and the level of comorbidity[120]. The present sample comprised post-menopausal women aged 50-79 years; hence, it may be possible that findings would differ among men, older or younger participants or populations with different health status or racial composition.

There are limitations to the present research. The study was not able to determine the causes of anemia, which may have varying associations with cancer mortality. In addition, the study was not able to control for cancer treatment. Residual confounding may have occurred if important confounders such as renal disease were not adjusted for in the analysis, and, since the study is observational, further research would be needed to establish a causal link between anemia (prior to cancer diagnosis) and cancer mortality.

The study relied on an anemia definition based on hemoglobin level, rather than other indices such as serum transferrin receptor, ferritin, serum folate and vitamin B12[129]. Data on persistent anemia was only available for women enrolled in the OS. Women who had missing hemoglobin values had significantly different characteristics from those with hemoglobin measures. Results would therefore not be generalizable to this group of women, but they accounted for only 1% of the WHI population who had cancers on follow-up. This research was carried out in post-menopausal women aged 50 to 79 years, hence the findings may not be generalizable to men or younger women.

The study also had several strengths. In addition to the commonly used WHO definition of anemia, the association between other levels of hemoglobin concentration and cancer mortality were also determined to explore the association across the full range of hemoglobin. The large sample size of the Women's Health Initiative allowed for adjustment for important covariates, stratification by cancer history, exploration of the modification by race/ethnicity, and also facilitated a variety of sensitivity analyses. The study determined the hazard of cancer death using a competing risks approach which confirmed the increased hazard of total cancer death in anemic women without a history of cancer, and the increased hazard of total and colorectal cancer death in women with cancer history, even when deaths from other causes were considered.

In summary, anemia is associated with total cancer death in women without a history of cancer, and total and colorectal cancer deaths in women with cancer history. Further research is needed to confirm these associations before recommendations are made regarding anemia correction.

Tables and Figures

Table 9 Distribution of Predictors by Anemia Status and Hemoglobin Category

	Anemia Status				Hemoglobin Level			
	Anemia=No		Anemia=Yes		< 15 g/dl		>=15 g/dl	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age (years)	65.0	(59,70)	65.0	(59,70)†	64.0	(59,70)	65.0	(60,70)†
BMI (kg/m2)	27.1	(23.9,31.3)	26.5	(23.1,31.2)†	26.9	(23.8,31.2)	28.2	(24.7,32.5)‡
Dietary fiber (g)	15.0	(10.9,20)	14.5	(10.6,19.5)†	15.0	(10.9,20)	14.2	(10.3,19.3)‡
Total Intake								
Calcium (mg)	1047.8	(670.3,1554)	984.2	(591.5,1520.9)‡	1047.8	(666.1,1556.3)	995.9	(645.8,1489.7)†
Iron (mg)	16.4	(10.5,28.1)	16.9	(10.6,28.2)	16.4	(10.5,28.2)	16.0	(10.2,27.6)
Vitamin B12 (mcg)	8.8	(5.2,12.7)	8.5	(4.8,12.3)†	8.8	(5.1,12.7)	8.9	(5.3,13.1)
Folate (mcg)	412.9	(224.6,647.3)	441.9	(221.2,640.1)	418.2	(225.1,648.1)	370.0	(217.2,635.3)†
Vitamin C (mg)	161.0	(90.9,573.3)	158.3	(87.1,543.9)	161.5	(91.5,573.7)	150.0	(82.8,546.4)†
Zinc (mg)	15.2	(9.2,24.8)	15.2	(8.5,23.9)†	15.2	(9.1,27.8)	15.1	(9.3,24.6)
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Race/Ethnicity								
Non-Hispanic White	20063	95.5	935	4.5‡	19434	92.6	1564	7.4‡
Black	1411	85.8	233	14.2	1602	97.4	42	2.6
Other	1219	93.9	79	6.1	1200	92.4	98	7.6
Education								
Below College	13234	94.9	714	5.1	12870	92.3	1078	7.7‡
>= College	9361	94.7	521	5.3	9264	93.7	618	6.3
Income								
< $20, 000	3444	94.1	215	5.9	3318	90.7	341	9.3‡
$20, 000 - 34,999	5324	94.9	286	5.1	5172	92.2	438	7.8
$35, 000 - 49,999	4412	94.9	239	5.1	4311	92.7	340	7.3
>=$50,000	8167	95.2	412	4.8	8083	94.2	496	5.8
Smoking Status								
Never smoked	10214	94.2	625	5.8‡	10235	94.4	604	5.6‡
Past smoker	10171	94.9	547	5.1	10071	94.0	647	6.0

	Anemia Status				Hemoglobin Level			
	Anemia=No		Anemia=Yes		< 15 g/dl		>=15 g/dl	
Current smoker	2059	97.2	59	2.8	1683	79.5	435	20.5
Alcohol Use								
None	2066	93.1	153	6.9‡	2077	93.6	142	6.4†
< 1 drink/day	17363	94.7	975	5.3	17051	93.0	1287	7.0
>= 1 drink/day	3154	96.5	115	3.5	3000	91.8	269	8.2
Obesity								
No	15513	94.8	857	5.2	15316	93.6	1054	6.4‡
Yes	7040	94.9	378	5.1	6785	91.5	633	8.5
Hospitalization Overnight*								
No	18066	95.0	954	5.0‡	17664	92.9	1356	7.1
Yes	3335	92.9	256	7.1	3337	92.9	254	7.1
Cancer History								
No	19776	95.1	1017	4.9‡	19333	93.0	1460	7.0
Yes	2750	92.4	226	7.6	2740	92.1	236	7.9
CT Trial Assignment**								
No	12950	94.1	813	5.9‡	12841	93.3	922	6.7†
Yes	9798	95.7	436	4.3	9449	92.3	785	7.7
Region of Residence								
Northeast	5631	94.5	328	5.5‡	5545	93.1	414	6.9‡
South	5315	93.2	390	6.8	5428	95.1	277	4.9
Midwest	4968	95.4	238	4.6	4819	92.6	387	7.4
West	6834	95.9	293	4.1	6498	91.2	629	8.8
Diabetes (Screening)								
No	21336	94.9	1154	5.1†	20952	93.2	1538	6.8‡
Yes	1345	93.6	92	6.4	1274	88.7	163	11.3
Hypertension Ever								
No	14645	94.9	794	5.1	14513	94.0	926	6.0‡
Yes	7915	94.7	447	5.3	7601	90.9	761	9.1
Cardiovascular Disease								
No	17278	94.8	949	5.2	16985	93.2	1242	6.8‡
Yes	4032	94.1	251	5.9	3918	91.5	365	8.5

Hospitalization Overnight = Hospitalization Overnight in the Past 2 Years;*CT= Clinical Trial;
Key: † - $p < 0.05$; ‡ - $p < 0.001$
IQR = Interquartile range (25^{th} percentile, 75^{th} percentile)

Table 10 Anemia and High Hemoglobin Proportions in the Total Population and by Cancer Sub-Group

	Total Population				No cancer history				Cancer History			
	No		Yes		No		Yes		No		Yes	
Variable	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Anemia (Hb < 12 g/dl)	22748	94.8	1249	5.2	19998	95.1	1023	4.9	2750	92.4	226	7.6
Mild Anemia (Hb >= 11 & < 12 g/dl)	22932	95.6	1065	4.4	20140	95.8	881	4.2	2792	93.8	184	6.2
Moderate Anemia (Hb >= 8 & < 11 g/dl)	23819	99.3	178	0.8	20885	99.4	136	0.6	2934	98.6	42	1.4
Hb >= 15 g/dl	22290	92.9	1707	7.1	19550	93.0	1471	7.0	2740	92.1	236	7.9
Hb >= 16.5 g/dl	23914	99.7	83	0.3	20951	99.7	70	0.3	2963	99.6	13	0.4

Proportion of individuals with severe anemia (Hb < 8 g/dl) in the total population= 0.03%
Hb = Hemoglobin

Figure 3 Median Time to Cancer Incidence and Mortality (Years) in Women with and Without Cancer History, Stratified by Anemia Status

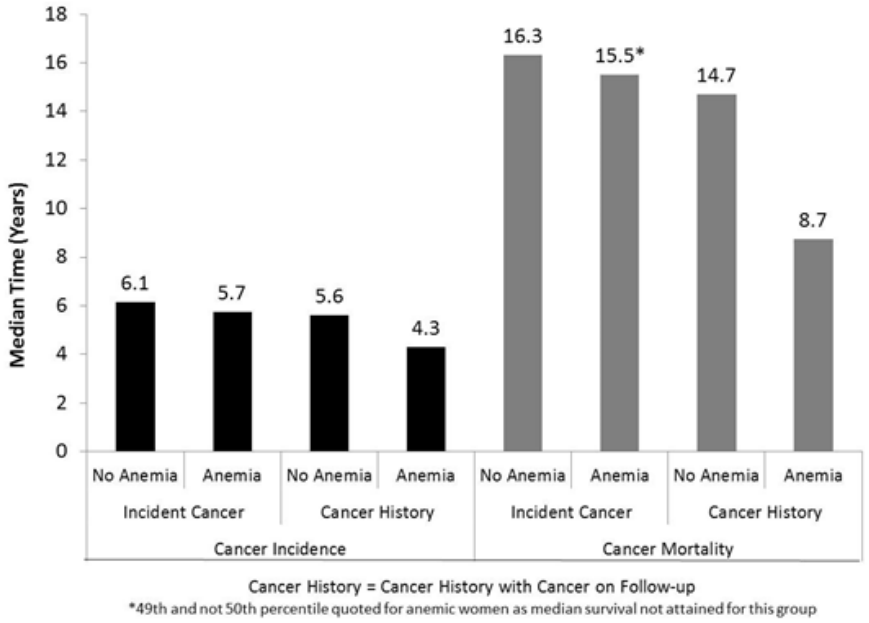


Table 11 Number of Cancer Deaths and Cancer Mortality Rate by Anemia Status, High Hemoglobin Category and Cancer Sub-Group

	Total Cancer Deaths (N=7348)				**Breast Cancer Deaths (N=849)**				**Colorectal Cancer Deaths (N=607)**			
	Anemia		**High Hemoglobin**		**Anemia**		**High Hemoglobin**		**Anemia**		**High Hemoglobin**	
	No	**Yes**	**< 15 g/dl**	**>= 15 g/dl**	**No**	**Yes**	**< 15 g/dl**	**>= 15 g/dl**	**No**	**Yes**	**< 15 g/dl**	**>= 15 g/dl**
Total Population												
No. of deaths	6781	461	6628	614	776	58	772	62	550	48	549	49
Person Years at Risk (10,000s)	24.98	1.26	24.44	1.80	24.98	1.26	24.44	1.80	24.98	1.26	24.44	1.80
Crude Mortality Rate (per 1,000 person-years)	27.14	36.66	27.12	34.09	3.11	4.61	3.16	3.44	2.20	3.82	2.25	2.72
Crude Mortality Rate Ratio	**1.35**	**(1.23, 1.48)**	**1.26**	**(1.16, 1.37)**	**1.48**	**(1.12, 1.94)**	1.09	(0.83, 1.41)	**1.73**	**(1.26, 2.33)**	1.21	(0.89, 1.62)
Without cancer history												
No. of deaths	5622	331	5443	510	469	24	454	39	469	33	462	40
Person Years at Risk (10,000s)	22.25	1.08	21.76	1.58	22.25	1.08	21.76	1.58	22.25	1.08	21.76	1.58
Crude Mortality Rate (per 1,000 person-years)	25.27	30.66	25.02	32.38	2.11	2.22	2.09	2.48	2.11	3.06	2.12	2.54
Crude Mortality Rate Ratio	**1.21**	**(1.08, 1.36)**	**1.29**	**(1.18, 1.42)**	1.05	(0.67, 1.59)	1.19	(0.83, 1.65)	1.45	(0.99, 2.07)	1.20	(0.84, 1.65)
Cancer History												
No. of deaths	1,159	130	1185	104	307	34	318	23	81	15	87	9
Person Years at Risk (10,000s)	2.73	0.18	2.69	0.23	2.73	0.18	2.69	0.23	2.73	0.18	2.69	0.23
Crude Mortality Rate (per 1,000 person-years)	42.40	72.99	44.12	46.09	11.23	19.09	11.84	10.19	2.96	8.42	3.24	3.99
Crude Mortality Rate Ratio	**1.72**	**(1.42, 2.06)**	1.04	(0.85, 1.28)	**1.70**	**(1.16, 2.43)**	0.86	(0.54, 1.31)	**2.84**	**(1.52, 4.97)**	1.23	(0.54, 2.45)

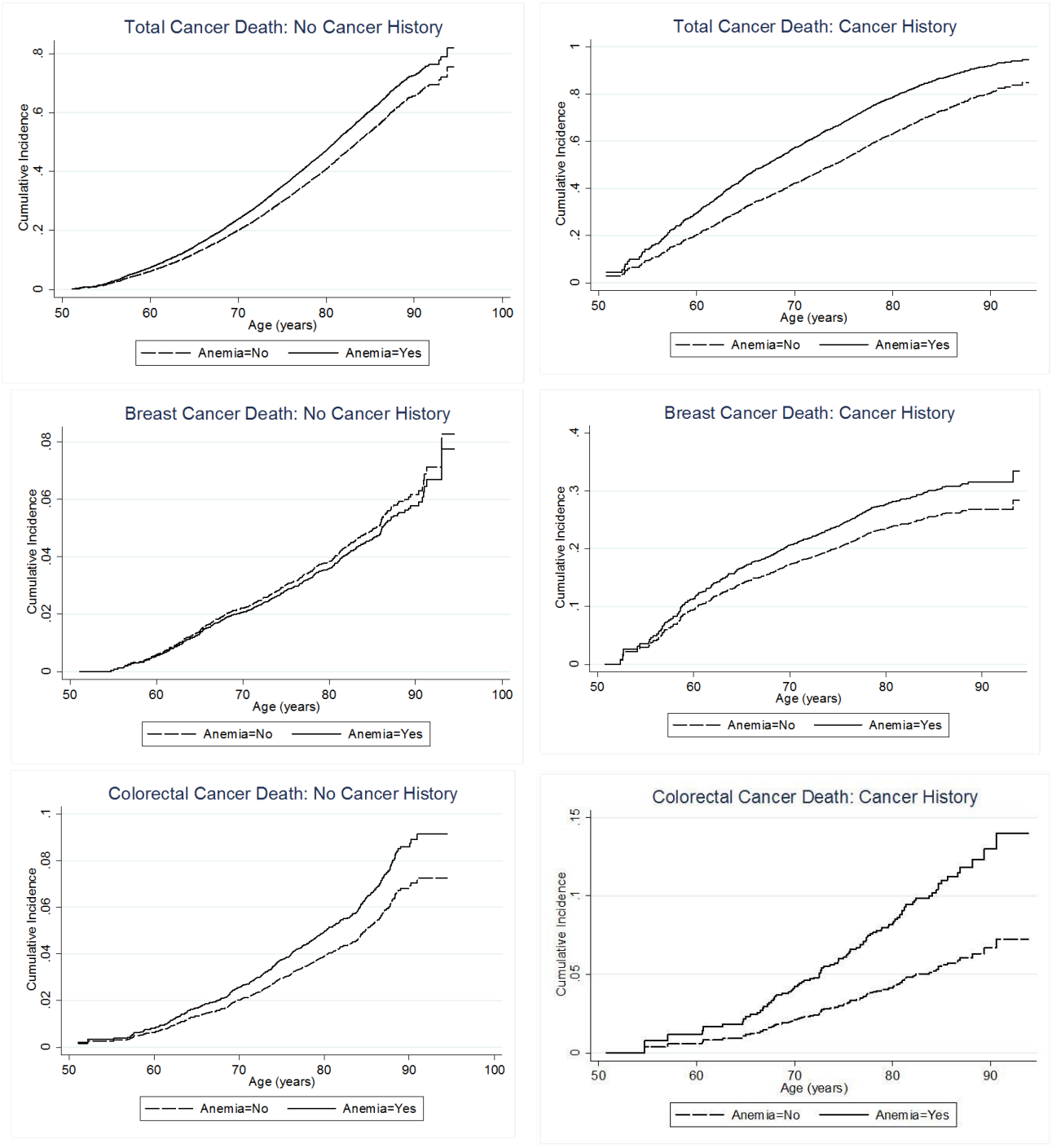
Significant crude mortality rate ratios highlighted in **bold** font

Table 12 Anemia and High Hemoglobin Sub-Hazard Ratios for Total, Breast and Colorectal Cancer Mortality by Cancer Group

	Total Cancer Death	**Breast Cancer Death**	**Colorectal Cancer Death**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Anemia[1]			
Without cancer history			
Age-adjusted Model	**1.20(1.07,1.34)**	1.01(0.67,1.52)	1.39(0.98,1.98)
Fully adjusted Model	**1.21(1.09,1.36)**	0.93(0.61,1.43)	1.27(0.88,1.83)
With cancer History			
Age-adjusted Model	**1.61(1.33,1.95)**	1.41(0.99,2.02)	**2.26(1.29,3.92)**
Fully adjusted Model	**1.55(1.27,1.89)**	1.21(0.84,1.76)	**2.01(1.09,3.72)**
High Hemoglobin[2]			
Total Population			
Age Adjusted Models	**1.20(1.10,1.30)**	1.02(0.79,1.32)	1.11(0.83,1.49)
Fully adjusted Model	0.99(0.91,1.09)	1.03(0.77,1.37)	1.14(0.83,1.56)

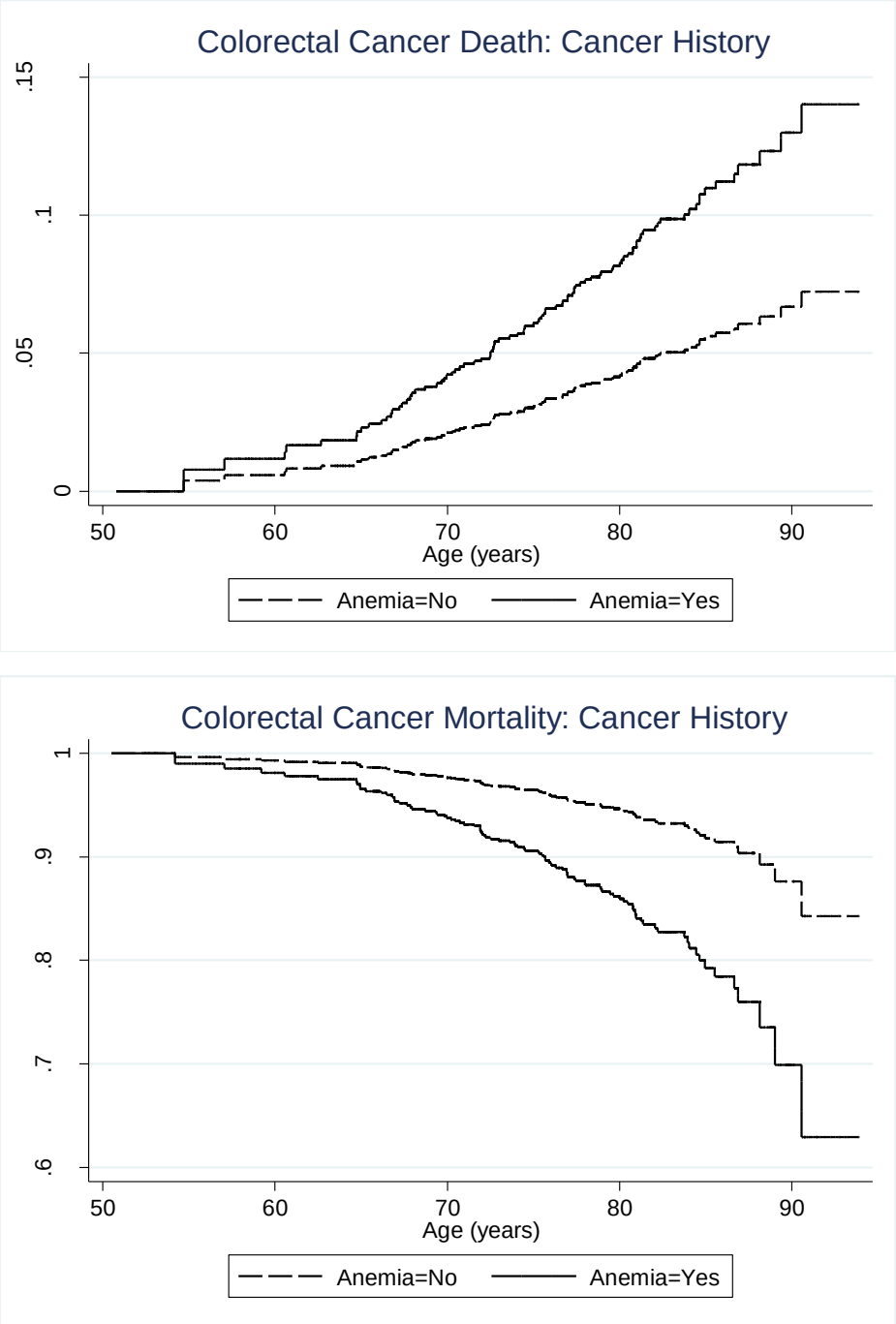
1. Anemia models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence and diabetes at screening
2. High hemoglobin models adjusted for body mass index, dietary fiber intake, race/ethnicity, education, income, smoking status, clinical trial assignment, cancer history, region of residence diabetes at screening and cardiovascular disease at baseline
3. Significant crude mortality rate ratios highlighted in **bold** font

Figure 4 Cumulative Incidence of Total, Breast and Colorectal Cancer Mortality by Anemia Status



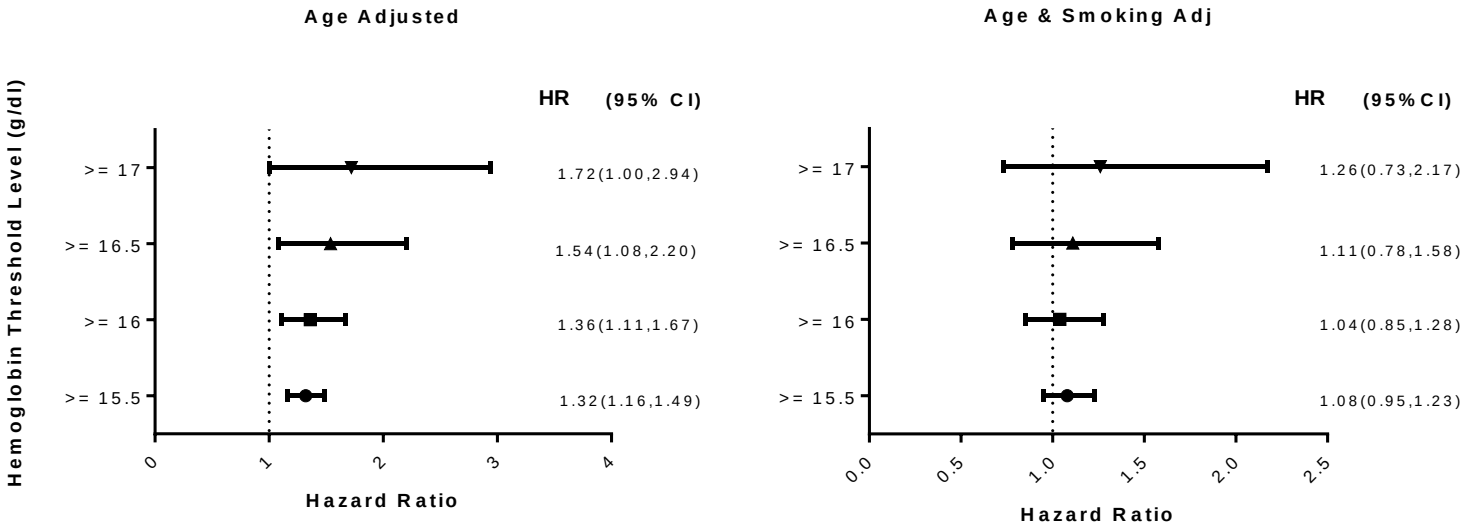
Models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence, diabetes at screening and dietary iron intake

Figure 5 Comparison of the Hazard of Colorectal Cancer Mortality Using Competing Risks and Cox Proportional Hazards Approaches



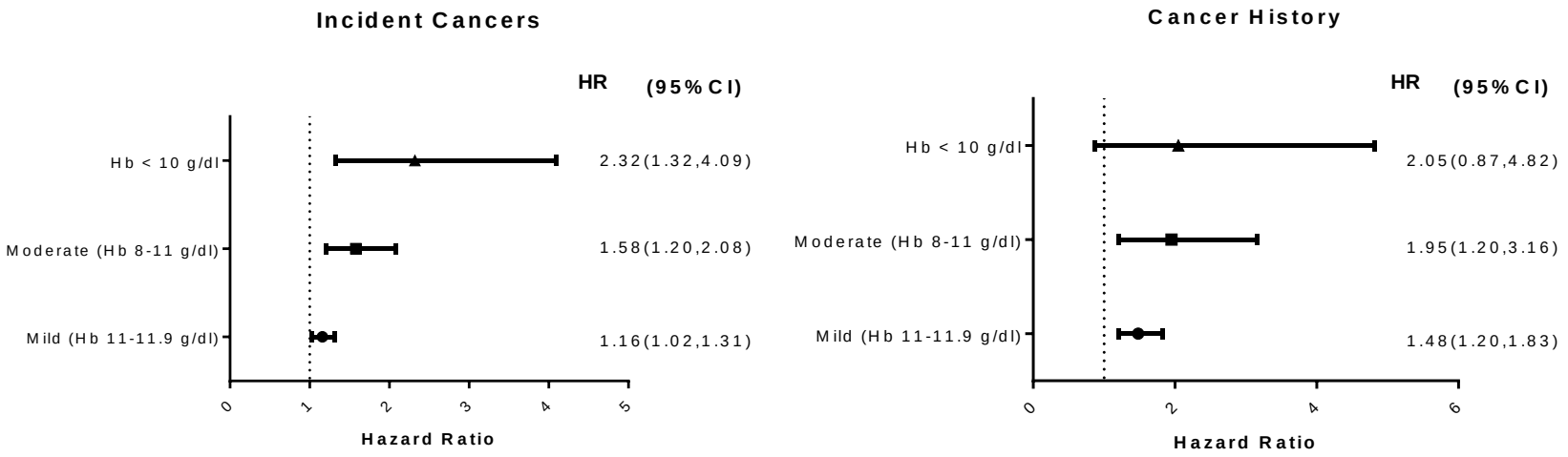
Models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence, diabetes at screening and dietary iron intake

Figure 6 Hazard of Total Cancer Death by High Hemoglobin Levels



High hemoglobin models adjusted for body mass index, dietary fiber intake, race/ethnicity, education, income, smoking status, clinical trial assignment, cancer history and region of residence

Figure 7 Hazard of Total Cancer Death by Cancer Group Using Other Low Hemoglobin Categorizations



Anemia models adjusted for body mass index, total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence and diabetes at screening

Table 13 Hazard of Cancer Mortality by Hemoglobin g/dl Increments and Quintiles in Women with Incident Cancers and Cancer History

	Hemoglobin Increments (g/dl)				
	< 12	**12.0-12.9**	**13-13.9**	**14-14.9**	**>= 15**
Without cancer history					
Total Cancer Death					
Age-adjusted	**1.25 (1.10, 1.41)**	1.00	1.01 (0.95, 1.09)	1.05 (0.97, 1.13)	**1.27 (1.14, 1.40)**
Fully-adjusted	**1.24 (1.10, 1.41)**	1.00	1.02 (0.95, 1.10)	1.02 (0.94, 1.11)	1.07 (0.96, 1.20)
Breast Cancer Death					
Age-adjusted	1.08 (0.69, 1.69)	1.00	1.20 (0.95, 1.53)	0.91 (0.70, 1.20)	1.17 (0.81, 1.69)
Fully-adjusted	0.98 (0.61, 1.56)	1.00	1.19 (0.93, 1.53)	0.91 (0.68, 1.21)	1.31 (0.88, 1.96)
Colorectal Cancer Death					
Age-adjusted	1.30 (0.89, 1.93)	1.00	0.92 (0.72, 1.16)	0.91 (0.70, 1.17)	1.04 (0.72, 1.49)
Fully-adjusted	1.18 (0.79, 1.77)	1.00	0.94 (0.74, 1.20)	0.89 (0.68, 1.17)	1.11 (0.77, 1.61)
Cancer History					
Total Cancer Death					
Age-adjusted	**1.33 (1.07, 1.64)**	1.00	**0.80 (0.70, 0.92)**	**0.69 (0.59, 0.81)**	0.89 (0.71, 1.10)
Fully-adjusted	**1.28 (1.03, 1.59)**	1.00	**0.80 (0.69, 0.92)**	**0.68 (0.57, 0.80)**	**0.78 (0.62, 0.99)**
Breast Cancer Death					
Age-adjusted	1.09 (0.73, 1.62)	1.00	**0.73 (0.56, 0.96)**	**0.66 (0.48, 0.90)**	0.68 (0.43, 1.06)
Fully-adjusted	0.96 (0.64, 1.43)	1.00	**0.73 (0.56, 0.96)**	**0.68 (0.49, 0.93)**	0.65 (0.40, 1.06)
Colorectal Cancer Death					
Age-adjusted	**2.23 (1.14, 4.38)**	1.00	0.91 (0.52, 1.59)	1.03 (0.56, 1.91)	1.29 (0.59, 2.83)
Fully-adjusted	**2.23 (1.08, 4.61)**	1.00	1.07 (0.60, 1.92)	1.19 (0.62, 2.27)	1.50 (0.62, 3.62)

Models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence, diabetes at screening and cardiovascular disease at baseline

Table 14 Modification of the Hemoglobin versus Cancer Mortality Association by Cancer Group

	Without Cancer History			With Cancer History			
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**	**p-interaction[1,2]**
Using A Cox Model							
Total Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	
Yes	**1.22**	**1.09**	**1.37**	**1.66**	**1.38**	**2.00**	**0.0018**
High Hemoglobin‡							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>= 15 g/dl	1.02	0.93	1.13	0.96	0.77	1.20	**0.0707**
Breast Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	-
Yes	0.98	0.64	1.50	1.48	1.03	2.13	**0.0842**
High Hemoglobin‡							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>= 15 g/dl	1.18	0.82	1.69	0.84	0.52	1.35	0.1541
Colorectal Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	-
Yes	1.32	0.92	1.91	**2.68**	**1.52**	**4.73**	**0.0173**
High Hemoglobin‡							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>=15 g/dl	1.17	0.83	1.65	0.97	0.41	2.27	0.4608
Using A Competing Risks Model							
Total Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	

	Without Cancer History			With Cancer History			
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**	**p-interaction[1,2]**
Yes	**1.21**	**1.09**	**1.36**	**1.55**	**1.27**	**1.89**	**0.0219**
High Hemoglobin[‡]							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>= 15 g/dl	1.01	0.91	1.12	0.95	0.77	1.18	**0.0864**
Breast Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	-
Yes	0.93	0.61	1.43	1.21	0.84	1.76	0.2725
High Hemoglobin[‡]							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>= 15 g/dl	1.18	0.82	1.70	0.82	0.50	1.32	0.1511
Colorectal Cancer Death							
Anemia							
No	1.00	-	-	1.00	-	-	-
Yes	1.27	0.88	1.83	**2.01**	**1.09**	**3.72**	0.1091
High Hemoglobin[‡]							
< 15 g/dl	1.00	-	-	1.00	-	-	-
>=15 g/dl	1.18	0.84	1.65	0.99	0.40	2.40	0.5160

1 tested at the 0.10 significance level

2 interaction terms tested in fully adjusted models

Anemia models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence and diabetes at screening

High hemoglobin models adjusted for body mass index, dietary fiber intake, race/ethnicity, education, income, smoking status, clinical trial assignment, cancer history, region of residence, diabetes at screening and cardiovascular disease at baseline

Table 15 Modification of the Hemoglobin versus Cancer Mortality Association by Race/Ethnicity

	White non-Hispanic			Black/African American				Other Race/Ethnicity			
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**	**p-Interaction[1]**	**HR**	**LCI**	**UCI**	**p-Interaction[1,2]**
Anemia											
Without cancer history											
No	1.00	-	-	1.00	-	-	-	1.00	-	-	-
Yes	**1.20**	**1.05**	**1.37**	1.19	0.92	1.54	0.9690	1.47	0.97	2.25	0.4100
Cancer History											
No	1.00	-	-	1.00	-	-	-	1.00	-	-	-
Yes	**1.51**	**1.22**	**1.86**	1.65	0.89	3.04	0.3456	1.15	0.40	3.35	0.9924
High Hemoglobin											
Total Population											
< 15 g/dl	1.00	-	-	1.00	-	-					
>= 15 g/dl	0.99	0.90	1.09	1.19	0.75	1.89	0.6509	0.95	0.61	1.48	0.8420

1 tested at the 0.10 significance level

2 interaction terms tested in fully adjusted models

Anemia models adjusted for total calcium intake, race/ethnicity, smoking status, hospitalization overnight within the past 2 years, clinical trial assignment, region of residence and diabetes at screening

High hemoglobin models adjusted for body mass index, dietary fiber intake, race/ethnicity, education, income, smoking status, clinical trial assignment, cancer history, region of residence diabetes at screening and cardiovascular disease at baseline

CHAPTER 6: HEMOGLOBIN LEVEL AND INFLAMMATORY BIOMARKERS IN POST-MENOPAUSAL WOMEN

Introduction

Anemia is an important condition in the elderly population, as it is indicative of a number of negative health consequences, including mortality[147]. Pro or anti-inflammatory cytokines may affect the development of anemia by affecting erythropoiesis through regulation of the production of hepcidin[41], inhibition of the growth of erythroid precursors[18,41,47], inhibition of erythropoietin production[47,148,149], stimulation of ferritin[18,41] or transferrin expression[18,48] or enhancement of the effect of other hematopoietic growth factors[150]. Significant differences in interleukin-6 (IL-6)[32], C-reactive protein (CRP)[32,151], fibrinogen[50,151] and tumor necrosis factor- alpha (TNF-alpha)[32] levels have been observed between anemic or iron deficient and non-anemic individuals in observational studies involving older individuals. There are few observational studies investigating the association between hemoglobin level and inflammatory markers in older populations. In addition, the relationship between hemoglobin level and other biomarkers remains to be studied.

The role of inflammatory biomarkers in erythropoiesis may be altered by obesity status or aging. Higher levels of the inflammatory cytokines CRP[152,153], IL-6[153] and TNF-alpha[153] have been reported in over versus normal weight individuals in observational studies. In addition, obesity may be associated with iron deficiency[152,154]. These associations have been reported primarily in younger populations. However, Lecube and colleagues (2006) reported that obese post-menopausal women had higher serum transferrin levels when compared with their normal weight controls[154]. In addition, levels of IL-6 and TNF-alpha may increase with age in the absence of chronic disease[155,156] negatively affecting erythropoiesis[156].

Although associations between inflammatory biomarkers and anemia status have previously been demonstrated, differences by race/ethnicity have not been fully explored, despite reported variations in anemia prevalence[2]. Other questions which remain unanswered are whether the associations between anemia and inflammatory markers are modified by age[155], and whether other markers of inflammation are associated with anemia. The present study therefore seeks to investigate the association between anemia status and/or hemoglobin level with inflammatory biomarkers, and to determine whether these associations are modified by age, race/ethnicity or obesity status. As a secondary objective we seek to determine the association between all biomarkers measured on a multiplex platform and anemia or hemoglobin level.

Methods

Study Population

The study population comprised 1,001 women who had biomarker data from the Biomarkers and Genetic Factors Related to Sarcopenia, an Ancillary Study of the Women's Health Initiative (WHI). Details regarding the study design of the WHI have been previously published[102,100]. In brief, women were recruited from 40 centers in twenty-four states[102] between 1993 and 1998[103]. Ten of the forty sites were selected as minority recruitment sites[102]. The CT comprised four different components, including: (1) two hormone therapy versus placebo trials, which investigated reduction in cardiovascular risk (2) a dietary modification trial in which a low fat diet was predicted to reduce breast and colorectal cancer risk and (3) a calcium and Vitamin D trial to investigate fracture risk[100]. Women were entered into the OS if they were interested in the dietary modification or hormone therapy trials, but were unwilling or ineligible to participate in the CT[100]. Main eligibility criteria for both the CT and OS were post-menopausal status, willingness to provide informed consent, and intention to reside in the area of study for a minimum of three years[102]. Exclusion criteria for both the OS and CT included medical conditions which adversely affect three-year survival, as well as other conditions which were likely to affect adherence or safety[102]. The hormone therapy trials

of estrogen alone or estrogen plus progestin were stopped in 2004 and 2002 respectively, but participants were followed without intervention until the end of the study time period. Informed consent was obtained from participants at each recruitment site[129].

Women were selected exclusively from the WHI OS for the Biomarkers and Genetic Factors Related to Sarcopenia Study at 2 centers that measured Dual-energy X-ray absorptiometry **(**DXA). These were the Universities of Arizona, Pittsburgh and Alabama, Birmingham. Participants for the WHI-OS DXA cohort were located in the communities where DXA scans were carried out. Two-thousand and eight hundred (2,800) Hispanic and non-Hispanic women were randomly selected from a possible pool of 5,428 eligible women in the WHI OS-DXA cohort to form a genetic study cohort. One thousand women (1,000) women were then randomly selected from the genetic cohort for determination of biomarker assessments[114]. Analysis was conducted using all biomarker measures.

Anemia Assessment

Hemoglobin levels were determined from a complete blood count (CBC) which was assessed from 12 hour fasting samples[129]. Anemia was defined using the World Health Organization definition of a hemoglobin level < 12 g/dl[19,130]. Participant hemoglobin levels were only available at year baseline and year 3, hence persistent anemia was defined as having a hemoglobin level < 12 g/dl. Transient anemia was defined as being anemic at year 0 only.

Biomarker Assessment

Blood was collected after a 12 hour fast[157]. Serum was collected and stored at -70^{o} C until the time of analysis[157]. Eighty-nine biomarkers were measured using bead-based multiplex suspension assays (Human Multi-Analyte Profile (MAP) version 1.6, Rules Based Medicine (RBM), Austin,TX)[157] and the resultant data was interpreted using RBM proprietary software. Validation was built into assays for each analyte[157]. The full list of

biomarkers measured, with corresponding Least Detectable Dose (LDD) are listed in Appendix 1.

Other Covariates

Age, smoking status, race/ethnicity, education, supplement use, income, hospitalizations within the past 2 years, cancer history and history of hypertension, diabetes and arthritis were assessed at baseline using self-administered and interviewer-administered questionnaires[158]. Dietary intake and alcohol intake were assessed using a semi-quantitative food frequency questionnaire developed by the Fred Hutchinson Cancer Center[129]. Total intake of calcium, folate, iron, vitamin B12 and vitamin C were determined by summing dietary and supplemental intake. Weight was measured using a balance beam scale, while height was measured using a stadiometer[158]. Body mass index was defined as weight (kg) divided by squared height (m^2).

Statistical Analysis

Hemoglobin values below 5 g/dl and greater than 20 g/dl were set to missing (N=20). Hemoglobin levels were analyzed using anemia status and continuous hemoglobin concentration as the two main exposures. Biomarkers were modeled as continuous variables, and were log transformed and standardized prior to analysis. Low/undetectable biomarker values were imputed as half the lowest value of each biomarker. Descriptive statistics were presented as frequencies (%) or medians (interquartile range). Differences in baseline characteristics and/or CRP levels by anemia status were assessed using t-tests or chi-squared tests.

Logistic regression was used to model the association between biomarkers and anemia status, while linear regression was used to model the association with continuous hemoglobin concentration. The main analysis focused on the association of anemia and/or hemoglobin level with selected biomarkers. These were CRP, fibrinogen, interferon-gamma (IFN-gamma), interleukin-1 alpha (IL-1 alpha), interleukin-1 receptor

antagonist (IL-1 ra), IL-6, IL-10, leptin, TNF-alpha and tumor necrosis factor beta (TNF-beta). However, as a secondary aim, the association with all biomarkers measured on the RBM platform was assessed. Seven markers which had less than 200 observations were excluded from analysis. These were endothelin-1, interleukin-12p70 (IL-12p70), interleukin-1β (IL-1β), lymphotactin, MMP-9, prostate specific antigen-free and tissue factor. Models presented both nominal ($p < 0.05$) and adjusted p values. Adjusted p values were Bonferroni-corrected for multiple comparisons and tested at a 0.0006 significance level. Modification of the hemoglobin versus biomarker association by age, ethnicity and obesity was explored for biomarkers of interest, as well as for other biomarkers which were significant in anemia or hemoglobin models. Confounders were identified from predictors which were significantly associated with anemia or hemoglobin. Although anemia was only associated with alcohol intake, models adjusted for age (years), race/ethnicity (White non-Hispanic, Hispanic), total body fat mass (kg), alcohol use (none, < 1 drink/day, >= 1 drink/day) and dichotomized smoking status (never smoked, past/current smoker) based on the association of these factors with hemoglobin level in previous literature. Dichotomized smoking status was used in anemia models due to the small number of anemic individuals who were current smokers. The associations of biomarkers with persistent and transient anemia were also determined using logistic regression models adjusting for the same covariates as listed above. Linear regression models adjusted for age (years), race/ethnicity (White non-Hispanic, Hispanic), total body fat mass (kg), alcohol use (none, < 1 drink/day, >= 1 drink/day), smoking status (never smoked, past smoker, current smoker), income (<$20,000, $20,000-34,999, $35,000-49,999, >=$50,000), diabetes status at screening (no, yes) and hypertension status at baseline (no, yes).

Forward stepwise regression was used to determine biomarkers which were significantly associated with hemoglobin level or anemia status. Stepwise models used a probability of entry of 0.050 and a probability of removal of 0.0501. Only biomarkers were considered

for inclusion. Pearson's correlation coefficients were determined for the association between biomarkers before entry into stepwise models.

Biomarkers were treated using a tiered approach according to data quality in a sensitivity analysis to assess the effect of data quality on results. An LLOQ (Lower Limit of Quantitation) value was provided on biomarker assessment which is 'the concentration of an analyte at which the coefficient of variation of replicate standard samples is 30%'[159]. Other concentrations corresponding to lower coefficient of variation values were provided. Hence, the concentration corresponding to a 20% coefficient of variation of replicate samples was used as a quality control cutpoint, and data falling above and below the cutpoint were treated as follows:

1. > 90% of values above the cutpoint: data was treated as continuous. Missing data was imputed as ½ the Least Detectable Dose (LDD);
2. 80 – 90% of values above the cutpoint: missing data was imputed as ½ the LDD then values were categorized into deciles;
3. 50-80% of values above the cutpoint: missing data was imputed as ½ the LDD then values were categorized into quintiles;
4. 10-50% of values above the cutpoint: data was dichotomized into values above and below the quality control cutoff point;
5. < 10% of values above the cutpoint: the biomarker was excluded from the analysis.

A full list of the biomarkers used, as well as the data treatment approach used in the sensitivity analysis for each biomarker, is given in Supplemental Table 5. Analyses were conducted using STATA 12.0 (Stata Corporation, College Station, TX).

Results

Characteristics of Participants

The median age of participants was 63 years (Table 16). Six-hundred and eighty-three (68%) participants were white non-Hispanic, while three hundred and eighteen women (32%) were Hispanic/Latino. Over half of participants (56%) were never smokers, and

six percent (6.1%) were current smokers (Table 16). The majority of participants (78%) drank less than one drink per day. Median body mass index was 26 kg/m^2 (Table 16). Few potential covariates differed by anemia status (Table 16); when compared with women who drank alcohol, a significantly higher proportion of non- drinkers had anemia (12% versus 5%, p=0.003). Anemic women had significantly higher median CRP (p=0.026), IL-1ra (p=0.046) and TNF-alpha (p=0.006) levels than women who were not anemic.

Age at screening (p=0.016), an income of $20,000-34,999 (p=0.015), current smoking status (p < 0.001), alcohol intake of < 1 drink/day (p=0.007), hypertension (p=0.002) and diabetes status (p=0.005) were significant predictors of continuous hemoglobin concentration (Table 17). Age at screening was positively associated with hemoglobin level (Table 17). Women with an income of $20,000-34,999 per year had higher intercepts than women who earned < $20,000 per year. Women who were current smokers and drank < 1 drink per day had higher hemoglobin intercepts when compared with their respective reference categories (Table 17). Participants who had hypertension or diabetes at baseline had higher hemoglobin intercepts than women who did not have these conditions (Table 17).

Level of Inflammation in the Population and In Obese Women

Fourteen percent of participants (14.5%) had CRP levels greater than or equal to 10 μg/ml while 1 in 3 participants (33%) had CRP levels >= 5 μg/ml (Figure 8). Between 26% - 43% of anemic women had elevated CRP levels, depending on the CRP threshold used (Figure 4). Anemic women had higher proportions of individuals with CRP levels >= 10 μg/ml than women who were not anemic (p=0.010). Median CRP, IL-1ra, IL-6 and TNF-alpha values were significantly higher in obese women when compared with their non-obese counterparts (Figure 11).

Biomarkers Significantly Associated with Anemia

Biomarkers which were significantly associated with anemia status were CRP (OR 1.44; 95% CI: 1.04, 2.00), TNF-alpha (OR 1.53; 95% CI: 1.11, 2.10), TNF-beta (OR 1.39; 95% CI: 1.03, 1.88), beta-2 microglobulin (OR 1.68; 95% CI: 1.29, 2.18), erythropoietin (OR 1.89; 95% CI: 1.37, 2.60) and TNF R2 (OR 1.68; 95% CI: 1.29, 2.20) (Table 18). All significant markers were positively associated with anemia status (Table 18). A one standard deviation change in erythropoietin level was associated with the largest increased odds (89%) of anemia (Table 18).

Biomarkers Significantly Associated with Persistent and Transient Anemia

CRP was associated with increased odds of persistent (Table 19, OR 2.39; 95% CI: 1.46, 3.90) but not transient (OR 0.95; 95% CI: 0.60, 1.52) anemia (OR 1.14; 95% CI: 0.82, 1.59). IL-1 alpha (OR 1.68; 95% CI: 1.05, 2.71) was also associated with persistent anemia, while TNF-beta (OR 1.99; 95% CI: 1.21, 3.29) was positively associated with transient anemia. No biomarker had a significant association with persistent or transient anemia at the Bonferroni-corrected level of 0.0006 (Table 19). However, persistent anemia was significantly associated with a larger number of biomarkers at the 0.05 level, including the acute phase/inflammatory markers alpha-1 antitrypsin (OR 1.65; 95% CI: 1.07, 2.52), beta-2 microglobulin (OR 1.73; 95% CI: 1.19, 2.50), haptoglobin (OR 1.85; 95% CI: 1.04, 3.30) and TNFR2 (Table 19, OR 1.56; 95% CI: 1.04, 2.36).

Biomarkers Significantly Associated with Hemoglobin Concentration

IL-1 alpha (p=0.0295), IL-10 (p=0.0139), leptin (p=0.0023), brain derived neurotrophic factor (BDNF, p=0.0003), erythropoietin (p <0.0001), and sex hormone binding globulin (SHBG, p=0.0002) were significantly associated with continuous hemoglobin concentration in fully adjusted models (Table 20). The p-value for the association between ferritin and hemoglobin (p=0.0006) fell just outside the threshold of the correction for multiple comparisons. IL-1 alpha (Coeff= -0.079), IL-10 (Coeff = -0.092), erythropoietin (Coeff = -0.155) and SHBG (Coeff = -0.141) were negatively associated

with hemoglobin concentration (Table 20), while leptin (Coeff = 0.152) and BDNF (Coeff=0.124) were positively associated (Table 20).

Modification of the Biomarker-Hemoglobin Concentration by Obesity and Age Category

There were few biomarkers in which the association with hemoglobin was modified by race/ethnicity (results not shown). These were complement C3 (p=0.054), IL-18 (p=0.096) and thrombopoietin (p=0.098). None of these markers were retained as significant predictors in anemia or hemoglobin models. Obesity modified BDNF (p-interaction=0.001), erythropoietin (p-interaction=0.003), ferritin (p-interaction <0.001) and leptin (p-interaction=0.015) associations with hemoglobin concentration (Table 21). In all cases the association between the biomarker and hemoglobin concentration was stronger in obese women (Table 21, Figure 9).

Age modified the associations of beta-2 microglobulin, BDNF, ferritin, IL-1ra (p-interaction – 70-79 years =0.055) and TNF-alpha with hemoglobin level (Table 22, Figure 10). The positive association between BDNF and ferritin with hemoglobin was strongest in the youngest women (Table 22, Figure 10). TNF-alpha was negatively associated with hemoglobin in women aged 50-59 and 60-69 years, but was positively associated with hemoglobin in women 70-79 years old (Table 22, Figure 10). The association between beta-2 microglobulin and hemoglobin was positive in women aged 60-69 years, in comparison with a negative association in other age categories. Levels of obesity differed significantly by age category, with younger women having the highest proportion of obesity (results not shown).

Sensitivity Analyses to Account for Biomarker Data Quality

Sensitivity analysis was conducted to identify the biomarkers that remained associated with anemia status when alternative methods of data treatment were used to account for differences in biomarker data quality. Significant predictors of anemia status were CRP (OR 1.44; 95% CI: 1.04, 2.00), IL-10 (OR 1.92; 95% CI: 1.00, 3.66), beta-2

microglobulin (OR 1.68; 95% CI: 1.29, 2.18) and TNFR2 (OR 1.68; 95% CI: 1.29, 2.20) (Table 23). Biomarkers which were significantly associated with continuous hemoglobin concentration were leptin (p=0.002), BDNF (p <0.001) and SHBG (p <0.001) (Table 24).

Multi-marker Predictors of Anemia Status and Hemoglobin Concentration

When biomarkers significant at the p=0.05 level were entered into a stepwise model, biomarkers which were associated with anemia status were erythropoietin (results not shown, HR: 1.73 95% CI: 1.28, 2.33), beta-2 microglobulin (HR: 1.58 95% CI: 1.23, 2.02) and IL-3 (HR: 1.73 95% CI: 1.25, 2.39). Biomarkers associated with hemoglobin level were SHBG (p=0.028), erythropoietin (p <0.001), ferritin (p=0.004), BDNF (p < 0.001), IL-3 (p < 0.001), alpha-fetoprotein (p=0.010), creatine kinase-MB (p=0.006) and insulin (p=0.011). Multi-marker models only explained 9% of the variance in continuous hemoglobin concentration (results not shown).

Discussion

In the present study anemic women had significantly higher levels of inflammation than non-anemic women. Anemia was associated with elevated levels of CRP, TNF-alpha, TNF-beta, beta-2 microglobulin, and TNFR2. Elevated CRP and IL-1 alpha were associated with persistent, but not transient anemia. Although biomarkers were not significant when adjusted for multiple comparisons, persistent anemia was associated with alpha-1 antitrypsin, beta-2 microglobulin, IL-1 alpha, IL-3, IL-7 and TNFR2. Biomarkers which were significantly associated with continuous hemoglobin concentration were IL-1 alpha, IL-10, leptin, BDNF, erythropoietin, and SHBG.

This is one of few studies which have examined the association of anemia with inflammatory markers in older individuals in the population setting. The Invecchiare in Chianti (InCHIANTI) study reported significantly elevated CRP levels in subjects with iron deficiency anemia and anemia of inflammation. TNF-alpha was elevated in anemia

of chronic kidney disease and anemia of inflammation[32]. The association of elevated CRP levels with anemia or hemoglobin levels have been reported in other populations of older individuals[2,3,160,151]. Similar to these studies, the present research found that elevated levels of CRP and TNF-alpha were associated at the 0.05 level with a greater risk of developing anemia in postmenopausal women.

TNF-alpha (or cachectin/differentiation inducing factor (DIF)) is produced primarily by activated macrophages and monoctyes[161], but also by neutrophils, fibroblasts, Kupffer cells and T and B cells, among others[162]. It may play a harmful or beneficial role in disease depending on the pathways activated, including resisting tumors and mediating response to infections[161]. There are numerous receptors in the TNF receptor family[163], but TNF-alpha is only able to bind to TNFR1 (or p55) and TNFR2 (or p75). TNFR1 expression occurs in most cells in the body, whereas the expression of TNFR2 occurs in only a few types of immune cells[164]. The binding of TNF-alpha to TNFR1 leads to apoptosis, but binding to TNFR2 generally leads to cell survival[164]. Despite the distinct roles of the two receptors, there is some overlap in the function, with the possibility of TNFR2 facilitating apoptosis and cell death[164]. Lymphotoxin alpha was formerly known as TNF-beta. It is primarily produced by beta cells and lymphocytes[165] and exists as a homotrimeric form, $LT\alpha_3$, or binds to lymphotoxin beta to form $LT\alpha_1\beta_2$. $LT\alpha_3$ binds to TNF receptors, while $LT\alpha_1\beta_2$ binds to lymphotoxin beta receptor $LT\beta R$[166]. Lymphotoxin is essential for the development of the spleen and lymph nodes and plays a critical role in the immune response to infection as well as in regulation of intestinal microbiota[166].

TNF-alpha affects erythropoiesis by inhibiting the growth of erythroid precursors[18,42,167] and inhibiting erythropoietin expression[18, 44, 168], both actions which impair the survival of red blood cell precursors. In addition, TNF-alpha stimulates ferritin expression[18,46], which leads to increased storage of iron in the mononuclear phagocyte system and subsequent reduction of available iron for erythropoiesis[18]. These actions are thought to play a role in the development of anemia of chronic disease[18], substantiating the positive association

observed between TNF-alpha and anemia in the present study. TNFR2 inhibits burst forming unit erythroid (BFU-E) colony growth in vitro, though the level of inhibition is less than that observed for TNF-alpha or TNFR1[167]. Similarly, recombinant lymphotoxin was demonstrated to inhibit BFU-E and colony forming unit erythroid (CFU-E)[42]. These observations suggest that elevated levels of TNFR2 or lymphotoxin could impair the proliferation of red blood cell precursors leading to a positive association with anemia.

C-reactive protein is the main acute phase protein in humans, with levels capable of rising 1000-fold during infection[169]. It plays a role in innate immunity by acting as an opsonin, facilitating the removal of microbes and apoptotic cells[170]. It is a non-specific marker of inflammation, and levels are elevated in infection, trauma and malignancy[171]. CRP does not appear to play a direct role in erythropoiesis; hence elevated levels may reflect other inflammatory conditions associated with anemia.

IL-1 alpha was inversely associated with continuous hemoglobin level. Interleukin-1 alpha belongs to the interleukin-1 family which comprises interleukin-1 alpha, interleukin-1 beta and interleukin-1 receptor antagonist[172]. IL-1 alpha and beta have similar functions, but IL-1 alpha acts within the cell, while IL-1 beta is largely secreted extra-cellularly[172,173]. IL-1 alpha is mainly produced by phagocytes in response to inflammation [172]. The role of IL-1 in disease is multifactorial, including the development of atherosclerotic plaques[172] and destruction of pancreatic beta cells[172], and levels may be up-regulated in many types of solid tumors[173]. IL-1 receptor antagonist inhibits the action of IL-1 alpha by competitively binding to the interleukin 1 receptor without producing a response[173,174]. Similar to TNF-alpha, IL-1α inhibits erythropoietin progenitor growth[43], retards erythropoietin production[18,44,168] and stimulates ferritin expression[45], explaining the positive association with anemia. IL-1 alpha is not normally found in the circulation except in cases of severe disease[174], hence elevated levels may reflect diseased states also associated with anemia. There appear to be few observational studies which have reported on the association between anemia and IL-1 alpha levels in older individuals.

Elevated biomarkers were significantly associated with persistent, but not transient anemia, suggesting that inflammation in these individuals may be chronic, rather than acute. Analysis of CRP levels indicated that between 20-40% of anemic women may have associated inflammation, and that anemic women had higher levels of inflammation than their non-anemic counterparts. Elevated inflammatory markers were associated with lower hemoglobin levels, reinforcing previously observed associations between inflammation, inflammatory markers and anemia. The cross-sectional design in the main analysis only allowed for determination of the baseline biomarker and hemoglobin level, and not the evaluation of incident disease as potential confounders.

In addition to inflammatory markers, erythropoietin was associated with both anemia status and hemoglobin level in the present study. The biomarker is a hormone produced by the kidneys in the presence of hypoxia. It binds to receptors on erythroid precursors promoting cell proliferation[175] and preventing apoptosis[176]. The inverse relationship between hemoglobin and erythropoietin has been reported previously[149,177]. There was a significant interaction between erythropoietin and obesity, with obese women having a stronger negative association between hemoglobin and erythropoietin levels. Obesity is associated with increased levels of inflammatory markers, and this was observed in the present population, where women with a BMI greater than or equal to 30 kg/m^2 had higher median levels of CRP, IL-6, and TNF-alpha. Ferrucci and colleagues (2005) found that inflammation led to an increase in erythropoietin levels in non-anemic individuals, while there was a blunted erythropoietin response in individuals with anemia[149]. The findings of the present study are in keeping with the compensatory rise of erythropoietin observed in non-anemic individuals, given that approximately ninety-five percent (95%) of the present population would be non-anemic.

Ferritin is one of the major iron-binding proteins in the body[178,179] and has two subunits, ferritin-H and ferritin-L[179]. The major function of ferritin is iron sequestration[179]. However, it also has the ability to oxidize $Fe2^+$ to $Fe3^+$, which is attributed to the H

subunit[179]. The marker is an acute phase reactant[180], in which levels are increased in chronic inflammatory conditions such as obesity[181,182] as well as in iron overload[179]. Ferritin levels appear to be positively associated with hemoglobin concentration[183,184,185], though there is limited literature on this association. Similar to previous findings[183,184,185], ferritin levels were positively associated with hemoglobin level in the present population. This association appeared to be stronger in obese individuals, and to decline with age. The modification of the hemoglobin-ferritin association by obesity may be explained by increased ferritin levels in the presence of chronic inflammation and obesity[181, 182], and the corresponding positive association between ferritin and hemoglobin levels.

In addition to well-established markers of inflammation or erythropoiesis, the study also found an association between anemia and elevated levels of beta-2 microglobulin, which is a polypeptide chain associated with the human leukocyte antigen class I molecule[175,186]. Beta-2 microglobulin has been found to be inversely associated with hemoglobin level or positively associated with anemia status in individuals with Hodgkin's lymphoma[187], Systemic Lupus Erythematosus (SLE)[188] and chronic kidney disease[189], and is a marker of poor survival in a number of diseases including diabetes[190], chronic kidney disease[189], non-Hodgkin's lymphoma [191] and multiple myeloma[192]. In animal models beta-2 microglobulin knockout mice develop iron overload, mimicking hereditary hemochromatosis[193]. Beta-2 microglobulin has been described as a chaperone to the hemochromatosis protein[194], HFE, and the beta-2 microglobulin bond is necessary for proper cell expression of HFE[195]. HFE has been found to inhibit intestinal iron uptake in vitro[196]. Studies evaluating the prognostic significance of beta-2 microglobulin have found elevated levels in anemic patients[197]. However, there are few observational studies which have reported on an association between beta-2 microglobulin and anemia in a relatively healthy population of older individuals.

There is limited published data on the association between leptin with hemoglobin level. Togo et. al (1999)[198] and Tungtrongchitr et. al (2000)[199] reported negative associations

between leptin level and hemoglobin level in Japanese[198] and Thai populations[199]. However, other studies have found no association[200] or a positive association in patients with renal failure[201] or ovarian cancer[202]. It is notable that the present study which is comprised of postmenopausal women, also found a positive association between leptin and hemoglobin level, similar to findings in a population of women with ovarian cancer[202], as there appears to be differences in leptin levels by sex[199]. Obesity was found to be a modifier of the hemoglobin-leptin association, with a stronger positive association being observed for obese women. Leptin enhances the growth of hematopoietic progenitors in vitro[203,204], supporting the observation of higher hemoglobin levels in obese individuals who are also likely to have higher leptin levels[205]. However, leptin may induce hepcidin expression[206] which promotes iron storage[18], suggesting that the influence of leptin on erythropoiesis is not straightforward. Further research is therefore needed to clarify the association between leptin and hemoglobin level in populations of different age groups, gender and obesity status.

SHBG was inversely associated with hemoglobin concentration. This association might be due to the effect of testosterone on hemoglobin level, as SHBG is a sex steroid binding protein which binds to dihydrotestosterone and testosterone with high affinity, and estradiol with lower affinity[207]. Testosterone is produced in the adrenal glands and ovaries in women, and levels fall with age up to the menopause, when the decline plateaus, and there may be an increase in free testosterone due to a reduction in SHBG levels[208]. In young women approximately 65% of testosterone is bound to SHBG, up to 2% is free and the remainder is bound to albumin[208]. Low testosterone has been associated with low hemoglobin level or anemia in both men[209,210] and women[209], and the findings of Ferrucci et. al (2006) suggest that this association may be stronger in women.

Hemoglobin level was also associated with BDNF. BDNF belongs to the neurotrophin family and plays a critical role in neuronal development[211] and may be associated with Alzheimer's or Parkinson's diseases, depression[211], and obesity[212]. Levels appear to be

positively correlated with body mass index[213], and are elevated in obese individuals[213]. There is a paucity of literature exploring the association of BDNF with hemoglobin level. In the present study BDNF was positively associated with hemoglobin, and the association was stronger in obese individuals. Further research is needed to confirm these associations.

The study had a number of limitations. Although anemia and hemoglobin level were associated with a number of biomarkers the coefficient of determination (R^2) for multivariate hemoglobin models was 0.09, indicating that the biomarkers studied did not explain much of the variance in hemoglobin level. Although a number of potential confounders were explored, the low coefficient of determination in multivariate models even after adjusting for covariates in hemoglobin models suggests the possibility of residual confounding. Anemia was only associated with alcohol intake in the present population, but anemia models adjusted for age, race/ethnicity, smoking status, alcohol intake and total body fat mass. This may raise the question of over-adjustment of anemia models. However, the biomarkers which were significant predictors of anemia were those that were also significant in crude and age adjusted models, indicating that fully adjusted models did not mask the association of these markers. The study was unable to account for renal disease as a potential confounder since renal function may affect the levels of hemoglobin as well as of biomarkers such as erythropoietin. Anemia was defined according to the WHO classification, and other indices such as ferritin or transferrin were not used to determine anemia type. This is important, given that inflammatory markers may be more likely to be associated with anemia of inflammation, iron deficiency anemia or anemia of chronic kidney disease[32].

Although sensitivity analysis explored the association of baseline biomarkers with persistent and transient anemia, the main analysis explored the cross-sectional association between inflammatory markers and hemoglobin. There are therefore limitations in inferring a causal association between the exposure and outcome. Biomarker

measurement was carried out on a multiplex platform in which 89 biomarkers were analyzed. Sensitivity analysis excluded the consideration of biomarkers which play an important role in erythropoiesis due to the level of data quality. However, the biomarkers which were associated with anemia or hemoglobin level in the main analysis had biologically plausible roles in erythropoiesis, supporting the validity of the findings.

The study also had a number of strengths. Eighty-nine biomarkers were measured on multiplex platform allowing for the exploration of a number of biomarkers with anemia and hemoglobin level. The volume of data collected in the WHI allowed for the exploration of the association with a number of important confounders. The study determined the association of biomarkers with both anemia status and hemoglobin concentration, and sensitivity analysis was carried out to determine the effect of data quality on study results.

In summary, anemia was associated with elevated levels of CRP, TNF-alpha, TNF-beta and TNFR2 while hemoglobin level was association with IL-1 alpha and IL-10. The findings confirm the association of pro and anti-inflammatory markers in a population of post-menopausal women. Anemia was also associated with beta-2 microglobulin and erythropoietin, while hemoglobin was associated with leptin, BDNF, erythropoietin and SHBG. Further research should be carried out to confirm the association of these markers in other elderly populations.

Tables and Figures

Table 16 Baseline Characteristics by Anemia Status

Baseline Characteristic	Total Population		By Anemia Status				p
			Anemia=No (N=921)		Anemia=Yes (N=53)		
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
Age at screening (years)	63.0	(57,69)	63.0	(57,69)	61.0	(57,68)	0.3665
BMI (kg/m2)*	26.1	(23.2,29.6)	26.1	(23.3,29.6)	26.3	(22.3,30.6)	0.9367
Total Body Fat Mass (kg)	28.2	(22.2,35.2)	28.2	(22.3,35.2)	28.7	(21.3,36.0)	0.9037
PA: MET hrs./week*	9.5	(3.5,20.2)	9.8	(3.5,20)	7.0	(2.3,19.8)	0.3834
Total Folate Intake (mcg)	308.8	(193.7,594.2)	308.9	(192.5,593.1)	279.8	(182.0,558.4)	0.3890
Total Iron Intake (mg)	16.5	(10.3,27.5)	16.3	(10.3,27.5)	15.3	(10.1,25.1)	0.4948
Total Vitamin B12 Intake (mcg)	7.9	(4.2,11.5)	7.8	(4.2,11.7)	7.2	(4.5,9.3)	0.3394
Total Vitamin C Intake (mg)	153.0	(82.6,542.0)	155.0	(83.9,551.9)	125.3	(73.8,256.5)	0.0872
	No.	**%**	**No.**	**%**	**No.**	**%**	
Race							
White non-Hispanic	683	100.0	628	95.0	33	5.0	0.3693
Hispanic/Latino	318	100.0	293	93.6	20	6.4	
Income							
< $20,000	267	100.0	243	93.8	16	6.2	0.5689
$20,000-$34,999	244	100.0	223	93.3	16	6.7	
$35,000-$49,999	159	100.0	144	94.7	8	5.3	
>=$50,000	235	100.0	222	96.1	9	3.9	
Education							0.5578
Less than College	694	100.0	638	94.5	37	5.5	
College or Above	293	100.0	272	95.4	13	4.6	
Smoking Status							
Never smoked	550	100.0	509	95.1	26	4.9	0.1833
Past smoker	370	100.0	336	93.1	25	6.9	
Current smoker	60	100.0	56	98.3	1	1.7	
Alcohol Intake							

Baseline Characteristic	Total Population		By Anemia Status				p
			Anemia=No (N=921)		Anemia=Yes (N=53)		
None	128	100.0	110	88.0	15	12.0	**0.0031**
< 1 drink/day	766	100.0	713	95.4	34	4.6	
>= 1 drink/day	92	100.0	83	95.4	4	4.6	
Stomach or Duodenal Ulcer							
No	892	100.0	821	94.8	45	5.2	0.2738
Yes	74	100.0	67	91.8	6	8.2	
Cardiovascular Disease ever							
No	791	100.0	730	95.2	37	4.8	0.3585
Yes	136	100.0	125	93.3	9	6.7	
Hypertension ever							
No	707	100.0	649	94.6	37	5.4	0.9069
Yes	275	100.0	255	94.8	14	5.2	
Diabetes ever							
No	935	100.0	861	94.7	48	5.3	0.3883
Yes	65	100.0	59	92.2	5	7.8	
Arthritis							
No	498	100.0	455	94.8	25	5.2	0.7282
Yes	481	100.0	445	94.3	27	5.7	
Cancer Ever							
No	890	100.0	819	94.6	47	5.4	0.6248
Yes	93	100.0	84	93.3	6	6.7	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**p**
CRP (ug/ml)	3.2	(1.3,6.4)	3.1	(1.3,6.3)	4.5	(2.4,10)	**0.0262**
Fibrinogen (mg/ml)	0.03	(0.02,0.04)	0.03	(0.02-0.04)	0.03	(0.02,0.04)	0.9778
IFN-gamma (pg/ml)	1.3	(0.5,2.4)	1.3	(0.5,2.4)	1.5	(0.5,2.8)	0.4988
IL-1alpha (ng/ml)	0.003	(0.002,0.004)	0.003	(0.002,0.004)	0.003	(0.002,0.004)	0.1098
IL-1ra (pg/ml)	102.5	(66.0,150.0)	101.0	(66.0,145.0)	123.0	(85.0,175.0)	**0.0462**
IL-6 (pg/ml)	0.4	(0.4,1.7)	0.4	(0.4,1.7)	1.0	(0.4,1.7)	0.4776
IL-10 (pg/ml)	5.3	(4.1,6.8)	5.3	(4.1,6.8)	5.8	(4.1,7.9)	0.2378
Leptin (ng/ml)	17.0	(10.0,27.0)	17.0	(10.0,27.0)	17.0	(8.4,27.0)	0.5410
TNF-alpha (pg/ml)	4.7	(2.7,7.7)	4.7	(2.7,7.5)	7.0	(3.9,11.0)	**0.0057**
TNF-beta (pg/ml)	5.3	(0.9,8.9)	5.2	(0.9,8.9)	7.0	(2.3,11.0)	0.0639

*BMI = Body Mass Index; PA= Physical Activity; p-significant predictors highlighted in **bold** font

Table 17 Baseline Characteristics Associated with Continuous Hemoglobin Concentration[1]

Baseline Characteristic	Coeff	SE	P
Age at screening (years)	0.011	0.004	**0.0161**
Body Mass Index (BMI), kg/m2	0.01	0.006	0.0890
Whole Body Fat Mass (corrected) (kg)	0.005	0.003	0.1228
Sqrt Physical Activity (MET hrs./week)	-0.003	0.016	0.8519
Log Total Folate Intake (mcg)	0.036	0.048	0.4624
Log Total Iron Intake (mg)	0.007	0.051	0.8964
Log Vitamin B12 Intake (mcg)	0.013	0.031	0.6637
Log Vitamin C Intake (mg)	0.033	0.028	0.2413
Race/Ethnicity			
White non-Hispanic	Ref	-	-
Hispanic/Latino	-0.108	0.070	0.1261
Income			
< $20,000	Ref	-	-
$20,000-34,999	-0.227	0.093	**0.0145**
$35,000-49,999	-0.068	0.106	0.5181
>=$50,000	-0.082	0.094	0.3828
Education			
< College	Ref	-	-
>= College	0.047	0.072	0.5143
Smoking Status			
Never smoked			
Past smoker	-0.016	0.070	0.8222
Current smoker	0.508	0.143	**0.0004**
Alcohol Intake (Shortened)			
None	Ref	-	-
< 1 drink/day	0.269	0.099	**0.0070**
>= 1 drink/day	0.241	0.144	0.0941
Stomach or duodenal ulcer ever			
No	Ref	-	-
Yes	-0.149	0.125	0.2352
Cardiovascular Disease ever			
No	Ref	-	-
Yes	-0.048	0.096	0.6212
Hypertension Ever			
No	Ref	-	-
Yes	0.233	0.073	**0.0016**
Diabetes ever			
No	Ref	-	-
Yes	0.371	0.132	**0.0052**
Arthritis ever			
No	Ref	-	-
Yes	0.086	0.067	0.1998
Cancer ever			

Baseline Characteristic	Coeff	SE	P
No	Ref	-	-
Yes	0.126	0.114	0.2685

1. Model = hemoglobin = intercept + baseline characteristic + ε
2. p-significant predictors highlighted in **bold** font

Figure 8 Proportion of Inflammation by Anemia Status

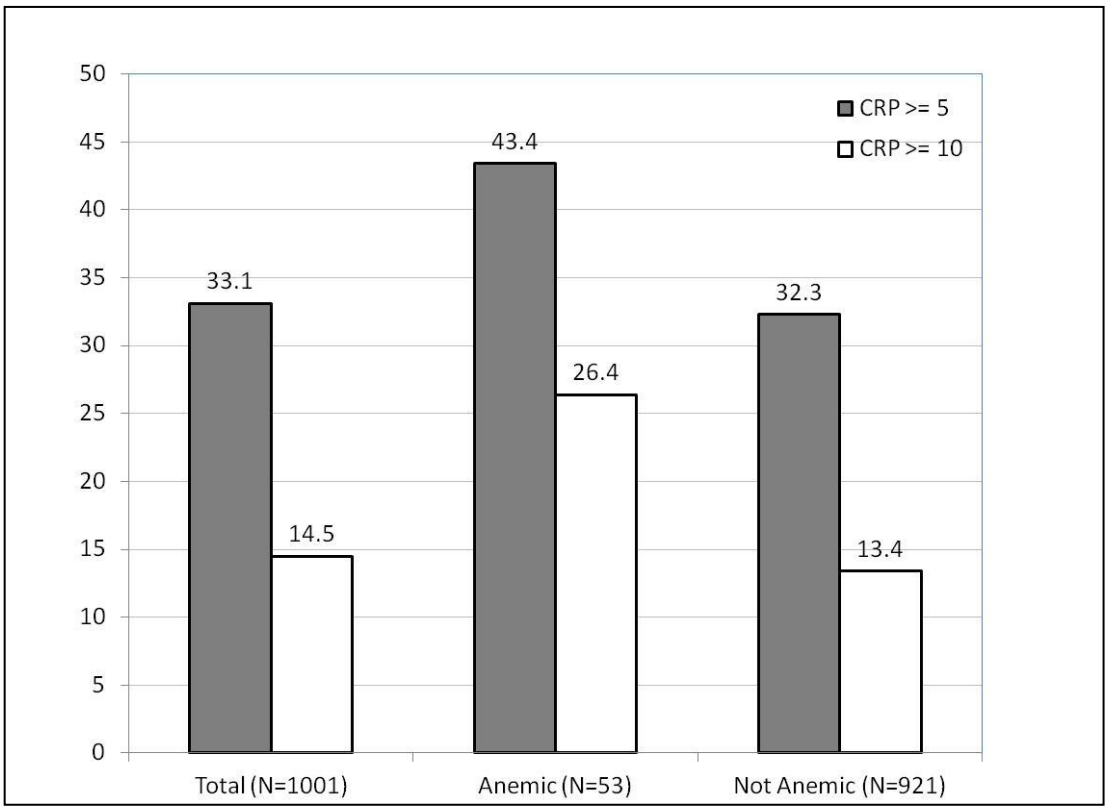


Table 18 Biomarkers Significantly Associated with Anemia Status

Biomarker	Crude				Model 1				Model 2			
	OR	**LCI**	**UCI**	**p**	**OR**	**LCI**	**UCI**	**p**	**OR**	**LCI**	**UCI**	**p**
Biomarkers of Interest												
C Reactive Protein	1.36	1.02	1.82	0.0351	1.36	1.02	1.82	0.0351	1.44	1.04	2.00	0.0271
Fibrinogen	0.97	0.73	1.28	0.8217	0.97	0.73	1.29	0.8403	0.98	0.74	1.30	0.8941
IFN-gamma	1.11	0.84	1.45	0.4628	1.10	0.83	1.44	0.5125	1.08	0.81	1.43	0.6014
IL-1alpha	1.28	0.95	1.72	0.0991	1.27	0.95	1.70	0.1123	1.36	0.99	1.85	0.0546
IL-1ra	1.23	0.91	1.66	0.1795	1.22	0.90	1.65	0.1937	1.24	0.91	1.70	0.1778
IL-6	1.08	0.83	1.41	0.5609	1.09	0.83	1.42	0.5455	1.07	0.80	1.42	0.6579
IL-10	1.19	0.89	1.58	0.2368	1.19	0.89	1.59	0.2372	1.26	0.94	1.68	0.1289
Leptin	0.84	0.65	1.08	0.1660	0.83	0.65	1.07	0.1484	0.74	0.55	1.01	0.0570
TNF-alpha	1.47	1.09	1.99	0.0118	1.50	1.11	2.04	0.0081	1.53	1.11	2.10	0.0089
TNF-beta	1.33	0.99	1.78	0.0550	1.33	0.99	1.78	0.0549	1.39	1.03	1.88	0.0338
Other Significant Biomarkers												
Alpha-1 Antitrypsin	1.42	1.06	1.90	0.0184	1.42	1.06	1.89	0.0185	1.40	1.03	1.90	0.0335
Beta-2 Microglobulin	1.58	1.24	2.01	**0.0002**	1.69	1.32	2.17	**<0.0001**	1.68	1.29	2.18	**0.0001**
ENA-78	1.42	1.07	1.87	0.0139	1.42	1.07	1.87	0.0147	1.40	1.05	1.87	0.0216
Erythropoietin	1.85	1.37	2.49	**0.0001**	1.86	1.38	2.51	**<0.0001**	1.89	1.37	2.60	**0.0001**
IL-3	1.66	1.20	2.30	0.0021	1.66	1.19	2.30	0.0025	1.69	1.21	2.36	0.0020
IL-7	1.34	0.97	1.84	0.0722	1.33	0.97	1.82	0.0799	1.47	1.05	2.05	0.0255
IL-8	1.29	0.99	1.69	0.0577	1.32	1.01	1.73	0.0396	1.26	0.96	1.67	0.0972
PAPP-A	1.27	1.01	1.60	0.0398	1.28	1.02	1.61	0.0345	1.29	1.02	1.63	0.0330
Stem Cell Factor	1.48	1.12	1.95	0.0062	1.49	1.12	1.97	0.0056	1.47	1.10	1.96	0.0090
SHBG	1.30	0.98	1.72	0.0703	1.30	0.98	1.72	0.0692	1.35	1.00	1.83	0.0499
TNFR2	1.58	1.23	2.02	**0.0003**	1.66	1.29	2.13	**0.0001**	1.68	1.29	2.20	**0.0001**

1. Coefficients represent a 1 standard deviation change in the biomarker
2. Model 1 adjusted for age
3. Model 2 models adjusted for age, race/ethnicity, total body fat mass, dichotomized smoking status and alcohol consumption
4. Significant biomarkers highlighted in **bold** font

Table 19 Biomarkers Associated with Persistent or Transient Anemia Status

Biomarker	**Persistent Anemia (Y0 & 3)**				**Transient Anemia (Year 0 Only)**			
	OR	**LCI**	**UCI**	**p**	**OR**	**LCI**	**UCI**	**p**
Biomarkers of Interest								
C Reactive Protein	2.39	1.46	3.90	**0.0005**	0.95	0.60	1.52	0.8438
Fibrinogen	1.05	0.73	1.53	0.7806	0.85	0.55	1.31	0.4686
IFN-gamma	0.90	0.59	1.38	0.6350	1.21	0.80	1.81	0.3664
IL-1alpha	1.68	1.05	2.71	0.0319	1.21	0.78	1.88	0.3918
IL-1ra	1.32	0.83	2.07	0.2389	1.17	0.75	1.82	0.4972
IL-6	1.21	0.80	1.82	0.3655	0.91	0.58	1.42	0.6619
IL-10	0.99	0.66	1.51	0.9754	1.43	0.95	2.16	0.0871
Leptin	0.69	0.40	1.19	0.1837	1.44	0.71	2.92	0.3105
TNF-alpha	1.39	0.90	2.15	0.1400	1.56	0.97	2.51	0.0690
TNF-beta	1.09	0.72	1.65	0.6945	1.99	1.21	3.29	0.0072
Other Significant Biomarkers								
Alpha-1 Antitrypsin	1.65	1.07	2.52	0.0221	1.24	0.79	1.93	0.3498
Adiponectin	1.38	0.89	2.15	0.1495	0.79	0.51	1.22	0.2884
Apolipoprotein A1	1.50	1.00	2.25	0.0481	1.05	0.69	1.59	0.8252
Beta-2 Microglobulin	1.73	1.19	2.50	0.0041	1.32	0.89	1.94	0.1640
BDNF	0.75	0.57	1.00	0.0486	1.01	0.68	1.51	0.9467
Eotaxin	0.58	0.40	0.84	0.0037	1.05	0.69	1.60	0.8192
Erythropoietin	1.85	1.21	2.82	0.0046	1.71	1.09	2.69	0.0193
Factor VII	1.47	0.91	2.38	0.1159	1.04	0.68	1.58	0.8690
Ferritin	0.96	0.64	1.45	0.8517	0.67	0.44	1.00	0.0489
GM-CSF	1.46	1.02	2.11	0.0408	1.03	0.68	1.57	0.8749
Haptoglobin	1.85	1.04	3.30	0.0371	0.98	0.64	1.49	0.9233
IGF-1	0.78	0.53	1.15	0.2102	1.06	0.69	1.61	0.7914
IL-12p40	0.88	0.60	1.31	0.5316	2.00	1.12	3.58	0.0189
IL-3	2.01	1.23	3.28	0.0054	1.57	0.98	2.54	0.0629
IL-7	1.69	1.02	2.79	0.0404	1.27	0.79	2.05	0.3221
MDC	0.69	0.49	0.96	0.0264	1.02	0.67	1.54	0.9340
RANTES	1.72	1.07	2.77	0.0241	1.03	0.69	1.54	0.8787
SHBG	1.89	1.21	2.96	0.0052	1.00	0.64	1.55	0.9864
TNFR2	1.56	1.04	2.36	0.0336	1.58	1.08	2.30	0.0178
Thrombopoietin	0.70	0.53	0.93	0.0155	1.11	0.71	1.74	0.6395
Apolipoprotein (a)	0.84	0.57	1.25	0.4016	1.01	0.66	1.54	0.9537

1. Coefficients represent a 1 standard deviation change in the biomarker
2. [†]Fully adjusted models adjust for age, race/ethnicity, total body fat mass, dichotomized smoking status and alcohol consumption
3. Significant biomarkers highlighted in **bold** font

Table 20 Biomarkers Significantly Associated with Hemoglobin Level

Biomarker	**Crude**			**Model 1**[2]			**Model 2**[3]			**Model 3**[4]		
	Coeff[1]	**SE**	**p**	**Coeff**[1]	**SE**	**p**	**Coeff**[1]	**SE**	**p**	**Coeff**[1]	**SE**	**p**
Biomarkers of Interest												
C-reactive Protein	0.015	0.033	0.6613	0.014	0.033	0.6834	-0.009	0.037	0.8055	-0.037	0.039	0.3511
Fibrinogen	0.045	0.033	0.1746	0.043	0.033	0.1960	0.043	0.033	0.1967	0.031	0.035	0.3752
IFN-gamma	-0.064	0.033	0.0517	-0.057	0.033	0.0857	-0.050	0.034	0.1384	-0.068	0.036	0.0583
IL-1alpha	-0.085	0.033	0.0094	-0.080	0.033	0.0152	-0.080	0.034	0.0189	-0.079	0.036	0.0295
IL-1ra	-0.009	0.033	0.7728	-0.005	0.033	0.8835	-0.027	0.034	0.4379	-0.036	0.037	0.3339
IL-6	0.015	0.033	0.6588	0.012	0.033	0.7105	0.003	0.035	0.9209	-0.012	0.037	0.7374
IL-10	-0.060	0.034	0.0774	-0.060	0.034	0.0783	-0.067	0.034	0.0512	-0.092	0.037	0.0139
Leptin	0.109	0.033	0.0008	0.115	0.033	**0.0004**	0.149	0.047	0.0015	0.152	0.050	0.0023
TNF-alpha	0.012	0.033	0.7097	0.001	0.033	0.9745	-0.011	0.034	0.7529	-0.017	0.036	0.6342
TNF-beta	0.004	0.033	0.9112	0.004	0.033	0.9013	-0.002	0.033	0.9508	0.013	0.035	0.7015
Other Significant Biomarkers												
Alpha-1 antitrypsin	-0.061	0.033	0.0663	-0.061	0.033	0.0666	-0.075	0.034	0.0273	-0.068	0.036	0.0560
Adiponectin	-0.102	0.033	0.0018	-0.119	0.033	**0.0004**	-0.118	0.035	0.0009	-0.077	0.038	0.0436
Alpha-Fetoprotein	0.093	0.033	0.0044	0.083	0.033	0.0125	0.069	0.034	0.0405	0.072	0.035	0.0443
Apolipoprotein A1	-0.110	0.033	0.0008	-0.114	0.033	**0.0005**	-0.105	0.034	0.0021	-0.089	0.036	0.0133
Apolipoprotein CIII	0.072	0.033	0.0283	0.067	0.033	0.0407	0.071	0.034	0.0341	0.073	0.036	0.0441
BDNF	0.115	0.033	**0.0005**	0.118	0.033	**0.0003**	0.119	0.033	**0.0003**	0.124	0.034	**0.0003**
Complement C3	0.116	0.033	**0.0004**	0.118	0.033	**0.0003**	0.117	0.036	0.0014	0.118	0.038	0.0021
CEA	0.073	0.033	0.0261	0.062	0.033	0.0618	0.026	0.035	0.4657	0.010	0.037	0.7905
CK-MB	0.093	0.033	0.0044	0.080	0.033	0.0165	0.082	0.034	0.0178	0.077	0.036	0.0345
Eotaxin	0.073	0.033	0.0264	0.065	0.033	0.0484	0.059	0.034	0.0788	0.077	0.036	0.0333
Erythropoietin	-0.133	0.033	**0.0001**	-0.136	0.033	**0.0000**	-0.137	0.034	**0.0001**	-0.155	0.035	**<0.0001**
Ferritin	0.136	0.033	**<0.0001**	0.129	0.033	**0.0001**	0.132	0.034	**0.0001**	0.122	0.036	0.0006
Growth Hormone	-0.082	0.033	0.0127	-0.087	0.033	0.0083	-0.088	0.036	0.0132	-0.066	0.038	0.0826
ICAM-1	0.119	0.033	**0.0003**	0.114	0.033	**0.0005**	0.097	0.034	0.0048	0.072	0.037	0.0494
IgA	0.057	0.033	0.0831	0.058	0.033	0.0743	0.069	0.034	0.0410	0.073	0.035	0.0396
IGF-1	0.073	0.033	0.0276	0.073	0.033	0.0258	0.074	0.034	0.0283	0.075	0.035	0.0333
IL-12p40	-0.048	0.033	0.1481	-0.050	0.033	0.1309	-0.055	0.033	0.1018	-0.077	0.035	0.0292
IL-15	-0.082	0.033	0.0128	-0.074	0.033	0.0244	-0.079	0.034	0.0195	-0.067	0.036	0.0619

Biomarker	Crude			Model 1[2]			Model 2[3]			Model 3[4]		
	Coeff[1]	SE	p	Coeff[1]	SE	p	Coeff[1]	SE	p	Coeff[1]	SE	p
IL-18	0.072	0.033	0.0277	0.072	0.033	0.0284	0.058	0.034	0.0873	0.038	0.036	0.2823
IL-2	-0.090	0.033	0.0063	-0.084	0.033	0.0108	-0.087	0.034	0.0114	-0.066	0.036	0.0706
IL-3	-0.119	0.033	**0.0003**	-0.113	0.033	0.0006	-0.111	0.033	0.0009	-0.107	0.035	0.0024
IL-5	-0.081	0.033	0.0135	-0.079	0.033	0.0155	-0.073	0.034	0.0309	-0.074	0.036	0.0395
IL-7	-0.107	0.033	0.0011	-0.104	0.033	0.0016	-0.112	0.034	0.0010	-0.111	0.036	0.0021
Insulin	0.133	0.032	**<0.0001**	0.140	0.032	**<0.0001**	0.164	0.039	**<0.0001**	0.137	0.044	0.0018
MDC	0.080	0.033	0.0152	0.079	0.033	0.0163	0.054	0.034	0.1212	0.042	0.036	0.2517
MIP-1beta	0.070	0.033	0.0330	0.063	0.033	0.0569	0.062	0.034	0.0648	0.074	0.035	0.0343
PAI-1	0.101	0.033	0.0021	0.103	0.033	0.0017	0.093	0.034	0.0059	0.098	0.035	0.0057
Prostatic Acid Phosphatase	0.092	0.033	0.0050	0.093	0.033	0.0043	0.081	0.033	0.0152	0.068	0.035	0.0567
Serum Amyloid P	0.128	0.033	**0.0001**	0.126	0.033	**0.0001**	0.128	0.035	**0.0003**	0.108	0.037	0.0038
SHBG	-0.153	0.033	**0.0000**	-0.154	0.033	**<0.0001**	-0.160	0.035	**<0.0001**	-0.141	0.037	**0.0002**
TIMP-1	0.109	0.033	0.0008	0.100	0.033	0.0025	0.088	0.034	0.0104	0.085	0.036	0.0169
VCAM-1	0.079	0.033	0.0160	0.065	0.034	0.0548	0.064	0.035	0.0633	0.054	0.037	0.1449
Apolipoprotein (a)	0.052	0.033	0.1154	0.050	0.033	0.1297	0.066	0.034	0.0511	0.092	0.036	0.0110

1. Coefficients represent a 1 standard deviation change in the biomarker
2. Model 1 adjusted for age
3. Model 2 adjusted models adjust for age, race/ethnicity, total body fat mass, smoking status and alcohol consumption
4. Model 3 adjusted for covariates in Model 2 plus income, hypertension status and diabetes status

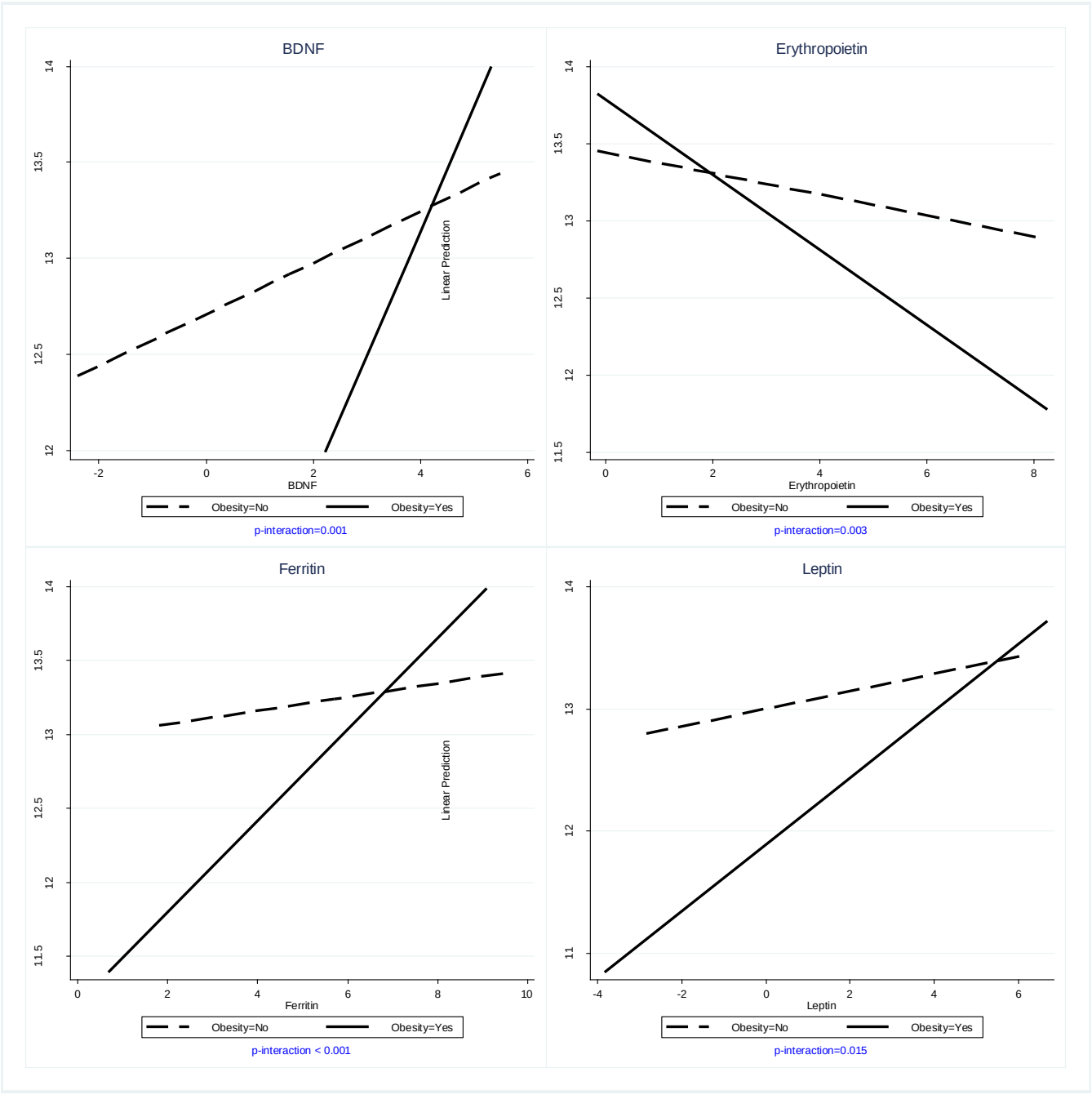
CEA= Carcinoembryonic antigen; CK-MB = Creatine Kinase-MB

Table 21 Modification of the Biomarker versus Hemoglobin Level Association by Obesity Status

Biomarker	**Obesity Status**									
	Total Sample (N=997)			**Not Obese (N=768)**			**Obese (N=229)**			
	Coeff	**SE**	**p**	**Coeff**	**SE**	**p**	**Coeff**	**SE**	**p**	**p-Interaction**
BDNF	0.125	0.034	0.0003	0.074	0.037	0.0479	0.353	0.085	0.0000	0.0014
Erythropoietin	-0.151	0.035	0.0000	-0.089	0.039	0.0226	-0.357	0.080	0.0000	0.0030
Ferritin	0.130	0.036	0.0003	0.058	0.039	0.1400	0.385	0.080	0.0000	0.0001
Leptin	0.133	0.040	0.0009	0.088	0.044	0.0466	0.298	0.094	0.0019	0.0146

1. Coefficients represent a 1 standard deviation change in the biomarker
2. Obesity models adjusted for age, race/ethnicity, smoking status, alcohol consumption, income, diabetes status and hypertension status; obesity combined models also included obesity as a covariate
3. Significant biomarkers highlighted in **bold** font

Figure 9 Modification of the Hemoglobin versus Biomarker Association by Obesity



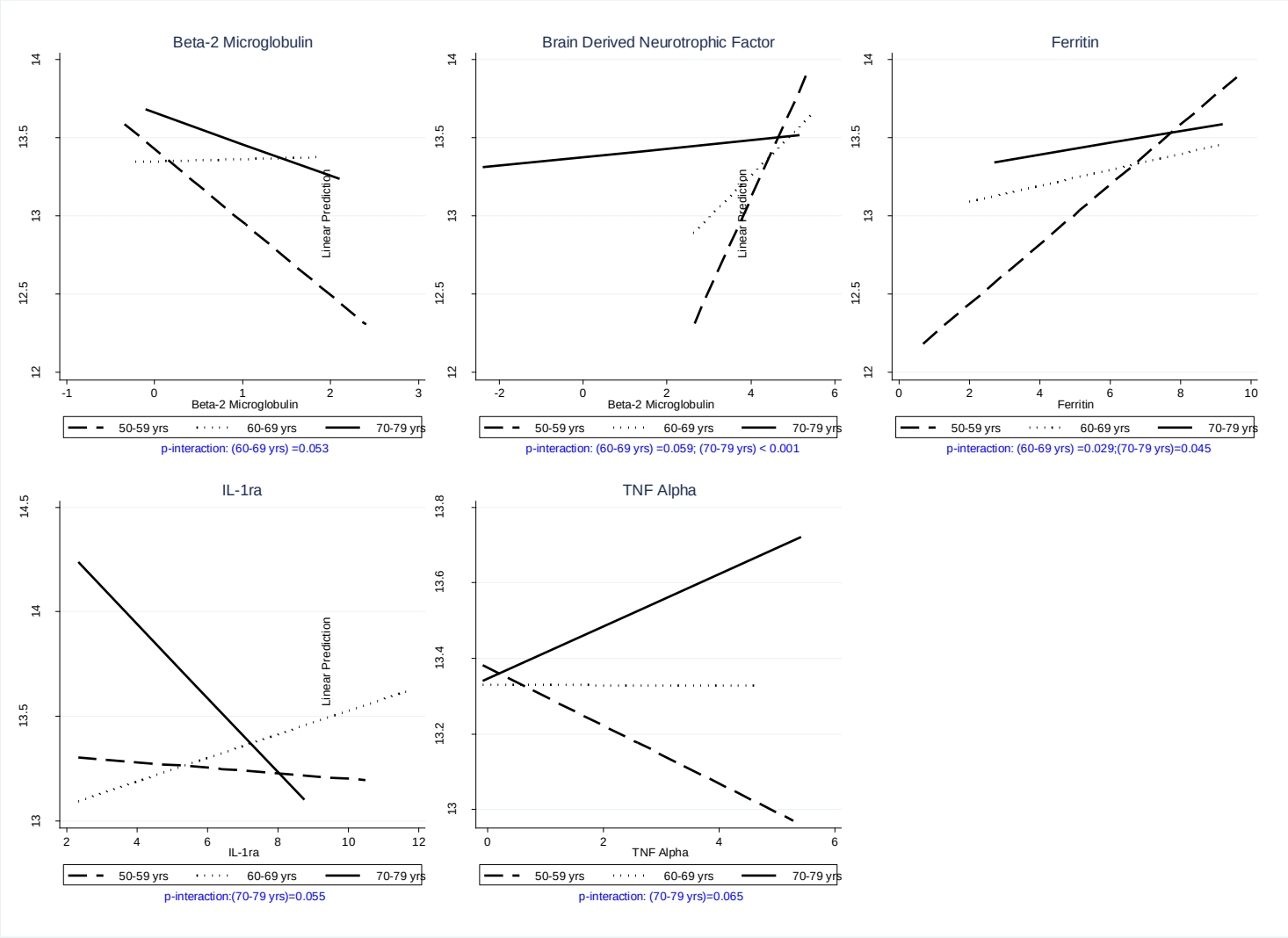
Obesity models adjusted for age, race/ethnicity, smoking status, alcohol consumption, income, diabetes status and hypertension status; obesity combined models also included obesity as a covariate

Table 22 Modification of the Hemoglobin versus Biomarker Association by Age Category

	Age Categories Combined			Age 50-59 Years			Age 60-69 Years				Age 70-79 Years			
Biomarker	**Coeff**	**SE**	**P**	**Coeff**	**SE**	**p**	**Coeff**	**SE**	**p**	**p-Interaction**	**Coeff**	**SE**	**p**	**p-Interaction**
Beta-2 Microglobulin	-0.070	0.038	0.0621	-0.169	0.066	**0.0110**	0.014	0.057	0.8013	**0.0533**	-0.107	0.073	0.1467	0.3449
BDNF	0.130	0.034	**0.0002**	0.287	0.071	**0.0001**	0.140	0.058	**0.0170**	**0.0590**	0.016	0.053	0.7676	**0.0005**
Ferritin	0.121	0.036	**0.0007**	0.235	0.061	**0.0001**	0.063	0.053	0.2290	**0.0290**	0.007	0.077	0.9240	**0.0452**
IL-1ra	-0.033	0.037	0.3808	0.014	0.072	0.8409	0.066	0.055	0.2305	0.3597	-0.223	0.071	**0.0020**	**0.0547**
TNF-alpha	-0.019	0.036	0.6044	-0.103	0.060	0.0878	-0.006	0.055	0.9196	0.2454	0.044	0.079	0.5754	**0.0646**

1. Coefficients represent a 1 standard deviation change in the biomarker
2. Fully models adjusted for age category, race/ethnicity, total body fat mass, smoking status, alcohol consumption, income, hypertension status and diabetes status
3. Significant biomarkers highlighted in **bold** font

Figure 10 Modification of the Hemoglobin versus Biomarker Association by Age Category



Fully models adjusted for age category, race/ethnicity, total body fat mass, smoking status, alcohol consumption, income, hypertension status and diabetes status

Table 23 Sensitivity Analysis: Biomarkers Significantly Associated with Anemia Using Alternative Methods of Biomarker Data Treatment

Biomarker	**OR**	**LCI**	**UCI**	**p**
Biomarkers of Interest				
C Reactive Protein	1.44	1.04	2.00	**0.0271**
Fibrinogen	-	-	-	-
IFN-y	-	-	-	-
IL-1alpha	-	-	-	-
IL-1ra (Categorical)	NA	NA	NA	NA
IL-6	-	-	-	-
IL-10 (Categorical)	1.92	1.00	3.66	**0.0490**
Leptin	0.74	0.55	1.01	0.0542
TNF-alpha	-	-	-	-
TNF-beta	-	-	-	-
Other Significant Biomarkers				
Alpha_1_antitrypsin	1.40	1.03	1.90	0.0335
Beta-2 Microglobulin	1.68	1.29	2.18	**0.0001**
ENA-78	1.40	1.05	1.87	0.0216
IL-13	-	-	-	-
quintile 1 (reference)	-	-	-	-
quintile 2	0.31	0.12	0.81	0.0163
quintile 3	0.40	0.24	0.94	0.0367
quintile 4	0.52	0.32	1.17	0.1121
quintile 5	0.53	0.33	1.24	0.1439
Stem Cell Factor	1.47	1.10	1.96	0.0090
SHBG	1.35	1.00	1.83	0.0499
TNFR2	1.68	1.29	2.20	**0.0001**

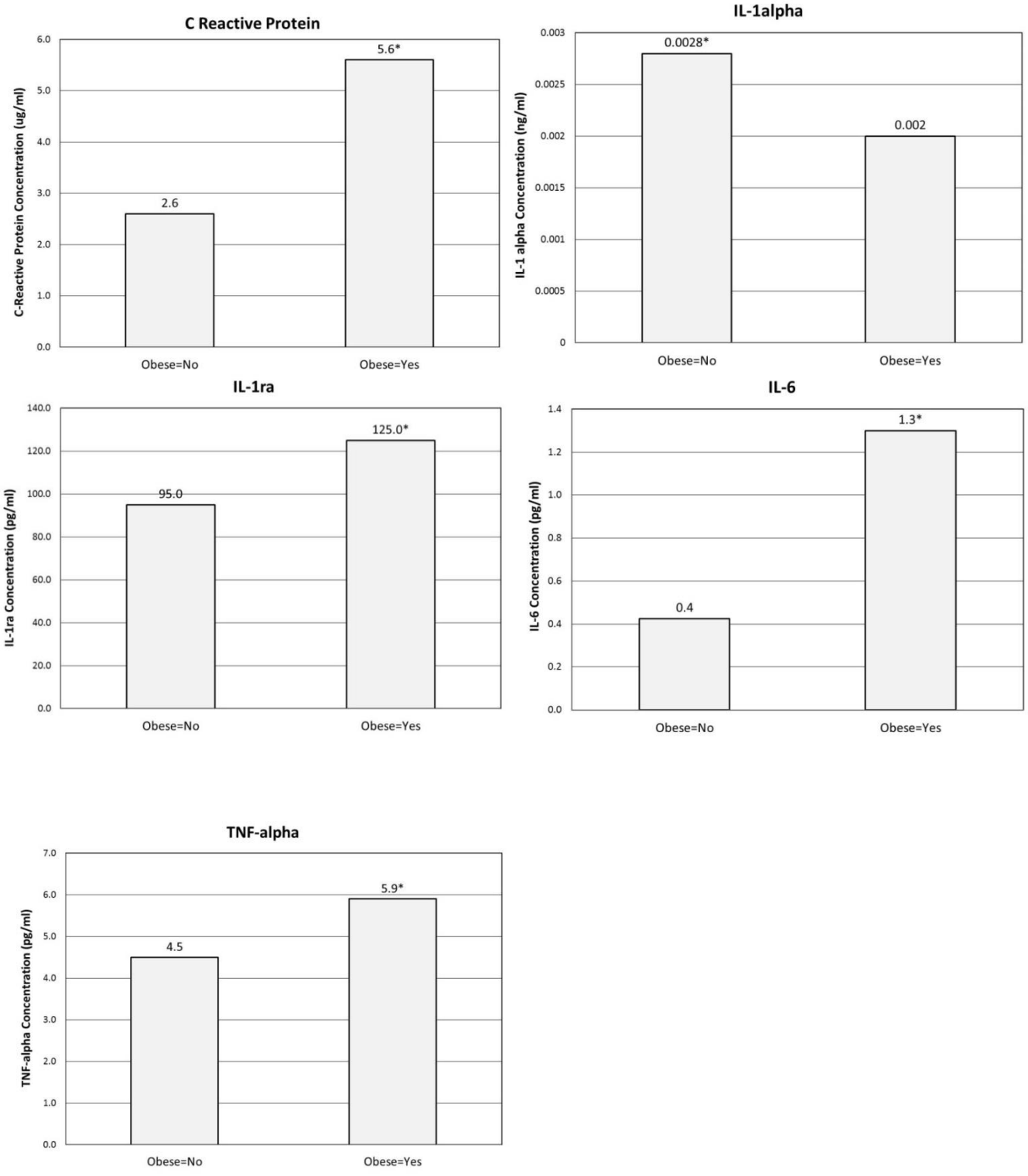
1. Coefficients represent a 1 standard deviation change in the biomarker
2. [†]Fully adjusted models adjust for age, race/ethnicity, total body fat mass, smoking status and alcohol consumption
3. Significant biomarkers highlighted in **bold** font

Table 24 Sensitivity Analysis: Biomarkers Significantly Associated with Hemoglobin Concentration Using Alternative Methods of Biomarker Data Treatment

Biomarker	**Coeff**	**SE**	**p**
Biomarkers of Interest			
C Reactive Protein	-0.037	0.039	0.3511
Fibrinogen	-	-	-
IFN-y	-	-	-
IL-1 alpha	-	-	-
IL-1ra (Categorical)	0.152	0.178	0.3923
IL-6	-	-	-
IL-10 (Categorical)	-0.172	0.093	0.0663
Leptin	0.153	0.050	**0.0022**
TNF-alpha	-	-	-
TNF-beta	-	-	-
Other Significant Biomarkers			
Adiponectin	-0.077	0.038	0.0436
Alpha-Fetoprotein (categorical)	0.166	0.080	0.0369
Apolipoprotein A1	-0.070	0.035	0.0468
Apolipoprotein CIII	0.073	0.036	0.0441
Brain-Derived Neurotrophic Factor	0.124	0.034	**0.0003**
Complement C3	0.118	0.038	0.0021
Ferritin			
quintile 1 (reference)	-	-	-
quintile 2	0.131	0.108	0.2235
quintile3	0.207	0.111	0.0637
quintile 4	0.289	0.111	0.0096
quintile 5	0.242	0.112	0.0318
ICAM-1	0.072	0.037	0.0494
Insulin (Categorical)	0.280	0.102	0.0064
MIP-1beta	0.074	0.035	0.0343
PAI-1	0.098	0.035	0.0057
Serum Amyloid P	0.108	0.037	0.0038
SHBG	-0.141	0.037	**0.0002**
TIMP-1	0.085	0.036	0.0169
Apolipoprotein (a)	0.092	0.036	0.0110

1. Coefficients represent a 1 standard deviation change in the biomarker
2. Fully adjusted models adjust for age, race/ethnicity, body mass index, smoking status and alcohol consumption
3. Biomarkers of interest were tested at the 0.05 level; Other significant biomarkers were tested at the Bonferroni-corrected p value of 0.0008.
4. Significant biomarkers highlighted in **bold** font

Figure 11 Median Inflammatory Marker Levels by Obesity Status



CHAPTER 7: HEMOGLOBIN LEVEL AND CANCER INCIDENCE IN POST-MENOPAUSAL WOMEN

Introduction

Anemia is defined as a hemoglobin level of less than 12 g/dl in women, and increases with age in women after the menopause[2]. The condition is common in cancer patients, resulting from cancer treatment, bone marrow infiltration, blood loss or nutrient deficiency[214]. Anemia may be an indicator of subclinical changes prior to the diagnosis of cancer, and hemoglobin levels may decrease years prior to cancer diagnosis[133]. It is considered an important symptom in the primary care setting for further investigation for colorectal cancer, having a positive predictive value (ppv) ranging from 2 to 41%[215,216], with a ppv of greater than 5% being highly predictive of the disease[216]. A population-based case control study using colorectal cancer cases from a British cancer registry reported odds of having a hemoglobin level of 10-11.9 g/dl of 4.3 when compared with controls[217]. The odds of having a hemoglobin level less than 10 g/dl was 13[217]. There appears to be limited evidence of an association between anemia and breast cancer risk.

Although the association between anemia and cancer risk may be non-causal, the quantification of the magnitude of risk in the general population is still important, as clinical-based studies would most likely overestimate any association observed. In addition, Hamilton et. al. (2005) have argued that the risk of colorectal cancer posed by symptoms remains 'largely unknown'[217]. The present study sought to determine the incidence of cancer in a population of post-menopausal women in a non-clinical setting. It also sought to determine whether the level of risk varies by age, hemoglobin level and race/ethnicity[2]. The risk of cancer for anemic women was determined for total, breast and colorectal cancers. The study determined whether persistent anemia (hemoglobin

<12g/dl at two time points) is more strongly associated with cancer incidence than transient anemia (hemoglobin <12g/dl at initial time point).

While the diagnostic value of hemoglobin level has been established in the clinical setting for certain cancers, there is limited research on whether the association between hemoglobin level and cancer incidence differs between the high and low range of hemoglobin concentration. Few studies have reported on cancer risk at higher hemoglobin levels above the anemia threshold. A case-control study identifying pre-diagnostic features of colorectal cancer reported that cancer cases had twice the odds of having hemoglobin levels of 12-12.9 g/dl when compared with controls[217]. Although the hemoglobin level was within the normal range[11], the increased odds of colorectal cancer suggest that risk may extend to levels outside of the standard definition of anemia. There is scarce literature on the association of breast or colorectal cancer incidence with high hemoglobin levels. The present study determined whether anemia and/or hemoglobin level at baseline is associated with cancer incidence.

Hemoglobin levels are affected by iron intake[129,184] and are positively, but weakly correlated with serum ferritin levels[184,185], an indicator of iron stores[218]. Significant associations have been found between dietary iron and risk of colorectal[15,219], and breast[14,220] cancers. A meta-analysis of risk of colorectal cancer from heme iron intake found an overall relative risk of 1.18[221]. However, the association of dietary iron with cancer risk is conflicting, with the reports of null associations with breast and colorectal cancers[16,17,222,223]. In addition, inverse associations with colorectal cancer have been reported[224,225]. The present study therefore aimed to clarify whether the hemoglobin-cancer incidence association is independent of dietary iron intake or is mediated by dietary iron intake levels.

The study used data from the WHI observational study and clinical trial. It investigated whether anemia or hemoglobin levels are associated with cancer incidence in

postmenopausal women, and explored the association of the full range of hemoglobin (high and low levels) with cancer risk. As there do not appear to be any studies which have investigated this association in a longitudinal cohort of relatively healthy postmenopausal women the findings will add to the body of scientific knowledge. The breadth of WHI data allowed important confounders to be controlled for and effect modification by age and race/ethnicity to be explored.

Methods

Study Population

The study population initially comprised 161,808 women who were enrolled in the Women's Health Initiative Observational Study (OS) or Clinical Trial (CT). Details regarding the study design and enrollment have previously been published[100,102]. Women were recruited from 40 centers in twenty-four states[102] between 1993 and 1998[103]. The CT comprised three different components, including: (1) two hormone therapy versus placebo trials, which investigated reduction in cardiovascular risk (2) a dietary modification trial in which a low fat diet was predicted to reduce breast and colorectal cancer risk and (3) a calcium and Vitamin D trial to investigate fracture risk[100]. Women were entered into the OS if they were interested in the dietary modification or hormone therapy trials, but were unwilling or ineligible to participate in the CT[100]. Main eligibility criteria for both the CT and OS were post-menopausal status, willingness to provide informed consent, and intention to reside in the area of study for a minimum of three years[102]. Exclusion criteria for both study components included medical conditions which adversely affect three-year survival, as well as other conditions which were likely to affect adherence or safety[102]. The hormone therapy trials of estrogen alone or estrogen plus progestin were stopped in 2004 and 2002 respectively, but participants were followed without intervention until the end of the study time period. Informed consent was obtained from participants at each recruitment site[129].

Exclusion criteria for the present study comprised women with cancer history (N=14,849), participants with extreme dietary energy intake values less than 600 and greater than 5000 kCal/day (N=4,598) and women with missing follow-up time (N=878) leaving 141,806 participants. Of these, the main analysis was restricted to women with baseline hemoglobin measures (N=140,402) Women who had hemoglobin values less than 5 and greater than 20 g/dl (N=133) were excluded from analysis, leaving a final sample of 140,269.

Length of Follow-Up

Recruitment for the WHI occurred between 1993-1998, and the WHI study ended on March 31, 2005. Closeout for data collection was April 8, 2005[107]. Women had a chance to be enrolled in the WHI Extension Study 1, which ended in September 2010, and the WHI Extension Study 2, which is ongoing and will conclude data collection in 2015. Outcomes occurring after the study close-out date for participants not enrolled in the Extension studies were censored at date of last contact[107].

Anemia Assessment

Hemoglobin levels were determined from a complete blood count (CBC) which was assessed from 12 hour fasting samples at baseline and year 3[129]. Anemia was defined using the World Health Organization definition of a hemoglobin level < 12 g/dl[130]. Mild anemia was defined as a hemoglobin level of 11-11.9 g/dl[130]. Moderate anemia was defined as a hemoglobin level of 8-10.9 g/dl and severe anemia was defined as a hemoglobin level < 8 g/dl[130]. High hemoglobin was defined as a hemoglobin level >= 16 g/dl. This categorization was based on clinical thresholds of the normal range of hemoglobin in women[11], as well as due to data distribution in which risk of cancer increased at the higher and lower end of hemoglobin concentration. Persistent anemia or high hemoglobin was defined as having anemia or a hemoglobin level >= 15 g/dl at both baseline and year 3. Transient anemia was defined as individuals having anemia or hemoglobin levels >= 15 g/dl at baseline only.

Outcomes Ascertainment

Outcomes of interest were incidence of any, breast and colorectal cancers. Cancer outcomes were centrally adjudicated and SEER coded up to September 17, 2012[107].

Dietary and Supplemental Intake

Intakes of dietary iron, folate, vitamin B12, vitamin C, energy, fat, alcohol, fiber, calcium and zinc were assessed by a food frequency questionnaire (FFQ) developed by the Fred Hutchinson Cancer Research Center[129]. The main section of the FFQ covered 122 food items or groups[226]. Questions on the FFQ related to frequency of intake and portion size, and assessed intake within the last 3 months[226]. Nutrient intake was computed using software developed by the Fred Hutchinson Cancer Research Center[129]. A validation study comparing intakes computed from the FFQ with 8 days of intake from 24 hour recalls and a food record reported energy adjusted correlation coefficients of 0.6 for iron, 0.2 for vitamin B12, 0.3 for vitamin C, 0.7 for dietary fiber, 0.7 for calcium and 0.4 for zinc[226]. Intake of supplements was assessed by an inventory system developed by the Fred Hutchinson Cancer Center[226]. Participants were asked to bring their supplements to the clinic where trained staff entered data on vitamin and supplement use directly into a computerized system[226].

Other Covariates

Age, parity, age at first and last regular period, menopausal hormone use, smoking status, race/ethnicity, education, income and medical history were assessed at baseline using self-administered and interviewer administered questionnaires[158]. Height and weight were measured by trained staff at clinic visit[103].Weight was measured on participants without shoes using a balance beam scale, while height was measured using a stadiometer[158]. Body mass index (BMI) was defined as the participant's weight in kilograms divided by the squared height in meters.

Statistical Analysis

Hemoglobin values below 5 g/dl and greater than 20 g/dl were set to missing (N=133), since previous literature indicated that individuals with a hemoglobin level less than 5 and greater than 20 g/dl were associated with poor survival[66,227]. Different categories of hemoglobin concentration were explored for use in modeling. These included the WHO definition of a hemoglobin level < 12 g/dl and hemoglobin 1 g/dl increments. The two cutpoints used in main models were < 12 g/dl, denoting anemia status, and high hemoglobin level, defined as a hemoglobin level >= 16 g/dl (using the upper limit of the hemoglobin normal range). Dietary iron, fat and energy intakes were log transformed, while red meat and fiber intakes were square root transformed prior to analysis.

Figure 12 Schematic of the Analyses Conducted for Chapter 7

Main Analysis	1. The association between anemia/hemoglobin level and cancer risk
	2. Effect modification by race/ethnicity and age category
Secondary analyses	1. The association between persistent anemia/hemoglobin level with cancer risk
	2. The association between dietary iron/red meat intake and cancer risk
Sensitivity Analyses	1. Main analysis, excluding first two years of follow-up time
	2. The association between hemoglobin and serum ferritin levels in a sub-sample of 890 women

Baseline characteristics were presented as medians (interquartile ranges) and numbers (%) on untransformed variables. Medians were presented due to the skewed distributions of body mass index and total intake variables. Differences in baseline characteristics by anemia status and high hemoglobin level were tested using Wilcoxon Rank Sum tests and chi-squared analysis. The number of incident cancers and age-adjusted incidence rates were computed for any, breast and colorectal cancers. Women who were lost to follow-up or did not have an event were censored at the date of last contact.

Figure 12 gives a schematic of the analyses carried out for the study. In the main analysis Cox proportional hazards models were used to determine the association between anemia or high hemoglobin level and cancer incidence. Models used age as the time scale, and used enrollment age as the entry time. Analysis was restricted to the first 15 years of follow-up. Confounders were identified from variables which were associated with the exposure and outcome, as well as *a priori* associations identified from literature. Anemia models adjusted for bmi (kg/m^2), age at last regular period (years), log total energy intake (kcal), ethnicity (white non-Hispanic, black/African-American, Hispanic/Latino, other ethnicity), income (<$20,000, $20,000-34,999, $35,000-49,999, >=$50,000), education (<college, college or above), smoking status (never smoked, past smoker, current smoker), alcohol intake (nondrinker, past drinker, < 1 drink/month, < 1 drink/week, 1 to < 7 drinks/week, 7+ drinks/week), diabetes status (no, yes), parity (never pregnant, never had term pregnancy, 1, 2, 3, 4, 5+), HRT usage (never used, past user, current user), clinical trial assignment (no, yes) and log dietary iron intake (mg). High hemoglobin models adjusted for bmi, age at last regular period, log total energy intake, ethnicity, income, education, HRT usage, smoking status, alcohol intake, diabetes status, and clinical trial assignment. Dietary iron was replaced with square root transformed red meat intake in models in order to determine the effect of adjustment for heme iron intake.

Effect modification by ethnicity was tested by inclusion of anemia/high hemoglobin-race/ethnicity interaction terms in models. Effect modification by age category was tested in models using time on study (years) as the time scale. Likelihood ratio testing was used to determine whether the addition of the interaction term significantly improved the model.

As a secondary analysis the association of persistent or transient anemia with cancer incidence was determined in women enrolled in the observational study (N=93,676). The full observational study data minus women with extreme energy values, cancer history or

missing hemoglobin values was used for the analysis with transient anemia (N=76,428). However, models including persistent anemia excluded the first three years of follow-up time and only included non-missing values for hemoglobin at both year 0 and 3 (N=60,790).

The associations of dietary iron and red meat intakes with cancer incidence were also determined to further clarify the roles of dietary iron and red meat in the hemoglobin versus cancer incidence associations. Linear regression models were used to assess the univariate association between hemoglobin concentration and continuous dietary iron or red meat intakes, while ANCOVA models estimated predicted hemoglobin levels by dietary iron or red meat intake quintiles, adjusted for age and ethnicity. Cox proportional hazards models were used to determine the associations between dietary iron and/or red meat intakes with cancer incidence. Dietary iron and red meat were entered in models as continuous variables or quintiles. Final dietary iron and red meat intake models adjusted for bmi, age at last regular period, ethnicity, income, education, smoking status, alcohol intake, diabetes status, parity, log dietary fat intake, HRT usage, log energy intake and clinical trial assignment. Contrasts were used to test for a linear trend in hazard ratios across dietary iron and red meat intake quintiles.

Two sensitivity analyses were also conducted: (i) incident cancers occurring within the first two years were excluded to determine the effect on hazard ratios and (ii) the association of hemoglobin level with serum ferritin was determined in a subsample of 890 women without cancer history who were participants in the *Biomarkers and Genetic Factors Related to Sarcopenia* ancillary study of the WHI. Women enrolled in this study were taken from a random sample of White non-Hispanic and Hispanic participants on whom Dual-energy X-ray absorptiometry (DXA) measurements were conducted. Serum ferritin levels were log transformed prior to analysis and associations were determined using linear regression and Pearson's correlation coefficients.

Schoenfeld residuals were used to test the proportional hazards assumption of Cox proportional hazards models, while Cox Snell residuals were used to assess model fit. Analyses were conducted using STATA 12.0 (Stata Corporation, College Station, TX).

Results

Descriptive Statistics

Anemic women had lower median bmi, age at last regular period, physical activity levels and dietary energy, fiber, iron, folate, vitamin B12, vitamin C, calcium and zinc intakes (Table 25). Anemia was more prevalent in black/African-American women, in women with low income or less than college education, in current users of menopausal hormones (HRT), never smokers, non- or past drinkers and in participants who had stomach ulcers, cardiovascular disease, hypertension, diabetes, or rheumatoid arthritis at baseline Table 25). Anemia prevalence was also higher in individuals who were enrolled in the observational study and in women who never had a term pregnancy (Table 25).

Women with high hemoglobin levels greater than or equal to 16 g/dl were older, had a higher BMI, lower age at last regular period, physical activity level, and dietary folate and vitamin C intakes, and higher dietary energy, vitamin B12 and zinc intakes (Table 25). High hemoglobin proportions were lowest in black/African American women, but higher in women of low income and who had less than college education (Table 25). High hemoglobin levels were highest in never users of HRT, in current smokers, and in women who had 7+ drinks/week. Proportions were higher in women with stomach ulcers, cardiovascular disease, hypertension and diabetes, when compared with women who did not have these conditions, as well as in women assigned to the clinical trial (Table 25).

Anemia Prevalence

Approximately 1 in 20 women (5.2%) were anemic (Table 26). Mild anemia accounted for 6,343 (88%) of 7,245 anemia cases. Less than one percent of women in the study

population had moderate (0.6%) or severe (0.01%) anemia. Six percent of women had hemoglobin levels greater than or equal to 15 g/dl, while less than one percent (0.7%) had hemoglobin levels greater than or equal to 16 g/dl.

The Association of Anemia and High Hemoglobin with Cancer Incidence

Breast cancer was the leading type of cancer occurring over the follow-up period in the WHI population, accounting for 10,348 of 25,341 (40.8%) cancers (Figure 13). This was followed by lung and colorectal cancers which accounted for 9.8% and 9.6% of cancers respectively (Figure 12). Breast and colorectal cancers accounted for 40.9% and 9.4% of total cancers in the study sample. Approximately twenty-one thousand (21,376) cancers occurred during follow-up, accounting for 1,520,810 person years of risk. There were 8,739 breast and 2,012 colorectal cancers.

The age-adjusted breast cancer hazard ratio in anemic versus non-anemic women (HR: 0.87; 95% CI: 0.78, 0.97) was the only significant hazard ratio in the exploration of the age-adjusted association between anemia status and cancer incidence (Table 27). However, the hazards of any and breast cancer incidence in women with hemoglobin levels above 15 or 16 g/dl were significantly higher than their respective reference groups (Table 27). High hemoglobin levels greater than or equal to 16 g/dl were associated with an increased hazard of any (HR: 1.57; 95% CI: 1.36, 1.81) and breast cancer (HR: 1.34; 95% CI: 1.04, 1.69) cancer incidence.

The hazard of cancer incidence was evaluated in models including the total sample, as well as in a population excluding individuals who had events or were lost to follow-up in the first two years of the study. Although hazard ratios were slightly elevated in models excluding follow-up time, results for the total population are reported, since the primary findings did not change (results not shown).

Anemia was not significantly associated with any (HR: 1.03; 95% CI: 0.96, 1.11), breast (HR: 0.96; 95% CI: 0.85, 1.07) or colorectal (HR: 1.08; 95% CI: 0.87, 1.35.) cancer incidence in fully adjusted models (Table 28). Women with moderate anemia had a 22% higher risk of any cancer incidence (Table 28, HR: 1.22; 95% CI: 1.01, 1.47), while those with a hemoglobin level less than 10 g/dl had a 54% greater risk of any cancer (results not shown, HR: 1.54; 95% CI: 1.01, 2.33) and an approximately 4 times greater risk of colorectal cancer than women with levels above the threshold (results not shown, HR: 3.77; 95% CI: 1.69, 8.43).

A hemoglobin level greater than 16 g/dl was associated with an increased hazard of any (HR: 1.37; 95% CI: 1.17, 1.60.) or breast (HR: 1.42; 95% CI: 1.10, 1.84.) cancer incidence (Table 28). This risk remained after adjustment for dietary iron or red meat intakes (Table 28) or for total iron intake, which also accounted for supplemental iron. The hazards of any and breast cancer incidence in models adjusting for total iron intake were 1.37 (95% CI: 1.17, 1.60) and 1.42 (95% CI: 1.10, 1.84) respectively (results not shown). Figure 14 shows the hazard of any, colorectal and breast cancer incidence by anemia and high hemoglobin status.

High hemoglobin levels were associated with a 37% increased risk of any cancer incidence and a 42% increased risk of breast cancer incidence. Examination of hemoglobin 1 g/dl increments also indicated that risk of any cancer was increased in individuals with hemoglobin levels of 15-15.9 g/dl (HR: 1.08; 95% CI: 1.01, 1.16) and greater than or equal to 16 g/dl (HR: 1.39; 95% CI: 1.18, 1.63) when compared with the reference category (Table 29). Women with hemoglobin levels greater than or equal to 16 g/dl had a 44% increased hazard of breast cancer incidence when compared with the reference (Table 29). The addition of dietary iron to any or breast cancer incidence models did not change the association with high hemoglobin level (Table 29).

Modification of the Hemoglobin-Cancer Association by Race/Ethnicity and Age Category

The association between anemia and any cancer incidence was modified by race/ethnicity (Table 30, p-interaction=0.028). Anemic black/African-American women had an 18% reduced risk of any cancer incidence when compared with their non-anemic counterparts (HR: 0.82; 95% CI: 0.68, 0.98). However, anemic non-Hispanic white women did not have a significant risk of any cancer (Table 30). Although the p-value associated with the z statistic indicated a significant high hemoglobin versus age interaction for colorectal cancer incidence (Table 30, p-interaction=0.073), this was not indicated in the p-value obtained from likelihood ratio testing (p-interaction=0.278). In addition, high hemoglobin was not significantly associated with colorectal cancer risk in age subcategories (Table 30).

Exploration of descriptive characteristics by race/ethnic group (Table 31) indicated that Hispanic/Latino women had the youngest mean age ($p < 0.001$), but black/African-American women were also younger than white non-Hispanic women, and women in other race/ethnic groups ($p < 0.001$). African American women had higher median body mass indices, and had a higher proportion of current smokers, hypertensive, diabetic and arthritic individuals, and participants with cardiovascular disease than women in other race/ethnic groups (Table 31). Black/African-American women had significantly lower mean hemoglobin concentrations (Table 31, $p < 0.001$). In addition, black and Hispanic women, and women of other ethnicities had lower intakes of anemia-associated nutrients such as dietary iron, dietary folate and vitamin C (Table 31) than white women.

The Association with Persistent and Transient Anemia

The associations between persistent and transient anemia and/or high hemoglobin level with cancer risk were also determined (Table 32). Although persistent and transient high hemoglobin levels greater than or equal to 15 g/dl were associated with a significantly increased risk of any cancer, this risk was attenuated in fully adjusted models (Table 32).

The Association with Dietary Iron and Red Meat Intakes

Approximately one in five women (26,892, or 19%) had intakes below the 8 mg recommended dietary allowance (RDA) for iron for women older than 50 years (results not shown). Sixty women (0.04%) reported intakes of dietary iron above the tolerable upper intake of 45 mg. Only women falling within the upper 5^{th} percentile reported red meat intakes of greater than 1.8 medium servings per day (results not shown).

Hemoglobin was weakly correlated with log dietary iron intake (Rho=0.02, $p < 0.0001$). In addition, mean hemoglobin levels increased with increasing dietary iron quintile (Figure 14). Although the change in hemoglobin level across dietary iron quintiles was significant ($p < 0.0001$), the difference in predicted mean hemoglobin level between the highest and lowest quintile was only 0.03 g/dl (Figure 14). Hemoglobin was also weakly associated with red meat intake (Rho=0.07, $p<0.0001$), but the difference in predicted mean hemoglobin levels between the first and fifth quintile was 0.21 g/dl (Figure 15).

There was an inverse association between dietary iron quintile and colorectal cancer risk with women in the second and third quintile having a 16% (HR: 0.84; 95% CI: 0.71, 0.99) and 19% (HR: 0.81; 95% CI: 0.67, 0.98) reduced risk of colorectal cancer when compared with the first quintile (Table 32). Women who reported red meat intakes which fell in the second (HR: 1.10; 95% CI: 1.02, 1.19) and fourth (HR: 1.10; 95% CI: 1.01, 1.20) quintiles had a 10% increased risk of breast cancer when compared with the reference (Table 33). Dietary iron was not associated with an increased hazard of any cancer incidence (Table 33). Similarly, there was no association between continuous or dietary iron quintile and breast cancer risk after adjustment for covariates (Table 33).

Sensitivity Analyses

As a sensitivity analysis to determine the relationship between hemoglobin level with stored iron, the association with serum ferritin in a sub-sample of 890 women with biomarker measures was determined. Hemoglobin was positively but weakly associated

with serum ferritin levels (results not shown, Rho = 0.14, p < 0.0001). When results were stratified according to C-reactive protein level, the association between hemoglobin level and serum ferritin was stronger in women with probable inflammation, indicated by C-reactive protein levels greater than or equal to 10 ug/ml (Rho=0.21, p =0.016). The removal of the first two years of follow-up time has been discussed previously in the results.

Model Testing

The significant high hemoglobin versus any cancer incidence model failed the proportional hazards assumption (p-global test of a non-zero slope of Schoenfeld residuals with time < 0.001). P-values associated with non-zero slopes of Schoenfeld residuals were obtained for bmi (p=0.049), age at last regular period (p <0.001), income >= $50,000 (p=0.010), college education or above (p=0.012), current hormone usage status (p=0.020) and current smoking status (p <0.001). The model was improved on categorization of age at last menstrual period into ten-year categories, and stratifying on income, education, smoking status, categorized age at last regular period and hormone use (p-global test =0.57), which yielded a hazard ratio of 1.33 (95% CI: 1.13, 1.56).

Discussion

The present research found no association between a hemoglobin level less than 12 g/dl and subsequent risk of any, breast or colorectal cancer in a population of postmenopausal women. However, anemic African-American women had a reduced risk of any cancer incidence. Women with moderate anemia had an elevated risk of any cancer incidence, while women with hemoglobin levels less than 10 g/dl had an increased risk of colorectal cancer. Elevated hemoglobin levels at baseline were associated with increased risk of any and breast cancers. The associations with high hemoglobin level remained after controlling for dietary iron and red meat intakes. Dietary iron intakes in the second and third versus the first quintile were associated with reduced risk of colorectal cancer

incidence, while red meat intakes in the second and third quintiles of intake were associated with increased breast cancer incidence when compared with the first quintile.

The prevalence of anemia in the current population was 5.2%. The low prevalence in relation to other populations of older individuals has been reported previously[129]. Close to 90% of anemia cases in the study population were mild, which concurs with findings using NHANES survey data[2], though a less stringent definition of mild anemia was used[2]. Thomson et. al (2011) have reported that anemic women in the WHI had lower intakes of anemia-associated nutrients when compared with their non-anemic counterparts, but the majority of women had adequate intakes of nutrients according to recommended intakes[129]. In the present analysis anemic women had lower median intakes of dietary iron than non-anemic women, but the intake of 11 mg was higher than the recommended dietary allowance (RDA) of 8 mg of iron, for women aged 51 years and older[228].

The study found no association between anemia level and cancer risk. However, when alternative anemia classifications were explored, moderate anemia as well as a hemoglobin level less than 10 g/dl were associated with risk of any and colorectal cancers respectively. Women with moderate anemia also had a marginally significant risk of any cancer. These findings are constrained by the small numbers of women who had low hemoglobin levels; of the 155 individuals with hemoglobin levels less than 10 g/dl, 6 developed colorectal cancer. Low hemoglobin levels may be associated with cancer risk, since hemoglobin levels decline prior to cancer diagnosis in selected cancers[9,133], and individuals with severe to moderate anemia would mostly likely be referred for further investigation[229,230]. The association is therefore unlikely to be causal, but may reflect sub-clinical changes or blood loss leading to lower hemoglobin levels which may precipitate cancer diagnosis. The elevated colorectal cancer incidence suggests that low hemoglobin levels may still be indicative of colorectal cancer risk in a population-based rather than clinical setting, though this would have to be confirmed by other studies with

a larger number of colorectal cancer cases in the low hemoglobin range. The 4 times greater risk of colorectal cancer observed in individuals with hemoglobin less than 10 g/dl in the present study is more conservative than the odds of 13 observed in general practice[217], which would be expected, given the non-clinical setting.

High hemoglobin concentration was associated with an increased risk of any or breast cancer. There appears to be no clear definition of a high hemoglobin threshold. However, if a range of two standard deviations from the reference mean is used, this would be equivalent to a level of 15.5 to 16.5 g/dl in women[66]. A concentration between 12-16 g/dl is considered the normal range for women[11]. In the present study two standard deviations from the mean hemoglobin concentration was equivalent to 15.4 g/dl. Health consequences of high hemoglobin concentration include increased blood viscosity, impaired oxygen transport, impaired cognition and risk of thromboembolic events[66].

The findings of this research are novel, as this appears to be the first study to report an association between high hemoglobin concentration and breast cancer risk. The significantly higher hazard of any cancer may be driven by breast cancer incidence, since breast cancers accounted for forty percent of total cancers occurring during follow-up. It is presently unclear why high hemoglobin level would be associated with increased cancer risk. The possibility that hemoglobin concentration was reflecting the positive association between iron or red meat intake and cancer incidence reported in previous studies[14,220] was tested by the inclusion of these variables as potential confounders in hemoglobin versus cancer incidence models. Although dietary iron and red meat intake were independently associated with colorectal and breast cancers, the associations with high hemoglobin concentration remained after adjustment for these variables. In addition, the correlations between hemoglobin concentration and dietary iron/red meat intakes in the present study were weak. Dietary iron accounts for only a small proportion of circulating iron stores which would be used for red blood cell production[231]. This suggests that other factors may be responsible for the increased cancer risk observed.

The study is limited by the lack of ferritin measures to further explore the association between hemoglobin level and iron stores. Iron overload (defined as a ferritin level > 200 ng/ml) is high in older populations, with the reported prevalence in two large U.S. based studies being approximately 10%[232,233]. Despite the potential association between hemoglobin and ferritin, there appears to be scarce evidence of an association between iron stores and breast cancer risk in U.S.-based populations[234]. A recent study conducted among female atomic bomb survivors reported an increased radiation-adjusted breast cancer risk with increasing ferritin level, but it should be noted that confounders such as alcohol, age at menarche and age at menopause were not controlled for in the analysis[235].

The association between anemia and any cancer risk was modified by race/ethnicity, with anemic African American women having a reduced risk of any cancer incidence when compared with their non-anemic counterparts. Similar to other studies[2], the prevalence of anemia in African Americans was significantly higher than women in other race/ethnic subgroups. African-American women had lower mean hemoglobin levels, were younger and had lower intakes of iron and folate than white non-Hispanic women. The reduced incidence of cancer in a sub-population with a high prevalence of anemia is supportive of a role for high hemoglobin level in cancer risk, as these women had a significantly lower proportion of the exposure. In addition, the observation of increased cancer risk with increasing hemoglobin g/dl increment is suggestive of a dose response effect. Although the use of age as the time scale was intended to control for the effect of age, it is possible that the reduced risk observed may be due to the younger age of African-American participants. In addition, black/African-American women had lower intakes of dietary iron which may also be associated with increased risk of cancer incidence.

An inverse association was observed between dietary iron intake and risk of colorectal cancer, and there was a positive association between red meat intake and breast cancer risk. Cross et al. (2000) reported a marginally significant inverse association between

dietary iron and colorectal cancer risk which was driven by colon, and not rectal cancer[236], while a population-based case control study reported that women in the highest versus lowest dietary iron quintile had significantly reduced odds of colorectal cancer risk[224]. This finding was not observed in male study participants[224]. Ashmore et al. (2003) have posited that the inverse association may be due to a reduction in available free iron in the gut lumen to compensate for increased iron needs due to menstrual losses and subsequent reduced formation of free radicals associated with cancer formation[224]. Although the women in the present study had attained menopausal status, this explanation may still be applicable due to the long latency period for development of colorectal cancer[92]. Another possibility may be that iron intake may be primarily derived from non-heme iron sources which are associated with other factors such as dietary fiber and antioxidants that are protective for colorectal cancer risk[224]. Dietary fiber was not considered in final models, but if models adjusted for fiber the inverse association remained (3^{rd} versus 1^{st} quintile: 0.83; 95% C.I.: 0.69, 0.99). Significant inverse associations were also observed on adjustment for fruit and vegetable intake (2^{nd} versus 1^{st} quintile: 0.84; 95% C.I.: 0.71, 0.99; 3^{rd} versus 1^{st} quintile: 0.81; 95% C.I.: 0.68, 0.98).

Previous evidence regarding the association between meat intake and breast cancer risk is conflicting; a meta-analysis exploring the association of indices of fat (including meat intake) and cancer risk reported a modest, increased risk of breast cancer with meat consumption[237]. However, a pooled analysis of data from eight large cohort studies reported no association between meat intake and breast cancer risk[238]. Findings of a recently conducted study suggest that the association may vary according to estrogen receptor status, with a stronger association occurring in ER+/PR+ tumors. The findings of the present study add to the debate. Red meat intake was used as a proxy for heme iron intake. However, it is possible that the positive associations observed may be due to other factors in red meat such as heterocyclic amines[238].

The study had a number of limitations. Firstly, the study is constrained by the lack of available serum ferritin measures for the total population. Iron stores are generally comprised of ferritin or hemosiderin[239], with serum ferritin being a good indicator of body iron stores, though its accuracy is compromised by inflammation[240]. The sensitivity analysis in a sample of 890 women indicated a modest association between hemoglobin levels and ferritin in women with elevated C-reactive protein levels, but the association was weak (Rho= 0.1) in the majority (80%) of women. However, previous literature does not provide evidence of a strong association between iron stores and breast cancer risk (one of the cancers associated with elevated hemoglobin level in the present population), hence there may be alternative explanations for the positive association between high hemoglobin level and breast cancer risk.

Another limitation is the inability to determine anemia cause, since colorectal cancer risk may be more strongly associated with iron deficiency anemia[241]. Red meat intake was used as a proxy for heme iron intake in the present study. However, red meat intake may be an imperfect measure of heme iron. Dietary intake variables were derived from a FFQ which may have inherent measurement error[129, 242]. A validation study of the FFQ used in the WHI reported correlations ranging from 0.2 to 0.7, with a correlation for dietary iron of 0.6[226]. Follow-up time differed according to women enrolled in the main WHI study versus the extension studies, with women not attaining cancer events being censored at study closeout. This may have led to more conservative estimates of cancer risk.

The strengths of the study were the large sample size which allowed for consideration of many potential confounders. Assessment of nutrient intake was conducted using a validated FFQ. Varying means of categorization of hemoglobin were used to establish a broad picture of the association with cancer incidence across the full range of hemoglobin concentration.

In summary, high hemoglobin concentration was associated with increased risk of any and breast cancer incidence after controlling for dietary iron and red meat intake. A hemoglobin level less than 12 g/dl was not associated with cancer incidence in the total population, but was associated with reduced risk of any cancer in African-American women. These findings should be confirmed by future research.

Tables and Figures

Table 25 Descriptive Characteristics by Anemia and High Hemoglobin Status

Variable Name	Anemia Status					High Hemoglobin Status				
	Anemia=No		Anemia=Yes			Hb < 16 g/dl		Hb >= 16 g/dl		
	Median	**IQR[1]**	**Median**	**IQR[1]**	**p**	**Median**	**IQR[1]**	**Median**	**IQR[1]**	**p**
Age at Screening (years)	63.0	(57,69)	63.0	(57,69)	**0.0018**	63.0	(57,69)	64.0	(58.5,69)	**0.0001**
Body Mass Index (kg/m2)	26.9	(23.8,31.1)	26.6	(23.4,31.1)	**0.0002**	26.9	(23.8,31.1)	28.5	(24.9,32.9)	**<0.0001**
Age at last regular period (years)	49.0	(44,52)	48.0	(42,51)	**<0.0001**	49.0	(43,52)	48.0	(42,52)	**0.0363**
Physical Activity (MET hrs./week)	8.3	(2.3,17.8)	7.5	(1.9,17.3)	**<0.0001**	8.3	(2.3,17.8)	5.5	(0.8,13.9)	**<0.0001**
Dietary Energy Intake (Kcal)	1547.1	(1194.3,1977.7)	1471.7	(1110.5,1908.9)	**<0.0001**	1542.9	(1189.8,1973.7)	1578.5	(1212.9,2081.1)	**0.0149**
Dietary Fiber Intake (g)	15.2	(11.1,20)	14.5	(10.5,19.3)	**<0.0001**	15.1	(11.1,20)	13.9	(10.2,18.9)	**<0.0001**
Dietary Iron (mg)	11.8	(8.8,15.4)	11.2	(8.3,14.9)	**<0.0001**	11.8	(8.8,15.4)	11.7	(8.5,15.4)	0.4868
Dietary Folate Equivalents (mcg)	456.8	(344.1,595.5)	432.8	(324.1,571.1)	**<0.0001**	455.6	(342.9,594.4)	441.7	(323.9,590.7)	**0.0135**
Dietary Vitamin B12 (mcg)	5.3	(3.7,7.5)	5.0	(3.3,7.5)	**<0.0001**	5.3	(3.6,7.5)	5.6	(3.9,8.1)	**0.0005**
Dietary Vitamin C (mg)	91.9	(58.2,129.4)	88.9	(55.8,127)	**0.0001**	91.8	(58.1,129.3)	84.8	(48.3,121.7)	**<0.0001**
Dietary Calcium (mg)	737.7	(509.1,1051.3)	657.7	(445.2,960.9)	**<0.0001**	734.0	(505.4,1046.8)	729.6	(498.6,1097.9)	0.7228
Dietary Zinc (mg)	10.0	(7.3,13.4)	9.1	(6.5,12.4)	**<0.0001**	9.9	(7.3,13.3)	10.4	(7.7,14.2)	**0.0029**
	No.	**%**	**No.**	**%**		**No.**	**%**	**No.**	**%**	**p**
Ethnicity										
White non-Hispanic	111752	95.9	4767	4.1	**<0.0001**	115666	99.3	853	0.7	**<0.0001**
Black/African American	10255	84.3	1911	15.7		12130	99.7	36	0.3	
Hispanic/Latino	5153	95.4	250	4.6		5375	99.5	28	0.5	
Other Ethnicity	5554	95.1	288	4.9		5812	99.5	30	0.5	
Income										
< $20,000	19761	93.6	1347	6.4	**<0.0001**	20886	98.9	222	1.1	**<0.0001**
$20,000-34,999	30171	94.8	1668	5.2		31615	99.3	224	0.7	
$35,000-49,999	25761	95.2	1294	4.8		26871	99.3	184	0.7	
>= $50,000	48588	95.3	2418	4.7		50749	99.5	257	0.5	
Education										

Variable Name	Anemia Status					High Hemoglobin Status				
	Anemia=No		Anemia=Yes			Hb < 16 g/dl		Hb >= 16 g/dl		
Less than College	79290	94.7	4405	5.3	**0.0289**	83026	99.2	669	0.8	**<0.0001**
>= College	52777	95.0	2777	5.0		55283	99.5	271	0.5	
HRT Usage Status										
Never used	57441	95.0	3049	5.0	**<0.0001**	59973	99.1	517	0.9	**<0.0001**
Past user	20673	95.6	951	4.4		21457	99.2	167	0.8	
Current user	54789	94.4	3237	5.6		57764	99.5	262	0.5	
Smoking Status										
Never smoked	67054	94.6	3792	5.4	**<0.0001**	70545	99.6	301	0.4	**<0.0001**
Past smoker	55075	94.8	3048	5.2		57817	99.5	306	0.5	
Current smoker	9233	97.0	284	3.0		9193	96.6	324	3.4	
Alcohol Intake (Original)										
Non drinker	13961	93.4	987	6.6	**<0.0001**	14879	99.5	69	0.5	**<0.0001**
past drinker	23715	93.3	1705	6.7		25215	99.2	205	0.8	
< 1 drink/month	16504	95.0	860	5.0		17228	99.2	136	0.8	
< 1 drink/week	27243	95.0	1438	5.0		28523	99.4	158	0.6	
1 to < 7 drinks/week	34871	95.7	1581	4.3		36239	99.4	213	0.6	
7+ drinks/week	15951	96.3	614	3.7		16407	99.0	158	1.0	
Stomach Ulcer (Baseline)										
No	123043	94.9	6546	5.1	**<0.0001**	128744	99.3	845	0.7	**0.0001**
Yes	8210	93.5	568	6.5		8690	99.0	88	1.0	
Cardiovascular Disease Ever (Baseline)										
No	103482	94.9	5516	5.1	**<0.0001**	108343	99.4	655	0.6	**<0.0001**
Yes	21367	93.9	1390	6.1		22535	99.0	222	1.0	
Hypertension Ever (Baseline)										
No	88210	95.1	4522	4.9	**<0.0001**	92265	99.5	467	0.5	**<0.0001**
Yes	43754	94.3	2646	5.7		45933	99.0	467	1.0	
Diabetes Status (Screening)										
No	125605	95.0	6615	5.0	**<0.0001**	131375	99.4	845	0.6	**<0.0001**
Yes	7342	92.2	623	7.8		7862	98.7	103	1.3	
Arthritis (baseline)										
No	70507	95.4	3386	4.6	**<0.0001**	73395	99.3	498	0.7	0.9768
Yes	61329	94.2	3785	5.8		64676	99.3	438	0.7	
Rheumatoid Arthritis (Baseline)										
No arthritis	70507	95.4	3386	4.6	**<0.0001**	73395	99.3	498	0.7	0.8999

Variable Name	Anemia Status					High Hemoglobin Status				
	Anemia=No		Anemia=Yes			Hb < 16 g/dl		Hb >= 16 g/dl		
Rheumatoid arthritis	5970	91.5	552	8.5		6481	99.4	41	0.6	
Other/Missing	55359	94.5	3233	5.5		58195	99.3	397	0.7	
Clinical Trial Assignment										
No	72147	94.4	4281	5.6	**<0.0001**	75952	99.4	476	0.6	**0.0080**
Yes	60877	95.4	2964	4.6		63369	99.3	472	0.7	
Parity										
Never pregnant	11985	94.6	685	5.4	**<0.0001**	12571	99.2	99	0.8	0.4321
Never had a term pregnancy	3331	93.3	239	6.7		3543	99.2	27	0.8	
1	11305	93.9	733	6.1		11967	99.4	71	0.6	
2	33054	94.8	1804	5.2		34640	99.4	218	0.6	
3	32280	95.4	1556	4.6		33612	99.3	224	0.7	
4	20467	95.3	1013	4.7		21332	99.3	148	0.7	
5+	19763	94.5	1149	5.5		20761	99.3	151	0.7	
Age at First Regular Period										
9 or less	740	93.7	50	6.3	0.3792	785	99.4	5	0.6	0.1516
10	3533	94.7	198	5.3		3705	99.3	26	0.7	
11	11130	94.8	608	5.2		11638	99.1	100	0.9	
12	23165	95.1	1183	4.9		24178	99.3	170	0.7	
13	29050	95.0	1533	5.0		30408	99.4	175	0.6	
14	19365	94.8	1053	5.1		20286	99.4	132	0.6	
15	9023	94.8	490	5.2		9453	99.4	60	0.6	
16	5073	94.5	296	5.5		5338	99.4	31	0.6	
17 or older	10394	95.0	546	5.0		10862	99.3	78	0.7	

[1] IQR = Interquartile Range (25^{th} percentile, 75^{th} percentile)

Figure 13 Leading Cancers Occurring Over the Follow-up Period in the WHI in 161,808 Women

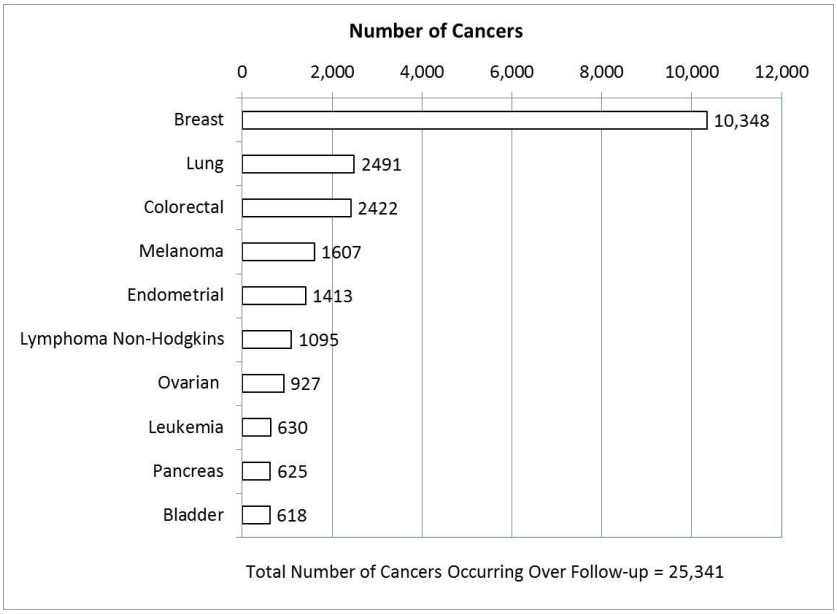


Table 26 Anemia and High Hemoglobin Proportions in the Total Population

	Total Population			
	No		Yes	
Variable	**No.**	**%**	**No.**	**%**
Anemia (Hb < 12 g/dl)	133,024	94.8	7,245	5.2
Mild Anemia (Hb >= 11 & < 12 g/dl)	133,926	95.5	6,343	4.5
Moderate Anemia (Hb >= 8 & < 11 g/dl)	139,384	99.4	885	0.6
Hb >= 15 g/dl	131,892	94.0	8,377	6.0
Hb >= 16 g/dl	139,321	99.3	948	0.7

Proportion of individuals with severe anemia (Hb < 8 g/dl) in the total population= 0.01%
Hb = Hemoglobin

Table 27 Age-Adjusted Hazard of Cancer Incidence

	Anemia Status		High Hemoglobin			
	Anemia=No	**Anemia=Yes**	**Hb < 15 g/dl**	**Hb >= 15 g/dl**	**Hb < 16 g/dl**	**Hb >= 16 g/dl**
Any Cancer						
No. Incident Cancers	20,002	992	19537	1457	20793	201
Person Years at Risk (per 10,000)	144.2	7.5	143.0	8.8	150.9	0.9
Incidence Rate (1000,s)	13.9	13.2	13.7	16.6	13.8	21.7
Incidence Rate Ratio	0.95	(0.89, 1.01)	**1.21**	**(1.15, 1.28)**	**1.57**	**(1.36, 1.81)**
Breast Cancer						
No. Incident Cancers	8203	374	8016	561	8506	71
Person Years at Risk (per 10,000)	148.5	7.8	147.2	9.1	155.3	1.0
Incidence Rate (1000,s)	5.5	4.8	5.4	6.2	5.5	7.3
Incidence Rate Ratio	**0.87**	**(0.78, 0.97)**	**1.13**	**(1.04,1.23)**	**1.34**	**(1.05, 1.69)**
Colorectal Cancer						
No. Incident Cancers	1888	111	1870	129	1983	16
Person Years at Risk (per 10,000)	152.5	7.9	151.0	9.4	159.4	1.0
Incidence Rate (1000,s)	1.2	1.4	1.2	1.4	1.2	1.6
Incidence Rate Ratio	1.13	(0.92, 1.36)	1.11	(0.92, 1.33)	1.29	(0.74, 2.10)

Table 28 Anemia and High Hemoglobin Hazard Ratios for Total Breast and Colorectal Cancer Incidence

	Any Cancer Incidence	Breast Cancer Incidence	Colorectal Cancer Incidence
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**
Anemia			
Age adjusted	0.95 (0.89,1.01)	**0.87 (0.79,0.97)**	1.13 (0.93,1.37)
Age, ethnicity adjusted	0.98 (0.92,1.04)	0.90 (0.81,1.00)	1.07 (0.88,1.30)
Model 1	1.03 (0.96,1.10)	0.96 (0.85,1.07)	1.08 (0.87,1.35)
Model 2	1.03 (0.96,1.11)	0.96 (0.85,1.07)	1.08 (0.87,1.35)
Model 3	1.03 (0.96,1.11)	0.96 (0.85,1.07)	1.08 (0.87,1.35)
Hemoglobin >= 16 g/dl			
Age adjusted	**1.54 (1.34,1.77)**	**1.32 (1.05,1.68)**	1.26 (0.77,2.06)
Age, ethnicity adjusted	**1.53 (1.33,1.76)**	**1.31 (1.04,1.66)**	1.28 (0.78,2.09)
Model 1	**1.37 (1.17,1.60)**	**1.42 (1.10,1.85)**	1.06 (0.60,1.88)
Model 2	**1.37 (1.17,1.60)**	**1.42 (1.10,1.84)**	1.06 (0.60,1.87)
Model 3	**1.37 (1.17,1.60)**	**1.42 (1.10,1.84)**	1.06 (0.60,1.87)

Anemia Models

Model 1: adjusted for bmi, age at last regular period, total energy intake, ethnicity, income, education, smoking status, alcohol intake, diabetes, parity, HRT usage and clinical trial assignment

Model 2: adjusted for variables in Model 1 plus dietary iron intake

Model 3: adjusted for variables in Model 1 plus red meat intake

High Hemoglobin Models

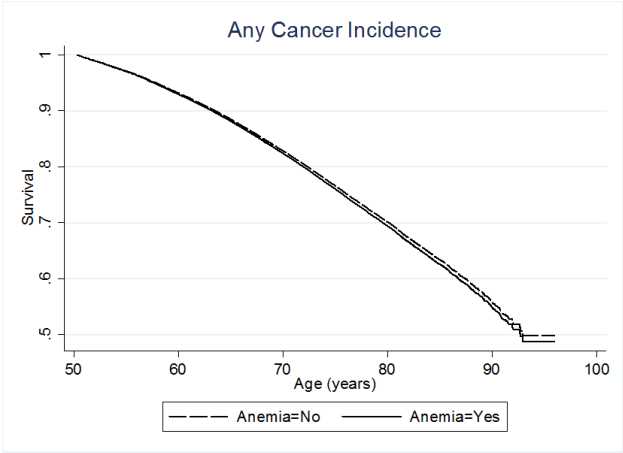
Model 1: adjusted for bmi, age at last regular period, total energy intake, ethnicity, income, education, HRT usage, smoking status, alcohol intake, diabetes and clinical trial assignment

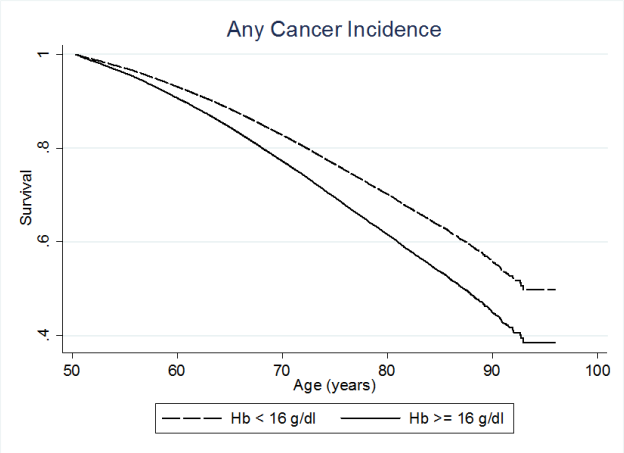
Model 2: adjusted for variables in Model 1 plus dietary iron intake

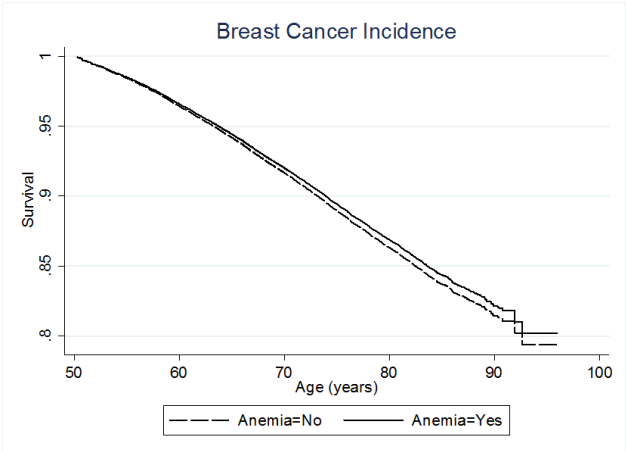
Model 3: adjusted for variables in Model 1 plus red meat intake

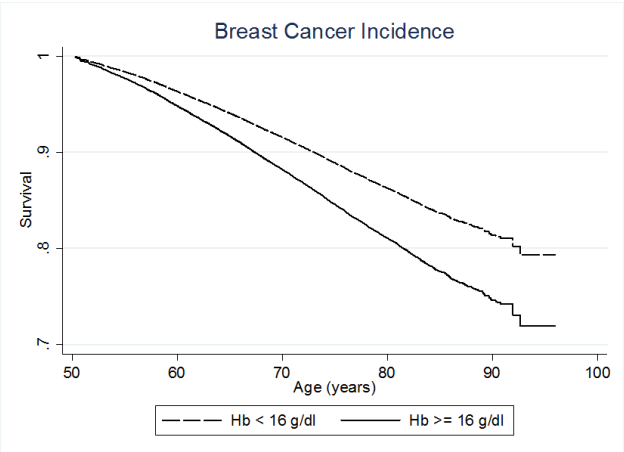
Figure 14 Hazard of Any, Colorectal and Breast Cancer Incidence by Anemia and High Hemoglobin Status

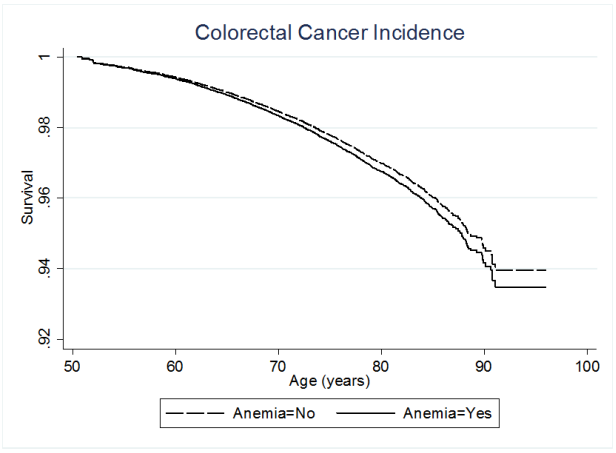
By Anemia Status *By High Hemoglobin Status*

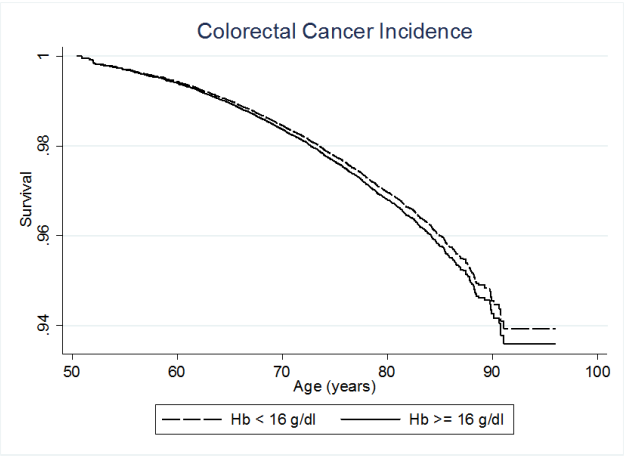












Models adjusted for covariates listed in Model 2 of Table 28

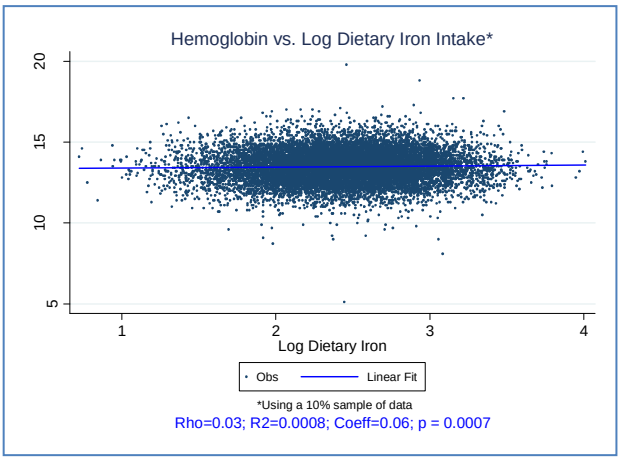
Table 29 Hazard of Cancer Incidence by Hemoglobin g/dl Increments

	Hemoglobin Increments (g/dl)					
	<12	**12.0-12.9**	**13-13.9**	**14-14.9**	**15-15.9**	**>=16**
Any Cancer						
Age-adjusted	0.98 (0.92, 1.05)	1.00	1.01 (0.97, 1.05)	**1.07 (1.03, 1.11)**	**1.16 (1.09, 1.24)**	**1.59 (1.38, 1.83)**
Age, Ethnicity adjusted	1.01 (0.94, 1.08)	1.00	1.00 (0.96, 1.03)	**1.06 (1.02, 1.10)**	**1.15 (1.08, 1.22)**	**1.58 (1.37, 1.82)**
Model 1	1.04 (0.96, 1.12)	1.00	0.99 (0.95, 1.03)	1.04 (0.99, 1.08)	**1.08 (1.01, 1.16)**	**1.39 (1.18, 1.63)**
Model 2	1.04 (0.96, 1.12)	1.00	0.99 (0.95, 1.03)	1.03 (0.99, 1.08)	**1.08 (1.01, 1.16)**	**1.39 (1.18, 1.63)**
Breast Cancer						
Age-adjusted	**0.89 (0.80, 0.99)**	1.00	1.01 (0.95, 1.06)	1.03 (0.97, 1.10)	1.10 (0.99, 1.22)	**1.34 (1.06, 1.70)**
Age, Ethnicity adjusted	0.91 (0.82, 1.02)	1.00	1.00 (0.94, 1.05)	1.02 (0.96, 1.09)	1.09 (0.99, 1.20)	**1.33 (1.05, 1.68)**
Model 1	0.97 (0.85, 1.09)	1.00	0.99 (0.93, 1.05)	1.04 (0.97, 1.11)	**1.13 (1.01, 1.27)**	**1.44 (1.11, 1.88)**
Model 2	0.97 (0.85, 1.09)	1.00	0.99 (0.93, 1.05)	1.04 (0.97, 1.11)	**1.13 (1.01, 1.27)**	**1.44 (1.11, 1.88)**
Colorectal Cancer						
Age-adjusted	1.13 (0.92, 1.39)	1.00	0.97 (0.86, 1.09)	1.03 (0.91, 1.17)	1.05 (0.85, 1.29)	1.26 (0.76, 2.08)
Age, Ethnicity adjusted	1.08 (0.87, 1.33)	1.00	0.99 (0.88, 1.11)	1.05 (0.93, 1.20)	1.07 (0.87, 1.32)	1.28 (0.78, 2.11)
Model 1	1.05 (0.83, 1.33)	1.00	0.96 (0.84, 1.10)	0.97 (0.84, 1.12)	0.96 (0.76, 1.21)	1.03 (0.58, 1.85)
Model 2	1.05 (0.83, 1.33)	1.00	0.96 (0.84, 1.09)	0.97 (0.84, 1.12)	0.96 (0.76, 1.21)	1.03 (0.58, 1.84)

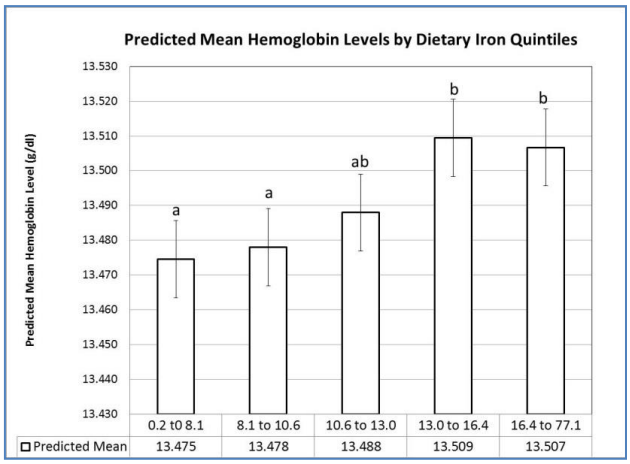
Model 1: adjusted for bmi, age at last regular period, total energy intake, ethnicity, income, education, smoking status, alcohol intake, diabetes, parity, HRT usage and clinical trial assignment

Model 2: adjusted for variables in Model 1 plus dietary iron intake

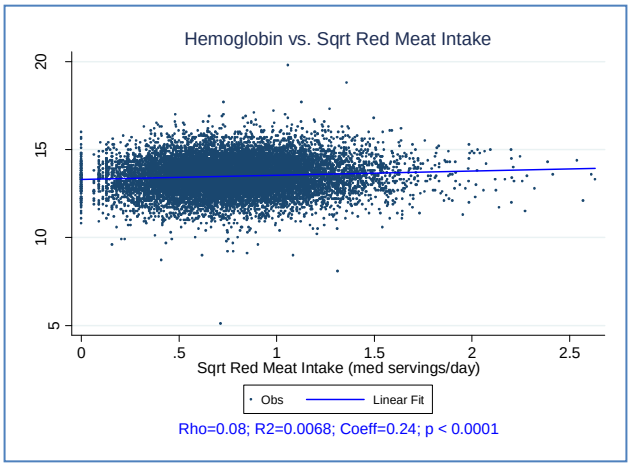
Figure 15 The Association between Hemoglobin Levels and Dietary Iron or Red Meat Intake



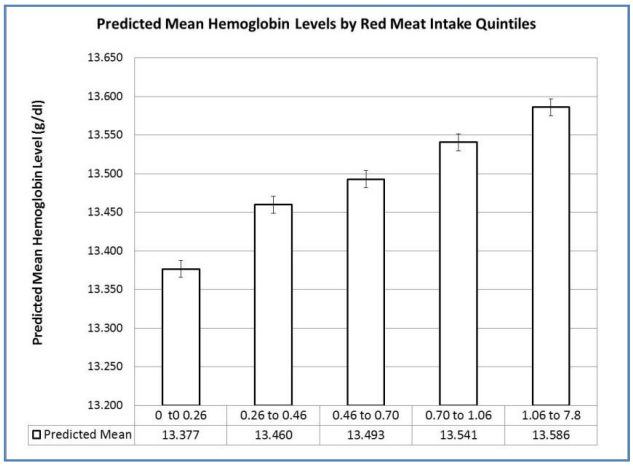
1. Analysis for Chart Determined from a 10% Sample of Women
2. Rho for the Total Sample of Women = 0.02 (p <0.0001)



1. $p > F < 0.0001$
2. Values presented as predicted means ± SEM
3. Models adjusted for age and race/ethnicity
4. Means for quintiles sharing a superscript letter are not significantly different at the 5% level.



1. Analysis for Chart Determined from a 10% Sample of Women
2. Rho for the Total Sample of Women =



1. $p > F < 0.0001$
2. Values presented as predicted means ± SEM
3. Models adjusted for age and race/ethnicity

Table 30 Modification of the Hemoglobin Level versus Cancer Incidence Association by Age Category and Race/Ethnicity

Race/Ethnicity

	White non-Hispanic			African American			p	p-Likelihood
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**	**Interaction**	**Ratio Test**
Anemia								
Any Cancer	1.05	0.97	1.14	**0.82**	**0.68**	**0.98**	**0.0284**	**0.0389**
Breast Cancer	0.96	0.84	1.09	0.87	0.66	1.15	0.6960	0.8911
Colorectal Cancer	1.15	0.88	1.49	0.95	0.61	1.48	0.4683	0.8794
High Hemoglobin >= 15 g/dl								
Any Cancer	**1.10**	**1.04**	**1.18**	0.86	0.56	1.33	0.2772	0.5198
Breast Cancer	**1.16**	**1.05**	**1.28**	0.69	0.30	1.54	0.1902	0.5407
Colorectal Cancer	NE	NE	NE	NE	NE	NE	NE	NE

Age Category[1]

	Age 50-59			Age 60-69			P	P-	Age >= 70			P	P-
	HR	**LCI**	**UCI**	**HR**	**LCI**	**UCI**	**Interaction**	**Likelihood Ratio Test**[2]	**HR**	**LCI**	**UCI**	**Interaction**	**Likelihood Ratio Test**[2]
Anemia													
Any cancer	1.11	0.96	1.27	0.98	0.88	1.09	0.1831	0.1988	1.07	0.94	1.23	0.6416	0.7384
Breast cancer	1.09	0.89	1.33	0.90	0.76	1.07	0.2011	0.4633	0.89	0.68	1.15	0.1980	0.4385
Colorectal cancer	1.06	0.65	1.72	0.92	0.65	1.30	0.4247	0.1576	1.35	0.95	1.92	0.6836	0.2142
High Hemoglobin >= 15 g/dl													
Any cancer	**1.17**	**1.04**	**1.33**	**1.13**	**1.04**	**1.23**	0.8892	0.5000	1.03	0.91	1.16	0.2531	0.1871
Breast cancer	1.09	0.90	1.32	**1.24**	**1.08**	**1.42**	0.2750	0.1370	1.05	0.85	1.29	0.7956	0.3007
Colorectal cancer	1.47	0.96	2.25	0.94	0.70	1.26	0.1435	0.7546	0.85	0.59	1.21	**0.0726**	0.2781

[1]Models assessing modification by age category used time on study (years) as the time scale

[2]For testing the addition of each interaction term to the full model

Anemia models adjusted for bmi, age at last regular period, log energy intake, ethnicity, income, education, smoking status, alcohol intake, diabetes status, parity, HRT usage status, clinical trial assignment and log dietary iron intake

High hemoglobin models adjusted for bmi, age at last regular period, log energy intake, ethnicity, income, education, HRT usage status, smoking status, alcohol intake, diabetes, clinical trial assignment and log dietary iron intake

Table 31 Descriptive Characteristics by Race/Ethnicity

Variable	Race/Ethnicity								
	White non-Hispanic		Black/African-American		Hispanic/Latino		Other Ethnicity		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p[1]**
Age at screening (years)	63.4[a]	7.2	61.4[b]	7.1	60.2[c]	6.7	62.8[d]	7.4	**<0.0001**
Hemoglobin level (g/dl)	13.6[a]	0.9	12.9[b]	1.0	13.4[c]	0.9	13.5[ad]	1.0	**<0.0001**
	Median	**IQR[2]**	**Median**	**IQR[2]**	**Median**	**IQR[2]**	**Median**	**IQR[2]**	**p[3]**
Body Mass Index (kg/m2)	26.6	(23.6,30.6)	30.2	(26.6,34.9)	28.2	(25,32.1)	25.3	(22.5,29)	**<0.0001**
Age at last regular period (years)	49.0	(44,52)	46.0	(40,50)	48.0	(42,51)	49.0	(44,52)	**<0.0001**
Physical Activity (Total met hours/wk)	9.0	(2.5,18.5)	5.0	(0.5,13.6)	5.5	(0.8,15)	8.3	(2.3,18)	**<0.0001**
Dietary energy intake (kcal)	1559.8	(1216.6,1976.1)	1445.2	(1060.2,1992.7)	1475.8	(1077.5,2024.3)	1411.7	(1057.8,1862.8)	**<0.0001**
Dietary fiber intake (g)	15.5	(11.5,20.2)	12.9	(9.1,18)	14.0	(9.9,19.3)	13.8	(10.2,18.6)	**<0.0001**
Dietary iron (mg)	11.9	(9,15.4)	10.8	(7.8,14.9)	10.9	(7.9,15.1)	10.9	(8,14.7)	**<0.0001**
Dietary folate intake (mcg)	462.0	(351,598.8)	400.3	(288.9,547.6)	431.3	(314.2,590.9)	444.5	(332.2,585.5)	**<0.0001**
Dietary vitamin B12 intake (mcg)	5.4	(3.7,7.5)	5.5	(3.5,8.5)	4.7	(3.1,7.1)	4.7	(3,7.1)	**<0.0001**
Dietary vitamin C intake (mg)	93.1	(59.3,130.1)	87.0	(53.6,127.1)	79.6	(48.4,120.4)	84.0	(52.4,123.5)	**<0.0001**
Dietary calcium intake (mg)	766.2	(538.5,1081.1)	532.2	(355.3,796.5)	674.1	(446.4,1003.5)	556.2	(369.8,813.6)	**<0.0001**
Dietary zinc intake (mg)	10.2	(7.6,13.5)	8.1	(5.6,11.7)	9.0	(6.3,12.7)	8.6	(6.1,11.9)	**<0.0001**
Red meat intake (med servings/day)	0.6	(0.3,0.9)	0.6	(0.3,1)	0.6	(0.3,1)	0.5	(0.3,0.9)	**<0.0001**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**p[4]**
Income									
< $20,000	15068	13.8	3284	29.2	1801	37.5	888	16.3	**<0.0001**
$20,000-34,999	26710	24.5	2746	24.4	1152	24.0	1154	21.2	
$35,000-49,999	23085	21.1	2069	18.4	796	16.6	1045	19.2	
>=$50,000	44322	40.6	3158	28.1	1057	22.0	2359	43.3	
Education									
< College	68087	58.8	7693	64.0	4173	78.6	3518	60.7	**<0.0001**
College or above	47695	41.2	4329	36.0	1138	21.4	2277	39.3	
HRT[5] Usage Status									
Never used	48013	41.2	7177	59.1	2735	50.7	2416	41.4	**<0.0001**
Past user	18125	15.6	1828	15.0	749	13.9	861	14.7	
Current user	50286	43.2	3142	25.9	1909	35.4	2562	43.9	
Smoking Status									

Variable	Race/Ethnicity								
	White non-Hispanic		Black/African-American		Hispanic/Latino		Other Ethnicity		
Never smoked	57691	50.1	5895	49.5	3320	62.6	3759	65.0	**<0.0001**
Past smoker	50019	43.4	4677	39.2	1594	30.1	1703	29.4	
Current smoker	7442	6.5	1345	11.3	389	7.3	324	5.6	
Alcohol Intake									
Non-drinker	10201	8.8	1991	16.6	1007	19.0	1707	29.4	**<0.0001**
Past drinker	18958	16.4	3927	32.7	1232	23.2	1235	21.2	
< 1 drink per month	14204	12.3	1593	13.3	707	13.3	823	14.2	
< 1 drink per week	24266	20.9	2244	18.7	1123	21.2	980	16.9	
1 to < 7 drinks/week	32876	28.4	1719	14.3	985	18.6	788	13.6	
7+ drinks/week	15466	13.3	531	4.4	256	4.8	281	4.8	
Stomach or Duodenal Ulcer Ever (Baseline)									
No	108003	93.8	10997	92.6	4896	93.6	5394	93.6	**<0.0001**
Yes	7169	6.2	878	7.4	334	6.4	369	6.4	
Cardiovascular Disease Ever (Baseline)									
No	90185	82.5	9297	81.0	4383	88.2	4861	85.8	**<0.0001**
Yes	19138	17.5	2175	19.0	587	11.8	806	14.2	
Hypertension Ever (Baseline)									
No	79674	68.9	5413	45.2	3753	70.2	3685	63.5	**<0.0001**
Yes	36017	31.1	6558	54.8	1593	29.8	2115	36.5	
Diabetes Ever (Baseline)									
No	111140	95.4	10520	86.6	4920	91.1	5331	91.4	**<0.0001**
Yes	5326	4.6	1633	13.4	479	8.9	505	8.7	
Arthritis Ever (Baseline)									
No	61318	53.1	5819	48.5	3047	57.4	3541	61.1	**<0.0001**
Yes	54250	46.9	6188	51.5	2260	42.6	2254	38.9	
Rheumatoid arthritis (Baseline)									
No arthritis	61318	53.1	5819	48.5	3047	57.4	3541	61.1	**<0.0001**
Rheumatoid arthritis	4897	4.2	982	8.2	315	5.9	308	5.3	
Other/missing	49353	42.7	5206	43.4	1945	36.7	1946	33.6	
Clinical Trial Assignment									
No	64313	55.2	5689	46.8	2798	51.8	3419	58.5	**<0.0001**
Yes	52206	44.8	6477	53.2	2605	48.2	2423	41.5	
Parity									
Never pregnant	10688	9.2	927	7.7	428	8.1	599	10.3	**<0.0001**

Variable	Race/Ethnicity								
	White non-Hispanic		Black/African-American		Hispanic/Latino		Other Ethnicity		
Never had term pregnancy	2565	2.2	694	5.8	141	2.7	160	2.8	
1	9173	7.9	1822	15.1	453	8.5	565	9.7	
2	29388	25.4	2780	23.1	1063	20.0	1541	26.5	
3	29101	25.1	2183	18.1	1097	20.6	1369	23.6	
4	18285	15.8	1458	12.1	872	16.4	811	14.0	
5+	16666	14.4	2177	18.1	1266	23.8	764	13.2	
Age at first regular period (years)									
9 or less	535	0.6	154	1.6	67	1.5	32	0.7	**<0.0001**
10	3005	3.1	371	3.8	168	3.8	177	3.6	
11	9494	9.7	1218	12.4	543	12.3	452	9.1	
12	20238	20.7	2100	21.4	934	21.2	1022	20.6	
13	26033	26.6	2366	24.1	986	22.4	1131	22.8	
14	17378	17.7	1524	15.5	676	15.3	790	15.9	
15	7861	8.0	755	7.7	426	9.7	456	9.2	**<0.0001**
16	4327	4.4	541	5.5	211	4.8	283	5.7	
17 or older	9103	9.3	803	8.2	396	9.0	615	12.4	

a,b,c,d superscript letters that differ indicate differences within outcomes between race/ethnic sub-categories, ANOVA, bonferroni corrected

1 Analysis of Variance (ANOVA)
2 IQR = interquartile range
3 Kruskal Wallis test
4 Chi-squared analysis
5 HRT = hormone replacement therapy

Table 32 Persistent and Year 0 Anemia and High Hemoglobin Hazard Ratios for Total, Breast and Colorectal Cancer Incidence

	Any Cancer Incidence		Breast Cancer Incidence		Colorectal Cancer Incidence	
	Persistent (Yr0 & Yr3)	**Year 0 Only**	**Persistent (Yr0 & Yr3)**	**Year 0 Only**	**Persistent (Yr0 & Yr3)**	**Year 0 Only**
	HR (95%CI)	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
Anemia						
Age adjusted	0.96 (0.83,1.12)	**0.86 (0.76,0.98)**	0.86 (0.67,1.11)	0.85 (0.69,1.04)	0.71 (0.39,1.29)	1.08 (0.73,1.61)
Age, ethnicity adjusted	1.00 (0.86,1.17)	**0.88 (0.77,0.99)**	0.89 (0.69,1.14)	0.86 (0.70,1.06)	0.67 (0.37,1.22)	1.05 (0.70,1.56)
Model 1	1.05 (0.88,1.24)	0.93 (0.80,1.07)	0.93 (0.70,1.24)	0.93 (0.74,1.16)	0.53 (0.25,1.12)	1.10 (0.70,1.71)
Model 2	1.05 (0.88,1.24)	0.93 (0.80,1.07)	0.93 (0.70,1.24)	0.93 (0.74,1.16)	0.53 (0.25,1.12)	1.10 (0.70,1.71)
Hemoglobin >= 15 g/dl						
Age adjusted	**1.23 (1.07,1.42)**	1.11 (0.99,1.23)	**1.30 (1.04,1.62)**	1.09 (0.92,1.29)	1.22 (0.75,1.97)	1.19 (0.84,1.67)
Age, ethnicity adjusted	**1.22 (1.06,1.41)**	1.10 (0.99,1.22)	**1.29 (1.04,1.61)**	1.08 (0.92,1.28)	1.23 (0.76,2.00)	1.21 (0.86,1.70)
Model 1	1.12 (0.96,1.31)	1.07 (0.95,1.20)	1.18 (0.92,1.52)	1.12 (0.93,1.34)	1.15 (0.70,1.91)	1.05 (0.72,1.55)
Model 2	1.12 (0.96,1.31)	1.06 (0.95,1.20)	1.18 (0.92,1.52)	1.12 (0.93,1.34)	1.15 (0.70,1.90)	1.05 (0.71,1.54)

Anemia Models

Model 1: adjusted for bmi, age at last regular period, total energy intake, ethnicity, income, education, smoking status, alcohol intake, diabetes, parity, HRT usage and clinical trial assignment

Model 2: adjusted for variables in Model 1 plus dietary iron intake

High Hemoglobin Models

Model 1: adjusted for bmi, age at last regular period, total energy intake, ethnicity, income, education, HRT usage, smoking status, alcohol intake, diabetes and clinical trial assignment

Model 2: adjusted for variables in Model 1 plus dietary iron intake

Table 33 Dietary Iron and Red Meat Intake Hazard Ratios for Total, Breast and Colorectal Cancer Incidence

Cancer Type	Dietary Iron Quintile (mg)						Continuous Dietary Iron[1]
	0.01-7.8	7.8-10.4	10.4-12.9	12.9-16.3	16.3-184.1	p-trend	
Any Cancer							
Age adjusted	Ref	1.00 (0.96, 1.05)	1.02 (0.98, 1.06)	1.04 (1.00, 1.08)	1.02 (0.98, 1.07)	0.0908	1.03 (1.00,1.06)
Age, ethnicity adjusted	Ref	0.99 (0.95, 1.03)	1.00 (0.96, 1.05)	1.02 (0.98, 1.07)	1.01 (0.97, 1.05)	0.3085	1.02 (0.98,1.05)
Fully Adjusted	Ref	0.98 (0.93, 1.04)	1.01 (0.95, 1.07)	1.02 (0.95, 1.08)	1.00 (0.93, 1.08)	0.6940	1.01 (0.95,1.07)
Breast Cancer							
Age adjusted	Ref	1.06 (0.99, 1.14)	**1.11 (1.04, 1.19)**	**1.11 (1.04, 1.19)**	**1.13 (1.05, 1.21)**	**0.0002**	**1.09 (1.03,1.14)**
Age, ethnicity adjusted	Ref	1.05 (0.98, 1.12)	**1.09 (1.02, 1.17)**	**1.09 (1.02, 1.17)**	**1.11 (1.04, 1.19)**	**0.0014**	**1.07 (1.02,1.13)**
Fully Adjusted	Ref	1.05 (0.97, 1.15)	1.09 (0.99, 1.19)	1.06 (0.96, 1.17)	1.09 (0.97, 1.22)	0.2121	1.02 (0.92,1.12)
Colorectal Cancer							
Age adjusted	Ref	**0.85 (074, 0.98)**	**0.86 (0.75, 0.98)**	0.89 (0.78, 1.02)	**0.84 (0.73, 0.97)**	0.0591	**0.88 (0.80,0.98)**
Age, ethnicity adjusted	Ref	**0.86 (075, 0.99)**	**0.87 (0.76, 0.99)**	0.91 (0.79, 1.04)	**0.85 (0.74, 0.98)**	0.0860	**0.90 (0.81,0.99)**
Fully Adjusted	Ref	**0.84 (071, 0.99)**	**0.81 (0.67, 0.98)**	0.86 (0.70, 1.06)	0.84 (0.67, 1.07)	0.2693	0.83 (0.68,1.02)
	Red Meat Intake Quintile (Medium Servings/Day)						Continuous Red Meat Intake[1]
	0-0.26	0.26-0.46	0.46-0.70	0.70-1.06	1.06-7.8	p-trend	
Any Cancer							
Age adjusted	Ref	**1.06 (1.01, 1.11)**	**1.09 (1.04, 1.13)**	**1.12 (1.07, 1.17)**	**1.09 (1.04, 1.14)**	**<0.0001**	**1.11 (1.06,1.16)**
Age, ethnicity adjusted	Ref	**1.05 (1.01, 1.10)**	**1.08 (1.03, 1.12)**	**1.11 (1.06, 1.16)**	**1.09 (1.04, 1.14)**	**<0.0001**	**1.11 (1.06,1.16)**
Fully Adjusted	Ref	1.02 (0.97, 1.08)	1.01 (0.96, 1.07)	1.03 (0.98, 1.09)	0.96 (0.90, 1.02)	0.3397	0.98 (0.91,1.04)
Breast Cancer							
Age adjusted	Ref	**1.12 (1.05, 1.20)**	**1.14 (1.06, 1.22)**	**1.17 (1.09, 1.25)**	**1.10 (1.02, 1.18)**	**0.0037**	**1.09 (1.02,1.17)**
Age, ethnicity adjusted	Ref	**1.12 (1.04, 1.20)**	**1.13 (1.05, 1.21)**	**1.16 (1.08, 1.24)**	**1.10 (1.02, 1.18)**	**0.0043**	**1.09 (1.02,1.17)**
Fully Adjusted	Ref	**1.10 (1.02, 1.19)**	1.06 (0.97, 1.15)	**1.10 (1.01, 1.20)**	1.00 (0.90, 1.10)	0.9652	0.98 (0.89,1.09)
Colorectal Cancer							
Age adjusted	Ref	1.00 (0.87, 1.15)	1.04 (0.91, 1.20)	1.08 (0.94, 1.25)	1.14 (0.99, 1.31)	**0.0291**	**1.23 (1.07,1.41)**

Age, ethnicity adjusted	Ref	1.01 (0.88, 1.16)	1.05 (0.91, 1.21)	1.09 (0.95, 1.26)	1.15 (1.00, 1.32)	**0.0272**	**1.23 (1.07,1.41)**
Fully Adjusted	Ref	0.91 (0.78,1.07)	0.93 (0.78, 1.10)	0.96 (0.80, 1.14)	0.92 (0.75, 1.12)	0.5951	1.05 (0.85,1.30)

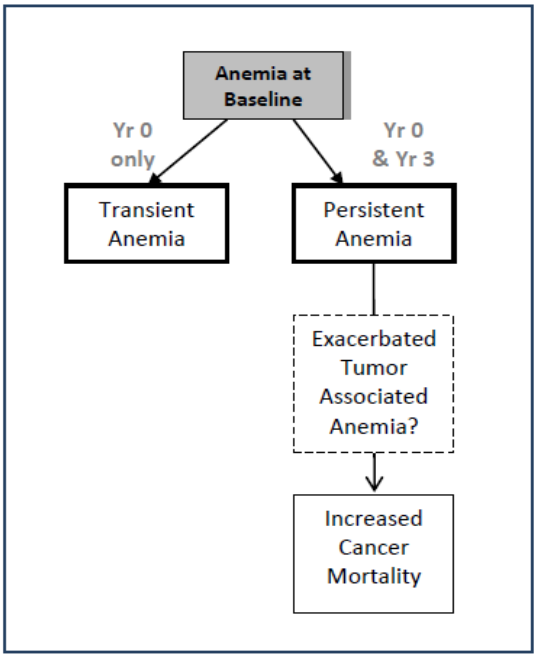
[1] dietary iron was log transformed and red meat intake square root transformed prior to analysis using the variable in the continuous form
Models adjusted for bmi, age at last regular period, ethnicity, income, education, smoking status, alcohol intake, diabetes, parity, dietary fat intake, HRT usage, energy intake and clinical trial assignment

CHAPTER 8: OVERALL CONCLUSIONS

These studies have shown that anemic women are at increased risk of total cancer mortality in women with no history of cancer, and total and colorectal cancer mortality in women with cancer history. Risk of cancer mortality was elevated in women with mild anemia. Anemia or hemoglobin level was associated with the inflammatory markers C-reactive protein, TNF-alpha and IL-1 alpha, and there was a higher proportion of inflammation in anemic versus non-anemic women. Anemia was not significantly associated with increased cancer incidence. However, a high hemoglobin concentration was associated with an increased risk of any or breast cancer incidence, after controlling for important confounders.

The findings of increased total or colorectal cancer mortality risk in women with anemia prior to cancer diagnosis are novel. It is unclear why cancer mortality would be associated with baseline hemoglobin measurements which were made several years prior to cancer diagnosis. Possible mechanisms to explain this association were discussed in Chapter 5. One possibility is that women with anemia at baseline would be starting at a low set point and would have persistently low hemoglobin levels continuing over follow-up, leading to an exacerbated tumor associated anemia once cancer diagnosis was attained (Figure 16). This hypothesis was supported by the finding that

Figure 16 Proposed Mechanism: Baseline Anemia vs. Cancer Mortality in Women with Cancer History



persistent, but not transient anemia was associated with total cancer mortality risk in women with no cancer history. This finding was not observed in women who reported a history of cancer. Participants with both persistent and transient anemia had increased mortality risk in this group, but findings were only significant in women with transient anemia. Women with cancer history had a higher prevalence of the exposure, and may have had other factors from the previous cancer diagnosis which predisposed the total cancer history sample (including those with persistent and transient anemia) to greater mortality risk.

It is possible that other factors such as tumor hypoxia may be contributing to cancer mortality. In addition, elevated inflammatory marker concentrations are also associated with cancer mortality[162,170]. The present research found elevated levels of CRP, IL-1 alpha and TNF-alpha in women with low hemoglobin levels. In addition, CRP, IL-1 alpha and TNFR2 were associated with persistent anemia. TNF-alpha has been suggested as a link between anemia and cancer[162]. Elevated levels are associated with anemia, but also occur in the tumor microenvironment and are associated with worse cancer staging and poor survival[162]. Biomarkers in the present analysis were measured at baseline, hence, it is unclear whether levels remained elevated up to the time of cancer incidence, or were ameliorated over time. In addition, the study only had biomarker measurements for one thousand women.

The prospect that anemia levels were reflecting the presence of other comorbidities which were associated with cancer mortality was considered by testing the association of baseline cardiovascular disease with cancer mortality, and adjusting for baseline diabetes status in final models. However, the study was constrained by the inability to measure renal disease, which may be an important confounder of the anemia-cancer mortality association. Finally, it may be possible that the observed association may be due to the effect of an unmeasured confounder.

Risk of total cancer mortality increased with lower hemoglobin levels, suggesting a dose response effect. In exploring the association between anemia and cancer mortality the causal criteria of temporality, dose response and biological plausibility have been met. However, the criterion of consistency would have to be satisfied by confirmation of findings in other populations.

Baseline CRP and TNF-alpha were associated with increased odds of anemia, reinforcing findings found in other populations. Despite the established role of these biomarkers in anemia development, there is still limited research exploring this association in elderly populations, particularly post-menopausal women. In addition to associations with the main biomarkers of interest, the study also found associations between beta-2 microglobulin, erythropoietin or TNFR2 and anemia, and BDNF, erythropoietin, ferritin, Il-1alpha or SHBG and hemoglobin concentration. The study also found that associations between hemoglobin concentration and BDNF, erythropoietin, ferritin or leptin increased in obese individuals. This research adds to the body of literature on these biomarkers as there have been limited studies on the associations of BDNF, leptin and SHBG with hemoglobin level, as well as the association of beta-2 microglobulin with anemia in a relatively healthy population of older individuals. Apart from leptin, the direction of the associations with these markers was consistent with previous findings. In addition, most markers which were significant predictors of anemia or hemoglobin level may play a biologically plausible role in erythropoiesis.

Anemia was not associated with increased cancer risk. However hemoglobin levels below 10 g/dl were associated with an increased risk of colorectal cancer. This was not likely to be due to a causal association but, rather, to blood loss or hemoglobin decrements which occur prior to cancer diagnosis. Although colorectal cancer risk has been determined in clinical practice the current findings suggest the utility of a low hemoglobin levels to identify colorectal cancer risk in a non-clinical setting. Some colorectal cancer clinical practice guidelines may already indicate that women with unexplained iron deficiency

anemia less than 10 g/dl be referred for further investigation for colorectal cancer[243,230]. However, the study is constrained by small numbers.

The research found a positive association between elevated hemoglobin level and cancer risk. This association remained after controlling for important confounders such as smoking status, alcohol intake, red meat or dietary iron intake which may independently affect cancer risk. There is a paucity of research on high hemoglobin level, hence, there are no standard definitions for high hemoglobin concentration used in studies, and there is limited knowledge of the association between high hemoglobin concentration and cancer incidence. In addition, there are no prevalence estimates for high hemoglobin level. Few women (0.7%) had hemoglobin levels beyond the normal range threshold of 16 g/dl, but six percent had a hemoglobin level greater than 15 g/dl.

Increased cancer mortality risk at lower hemoglobin levels and increased cancer incidence at high hemoglobin concentration raises the question of an optimal hemoglobin concentration. Although there is already a current debate on the normal hemoglobin range for different ethnic groups, this, and past research suggest that there is a tight range of hemoglobin concentration in which there is reduced risk of disease, and beyond which there are negative health outcomes.

Anemia or high hemoglobin levels may be modifiable risk factors, which would suggest that cancer deaths or incident cancers could be prevented if hemoglobin levels were corrected. However, hemoglobin correction may not be a straightforward process, since iron may be associated with increased cancer incidence, and folate may promote the growth of established neoplasms.

Future Directions

The present research found that persistent anemia was associated with total cancer mortality in women with no cancer history, and that baseline biomarkers of inflammation were associated with persistent anemia. Further analysis should be carried out in study populations which have available hemoglobin measures at more time points to further explore the effects of persistent anemia and high hemoglobin concentration. Anemia is a modifiable condition; hemoglobin levels may be improved by blood transfusion, oral or parenteral iron or recombinant erythropoietin. It is therefore possible that baseline hemoglobin levels changed with time. Hence, an analysis of the longitudinal associations between hemoglobin level and cancer risk would clarify mechanisms, especially if hemoglobin levels were available for several time points before and after cancer diagnosis. In addition, studies of the longitudinal associations between (i) biomarkers and hemoglobin level and (ii) biomarkers and cancer mortality would clarify whether the associations between hemoglobin levels and cancer risk and mortality are independent of biomarker concentration.

The association between biomarkers and hemoglobin level was determined in a sub-population of non-Hispanic white and Hispanic women. It would be important to replicate this analysis in other populations with different race/ethnic composition. Black/African American women have a higher prevalence of anemia and may have other factors associated with hemoglobin level which may alter associations observed.

Strengths and Limitations

The main strength of the study was the large sample size which allowed for stratification by cancer group, race/ethnicity and age. The study had a large number of variables which allowed for testing of important confounders. It used a validated food frequency questionnaire (FFQ), and reported on the reliability of instruments used. The present

research used clinical definitions of anemia which allowed for comparability with other studies.

Limitations included the lack of hemoglobin measures at several time points. In addition, the study was unable to distinguish between anemia types. Although the study had data on several important confounders, there was no data on kidney function which is an important variable, potentially affecting hemoglobin and biomarker levels, and cancer mortality and incidence.

Conclusions

Anemia was associated with total and colorectal cancer mortality. Baseline C-reactive protein, TNF-alpha and IL-1 alpha levels were associated with baseline anemia or hemoglobin level. Anemia was not significantly associated with increased cancer incidence in the total population. However, a high hemoglobin concentration of greater than or equal to 16 g/dl was associated with an increased risk of any or breast cancer incidence, after controlling for important confounders such as dietary iron or red meat intake. These findings should be confirmed by future studies.

APPENDIX A: LEAST DETECTABLE DOSES AND ALTERNATIVE TREATMENT APPROACHES USED IN SENSITIVITY ANALYSIS FOR CHAPTER 6

Biomarker	LDD	Data Treatment	Biomarker	LDD	Data Treatment
Alpha-1 antitrypsin (mg/ml)	0.01	Continuous	IL-16 (pg/ml)	16	Continuous
Adiponectin (µg/ml)	0.028	Continuous	IL-1alpha (ng/ml)	0.007	Exclude from Analysis
Alpha-2 Macroglobulin (mg/ml)	0.013	Continuous	IL-1beta (pg/ml)	1.1	Exclude from Analysis
Alpha-Fetoprotein (ng/ml)	0.13	Exclude from Analysis	IL-1ra (pg/ml)	44	Exclude from Analysis
Apolipoprotein A1 (mg/ml)	0.006	Continuous	IL-2 (pg/ml)	19	Exclude from Analysis
Apolipoprotein CIII (µg/ml)	3.3	Continuous	IL-3 ng/ml)	0.049	Exclude from Analysis
Apolipoprotein H (µg/ml)	2.6	Continuous	IL-4 (pg/ml)	34	Categorical
Beta-2 Microglobulin (µg/ml)	0.067	Continuous	IL-5 (pg/ml)	4	Exclude from Analysis
Brain-Derived Neurotrophic Factor (ng/ml)	0.023	Continuous	IL-6 (pg/ml)	1.8	Exclude from Analysis
Complement 3 (mg/ml)	0.005	Continuous	IL-7 (pg/ml)	59	Exclude from Analysis
Cancer Antigen 125 (U/ml)	6.5	Exclude from Analysis	IL-8 (pg/ml)	3.8	Exclude from Analysis
Cancer Antigen 19-9 (U/ml)	1.6	Quintiles	Insulin (uIU/ml)	0.56	Categorical
Calcitonin (pg/ml)	7.2	Exclude from Analysis	Leptin (ng/ml)	0.12	Continuous
CD40 (ng/ml)	0.022	Continuous	Lymphotactin (ng/ml)	0.23	Exclude from Analysis
CD40 Ligand (ng/ml)	0.013	Continuous	MCP-1 (pg/ml)	40	Continuous
Carcinoembryonic Antigen (ng/ml)	0.19	Quintiles	MDC (pg/ml)	12	Continuous
Creatine Kinase-MB (ng/ml)	0.91	Categorical	MIP-1alpha (pg/ml)	19	Continuous
C Reactive Protein (µg/ml)	0.007	Continuous	MIP-1beta (pg/ml)	21	Continuous
EGF (pg/ml)	3	Continuous	MMP-2 (ng/ml)	15	Exclude from Analysis
ENA-78 (ng/ml)	0.033	Continuous	MMP-3 (ng/ml)	0.066	Continuous
Endothelin-1 (pg/ml)	14	Exclude from Analysis	MMP-9 (ng/ml)	10	Exclude from Analysis
EN-RAGE (ng/ml)	0.023	Continuous	Myeloperoxidase (ng/ml)	32	Deciles
Eotaxin (pg/ml)	19	Categorical	Myoglobin (ng/ml)	2	Continuous
Erythropoietin (pg/ml)	14	Exclude from Analysis	PAI-1 (ng/ml)	1.1	Continuous
Fatty Acid Binding Protein (ng/ml)	0.71	Exclude from Analysis	Prostatic Acid Phosphatase (ng/ml)	0.021	Continuous
Factor VII (ng/ml)	0.57	Continuous	PAPP-A (mIU/ml)	0.011	Exclude from Analysis
Ferritin (ng/ml)	1.5	Quintiles	Prostate Specific Antigen-Free (ng/ml)	0.015	Exclude from Analysis
FGF Basic (pg/ml)	249	Exclude from Analysis	RANTES (ng/ml)	0.033	Continuous
Fibrinogen (mg/ml)	0.014	Exclude from Analysis	Serum Amyloid P (µg/ml)	0.16	Continuous
G-CSF (pg/ml)	3.4	Exclude from Analysis	Stem Cell Factor (pg/ml)	43	Continuous
Growth Hormone (ng/ml)	0.092	Categorical	SGOT (µg/ml)	2.5	Exclude from Analysis
GM-CSF (pg/ml)	39	Exclude from Analysis	SHBG (nmol/L)	2.3	Continuous
Haptoglobin (mg/ml)	0.026	Continuous	Thyroxine Binding Globulin (µg/ml)	0.33	Continuous
ICAM-1 (ng/ml)	2.1	Continuous	Tissue Factor (ng/ml)	0.31	Exclude from Analysis
IFN-gamma (pg/ml)	3.2	Exclude from Analysis	TIMP-1 (ng/ml)	4.1	Continuous
IgA (mg/ml)	0.043	Continuous	TNFR2 (ng/ml)	0.22	Continuous
IgE (ng/ml)	3.9	Categorical	TNF-alpha (pg/ml)	7	Exclude from Analysis
IGF-1 (ng/ml)	6	Categorical	TNF-beta (pg/ml)	18	Exclude from Analysis
IgM (mg/ml)	0.08	Deciles	Thrombopoietin (ng/ml)	4.4	Exclude from Analysis
IL-10 (pg/ml)	6.3	Categorical	Thyroid Stimulating Hormone (uIU/ml)	0.021	Continuous
IL-12p40 (ng/ml)	0.23	Exclude from Analysis	VCAM-1 (ng/ml)	1.5	Continuous
IL-12p70 (pg/ml)	26	Exclude from Analysis	VEGF (pg/ml)	17	Continuous
IL-13 (pg/ml)	16	Quintiles	von Willebrand Factor (µg/ml)	2.8	Continuous
IL-15 (ng/ml)	0.58	Exclude from Analysis	Apolipoprotein (a) (µg/ml)	3.3	Continuous

APPENDIX B: SUMMARY OF PREVIOUS RESEARCH EXPLORING THE ASSOCIATION BETWEEN HEMOGLOBIN LEVEL AND BIOMARKERS

Biomarker	Magnitude	P	Other Studies	Mechanism
Anemia	**OR**			
Beta-2 microglobulin (B2M)	1.68 Continuous Hb 0.014 in 60-69 yr olds vs. -0.169 in 50-59 yr olds (p-inter=0.053)	0.0001	**Positive association:** 1. Cheung et al. (2013) – Participants of NHANES III who had beta-2 microglobulin measures and who were older than 20 years[190]. 2. Liabeuf et al. (2012) – 150 Caucasian male and female patients with chronic kidney disease (CKD) mean age 66-68 years[189]. 3. Kim et al. (2010) – 100 patients with Systemic Lupus Erythematosus (SLE) and 50 controls (mean ages 30 & 33 years respectively)[188]. 4. Anagnostopoulos et al. (2006) –124 patients with Waldenström's Macroglobulinemia (a type of lymphoma) aged 28-89 years [197]. 5. Vassilakopoulos et.al 2002 –232 Hodgkin's lymphoma patients (median age 31 years)[187].	Unclear 1. B2M serves as chaperone to HFE, the hereditary hemochromatosis (HH) protein[194]. 2. No B2M (B2M knockout) leads to iron overload in mice[193] 3. HFE-B2M competitively binds with Transferrin Receptor (TfnR). This reduces Tfn levels and leads to less iron taken up by cells[194]. 4. Individuals with HH have lower hepcidin levels[244] 5. The binding of Tfn to TfnR1 leads to a Tf/HFE/TfnR2 complex which upregulates hepcidin expression [244]
CRP	1.44	0.0271	**Positive association (Primarily simple tests of differences in Table 1):** 1. Den Elzen et al. (2013) - Leiden 85 plus population-based study of 490 male and female participants aged 86 years[160]. 2. Succurro et al. (2011) – Cross sectional survey of 1039 white male and female participants (mean age 48 years) enrolled in the CATAMERIS study[151]. 3. Ferrucci et al. (2010) – 582 male and female Italian participants of the InCHIANTI study aged 65 years and older[32]. 4. Zakai et al. (2005) – 5888 male and female participants of the Cardiovascular Health study aged 65 years and older[3]. 5. Guralnik et al. (2004) – Data from 4199 and 2096 adults >= 65 years enrolled in the NHANES III phases 1 and 2[2].	1. CRP is the main acute phase reactant in humans. Levels are increased in response to acute or chronic inflammatory states [245]. 2. CRP does not appear to play a direct role in erythropoiesis, but may solely be a marker of the association between anemia and inflammation
Erythropoietin	1.89	0.0001	**Positive association:**	1. EPO is produced in the kidneys in

Biomarker	Magnitude	P	Other Studies	Mechanism
(EPO)			1. Ferrucci et al. (2005) – 1235 participants of the inCHIANTI study. The majority were adults >= 65[149]. 2. Ershler et al. (2005) – 143 male and female participants of the Baltimore Longitudinal Study on Ageing (median age 62 years)[177].	response to hypoxia[177]. 2. In non-anemic individuals inflammation leads to an increase in EPO to maintain hemoglobin levels[149] 3. Inflammation in anemic individuals is associated with a blunted erythropoietin response[149]
TNF-alpha	1.53 Continuous Hb 0.044 in 70-79 yr olds vs. -0.103 in 50-59 yr olds (p-inter=0.065)	0.0089	**Positive association:** 1. Ferrucci et, al (2010) – 582 subjects >= 65 years of the InCHIANTI study[32]. 2. Voulgari et al. . (1999) – the role of inflammatory cytokines in 232 anemic vs. non anemic patients with rheumatoid arthritis [246].	TNF-alpha: 1. inhibits hematopoietic progenitors[42,167] 2. inhibits EPO production[44] 3. enhances ferritin expression[46]
TNF-beta (also called lymphotoxin alpha)	1.39	0.0338	1. Limited studies	1. Inhibits erythroid progenitor growth [42]
TNFR2	1.68	0.0001	**Positive Association:** 1. Vaiopoulos et al. (1998) – The association between hemoglobin levels, cd23 and TNF receptors was determined in 56 Greek rheumatoid arthritis patients aged 29 to 76 years and 20 age and sex matched subjects[247]. 2. Kruezer et al. (1997) – the study examined the association between cytokines and erythropoietin response in 82 male and female HIV seropositive subjects with mean age of 37 years[248].	1. TNFR2 partially inhibited BFU-E colony growth[167]

Biomarker	Magnitude	P	Other Studies	Mechanism
Hemoglobin Level	**Coeff**			
BDNF	0.124 0.353 in obese vs. 0.074 in non-obese (p-inter=0.001) Levels also decline with age	0.0003	1. No apparent literature	1. Unknown
Erythropoietin (EPO)	-0.155 -0.357 in obese vs. -0.089 in non-obese (p-inter=0.003)	<0.0001	**Inverse association:** 1. Ferrucci et al. (2005) – 1235 participants of the inCHIANTI study. The majority were adults >= 65[149]. 2. Ershler et al. (2005) – 143 male and female participants of the Baltimore Longitudinal study on Ageing[177].	1. EPO is produced in response to hypoxia[177]. 2. In non-anemic individuals inflammation leads to an increase in EPO to maintain hemoglobin levels[149] 3. Inflammation in anemic individuals is associated with a blunted erythropoietin respons [149]
IL-1 alpha	-0.079	0.0295	1. Apparently limited observational studies	IL-1: 1. inhibits EPO production[44,168] 2. Recombinant IL-alpha stimulates early erythroid progenitors (BFU-E) but suppresses late stage progenitors (CFU-E)[43] 3. upregulates hepcidin production[40] 4. Induces ferritin expression[45]
IL-10	-0.092	0.0139	**Inverse association:** 1. Tilg et al. (2002) – 329 participants aged 18-65 yrs old with Crohn's disease who received IL-10 as treatment in a randomized controlled trial were assessed for hematological changes[48] **No association:** 1. Voulgari et al. . (1999) –232 anemic vs. non anemic patients (median ages 57-58 years) with rheumatoid arthritis[246].	IL-10: 1. increases ferritin transcription[48] 2. promotes TfR mediated uptake of iron into cells[49]

Biomarker	Magnitude	P	Other Studies	Mechanism
Leptin	0.152 0.094 in obese vs. 0.088 in non-obese (p-inter=0.015)	0.0023	**Inverse association:** 1. Togo et al. (1999) – Cross sectional survey of Japanese male workers with mean age of 45-48 yrs. No detail of sampling given[198]. 2. Tungtrongchitr (2000) – Cross sectional survey of 48 male and 166 female volunteers to a hospital outpatient dept. Patients stratified by overweight/obesity. Median age was 37-38 years[199]. **No association:** 1. Laharrague et al. (2000) – Compared leptin levels with hematogical parameters of 300 hospitalized European male and female (aged 53-56 yrs) patients with 70 healthy controls (aged 28-42 years)[200]. **Positive association:** 1. Nasri (2006) – cross sectional survey conducted on 36 chronic hemodialysis patients (mean age 44-53 years). This included diabetic and non-diabetic patients[201]. 2. Maccio et al. (2005) – Case control study matching 91 women with advanced untreated ovarian cancer (mean age 62 years) with healthy control volunteers[202].	**Negative association:** 1. Leptin induces hepcidin expression. Hepcidin promotes iron storage[206] **Positive association:** 1. Leptin is an adipokine, produced by adipose tissue. Serum leptin levels are strongly correlated with percent body fat[249] 2. Leptin enhances erythropoiesis/erythroid development[203,204,250]
SHBG	-0.141	0.0002	**Inverse association between SHBG and hemoglobin (Crude Association):** 1. Yeap et al. (2009) – Cross-sectional survey of 492 male participants aged 31 to 95 participants in the Busselton Health survey[210]. **Positive association between testosterone and hemoglobin:** 1. Ferrucci et al. (2006) – 905 male and female participants of the inCHIANTI study aged 65 years and older. Non-anemic participants with low versus normal testosterone were more likely to develop anemia over 3 years[209]. 2. Yeap et al. (2009) – Cross-sectional survey of 492 male participants aged 31 to 95 participants in the Busselton Health survey[210]. 3. Fonseca (1998) – hemoglobin levels of 64 men (median age 68 years) who received bilateral orchiectomy between 1993-1994 at the Mayo Clinic. There was a significant difference between pre and post-op hemoglobin level and hemoglobin fell below normal in 58% of patients[251]. 4. Coviello et al. (2008) – RCT of 61 young men (aged 18-35 yrs) and 60 older men (aged 60-75 yrs) randomized to receive a GnRH agonist plus 1 of 5 different levels of testosterone[252].	1. Testosterone is produced in the adrenal glands and ovaries in women, and levels fall with age up to the menopause[208] 2. Testosterone may stimulate growth of erythroid progenitors[209]. 3. Testosterone may stimulate EPO production[209].

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