

DO CURRENT MEDICAL PROCEDURES SUFFICIENTLY DIAGNOSE IRON
DEFICIENCY?

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DEDICATION

I dedicate this thesis to the loving memories my Grandma Bruschi, Stephen, and Kristin. The significance of their lives in providing motivation to finally complete this work and more importantly on the betterment of me as individual is immeasurable.

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ABSTRACT

Iron deficiency is the most common nutrient deficiency throughout the world, including the United States. Although it has been known for over 125 years that symptomatic iron deficiency can exist in individuals despite normal levels of blood hemoglobin and red blood cell indices, current standards of medical practice frequently rely on measures of hemoglobin, hematocrit, mean corpuscular volume (MCV), and red blood cell distribution width (RDW) to screen for iron deficiency. The objective of this research is to determine how frequently iron deficient individuals are undiagnosed using hemoglobin, hematocrit, MCV, and RDW as indicators of iron status.

Data from eight National Health and Nutrition Examination Surveys (NHANES) studies (1976-2010) were included in the analysis. Males and females with data values for serum ferritin, hemoglobin, hematocrit, MCV, and RDW, not known to be pregnant or to have given birth within a year of data collection, were included in the analysis. Blood values from 56,000 individuals were stratified by study, gender, age and serum ferritin levels. Iron status was determined through the use of serum ferritin. Individuals were considered to be iron deficient and “missed” if adjusted serum ferritin was <21 ng/ml, but the levels of hemoglobin, hematocrit, MCV, and RDW were within normal ranges provided by the Centers for Disease Control and Prevention (CDC). Nonanemic iron deficiency (NAID) in various female age groups was missed 34% to 82% of the time. Younger males were missed for NAID 52% to 81% of the time, and males 40 years and older from 21% to 26% of the time. Therefore, screening for iron deficiency using hemoglobin, hematocrit, MCV and/or RDW fails to detect a significant portion of iron deficient individuals and can result both in delayed diagnosis and/or misdiagnosis that adversely affects the quality of life of the individual and incurs unnecessary medical costs.

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LIST OF ABBREVIATIONS

¹²⁵ I	Iodine-125
ACTH	Adrenocorticotrophic hormone
ADD	Attention deficit disorder
ADHD	Attention deficit hyperactivity disorder
CBC	Complete blood count
CDC	Centers for Disease and Control and Prevention
CI	Confident interval
DNA	Deoxyribonucleic acid
Fe ²⁺	Ferrous iron
Fe ³⁺	Ferric iron
fL	femtoliter
g/dL	Grams per deciliter
H	Heavy ferritin subunit
HAMP	Hemochromatosis type 2B
Hct	Hematocrit
Hgb	Hemoglobin
HFE	HFE-hemochromatosis
HJV	Juvenile hemochromatosis
ID	Iron deficiency
IDA	Iron deficient anemia
L	Light ferritin subunit
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
NAID	Nonanemic iron deficiency
ng/mL	Nanograms per milliliter
NHANES	National Health and Nutrition Examination Survey
RBC	Red blood cell
RDW	Red blood cell distribution width
sFer	Serum ferritin
SLC40A1	Ferroportin disease
TfR2	Transferrin receptor 2 hemochromatosis
WHO	World Health Organization
µg/dL	microgram per deciliter

CHAPTER 1 – LITERATURE REVIEW

Iron, the fourth most abundant element in the earth, is essential for nearly all forms of life (Beard and Dawson 1997). While only present in minute amounts in the body, iron is an essential cofactor for or component of hundreds of enzymes and proteins (Beard and Dawson 1997). The importance of iron to human health was first recognized by the ancient Egyptian, Hindu, and Greek physicians who used iron containing compounds for the treatment of “weakness” (Ross 1958).

IRON IMBALANCE

A negative iron balance can be caused by inadequate iron in the diet, insufficient iron absorption, or excessive iron losses. These conditions lead to an iron deficiency (ID) (Hunt 2003, Johnson-Wimbley and Graham 2011). Similarly, a positive iron balance due to excessive iron consumption or disproportionate iron absorption may lead to iron overload (Vujic 2014).

Iron Deficiency

ID is the pathological state resulting from a relative or absolute lack of iron and is linked to numerous physiological signs and symptoms. This nutrient deficiency is the most common and widespread essential nutrient deficiency in world (CDC 2002). The World Health Organization [2014] indicates that “Iron deficiency affects more people than any other condition, constituting a public health condition of epidemic proportions. More subtle in its manifestations than, for example, protein-energy malnutrition, iron deficiency exacts its heaviest overall toll in terms of ill-health, premature death and lost earnings” [WHO Micronutrient deficiencies. <http://www.who.int/nutrition/topics/ida/en/> Accessed April 22, 2014]”.

The symptoms of ID represent a wide spectrum of disorders impacting all eleven systems of the body. Children and pregnant women are especially impacted by the consequences of ID. Developing fetuses are of special concern as ID in pregnant mothers has been linked to low birth weight, schizophrenia in the offspring, impaired cognitive development, neurobehavioral abnormalities, i.e. attention deficit hyperactivity disorder (ADHD), poor motor function, and reduced learning (Konofal et al. 2004, Scholl 2005, Shafir et al. 2006, Unger et al. 2007, Shafir et al. 2008, Riggins et al. 2009, Sorensen et al. 2010). ID children also may be at a greater risk for febrile seizures and breath holding spells (Zehetner et al. 2010, Sadeghzadeh, Khoshnevis Asl and Mahboubi 2012).

Iron Overload

Just as insufficient iron can result in serious health consequences, excess iron may detrimentally affect the body. Due to the limited ability of humans to excrete excess iron, iron homeostasis relies on the regulation of iron absorption. If more iron is absorbed than can be properly liganded or excreted, the labile iron may catalyze the production of highly reactive free radicals via the Haber-Weiss-Fenton reaction. These free radicals can interfere with biological processes as well as cause oxidative damage to molecules such as deoxyribonucleic acid (DNA) (Jomova and Valko 2011).

Iron overload can occur from over consumption of high iron foods, dietary supplements, blood transfusions, or, of greater concern, the presence of one or more of the hemochromatosis genes. There are presently five recognized forms of hereditary hemochromatosis: HFE-hemochromatosis (HFE), transferrin receptor 2 hemochromatosis (TfR2), juvenile hemochromatosis (HJV), hemochromatosis type 2B (HAMP) and ferroportin disease (SLC40A1) (McLaren and Gordeuk 2009). Hereditary hemochromatosis is most common in Caucasians of Northern European descent. The disease is estimated to affect 0.5% of the U.S. population (Pietrangelo 2004). The different mutations responsible for hereditary hemochromatosis function through interfering with hepcidin production or the response to hepcidin. Hereditary hemochromatosis can be effectively managed through periodic phlebotomy (Bacon et al. 2011). Although data are limited, it appears that for patients wanting to be an active participant in their disease management, selecting a lower iron bioavailability diet has been shown to provide some benefits (Moretti et al. 2013).

In many cases, iron overload signs and symptoms are similar to those of ID. When iron overload or hemochromatosis is not identified and treated in a timely fashion, then iron will be taken up by multiple body tissues and can lead to liver disease, heart problems, joint pains, reproductive problems, excessive darkening of skin color, and underactive thyroid or adrenal glands (Herbert 1992, Dolbey 2001). Conditions related to iron overload will not be covered in this thesis, but more information can be found at <http://www.nhlbi.nih.gov/health/health-topics/topics/hemo/signs.html>.

RECOGNITION OF IRON DEFICIENCY WITHOUT ANEMIA

The idea that ID symptoms only occur subsequent to anemia was first proposed by Hahn and Whipple in 1936. They noted that tissue iron enzymes were “inviolable” even during periods of high iron demand (as cited in Beutler 1960). This idea, however, was known to be inaccurate at the time and refuted by later research.

ID in the absence of anemia was first noted by Becquerel and Rodier in 1845. They noted that individuals with chlorosis (later classified as pallor), had normal blood when examined macroscopically (Becquerel and Rodier 1845, Stillman 1891). This was confirmed microscopically in 1883 by Laache who examined blood from chlorosis individuals (as cited in Beutler 1960).

Over a century ago, Sahli performed one of the first experiments to show that an ID symptom responded to iron supplementation before hemoglobin (Hgb) changes were observed. He found that women with chlorosis and normal or near normal Hgb had their symptoms alleviated with iron supplementation prior to observed changes in Hgb levels (as cited in Beutler 1960).

The role of ID in the absence of anemia in explaining other pathologies was explored by Waldenstrom and Kjellberg in 1939 (1939). They found that women with dysphagia but without anemia responded to iron supplementation. Twenty years later, Beutler et al., (1960) performed the first placebo controlled double-blind experiment showing that nonanemic women with fatigue responded favorably to iron supplementation. These results have been independently confirmed in at least four other experiments (Patterson, Brown and Roberts 2001, Verdon et al. 2003, Krayenbuehl et al. 2011, Vaucher et al. 2012).

Beutler (1960) compiled a history of experiments conducted on ID without anemia and made a compelling case to address this largely unrecognized medical issue. Shortly thereafter, Stafford and Kemp noted that in the clinical setting, ID had become synonymous with Hgb deficiency and that nonanemic ID was being overlooked (Stafford and Kemp 1964). To this day, this misunderstanding that ID is symptomless until anemia occurs has gone largely uncorrected despite extensive research on the many subclinical symptoms documented in nonanemic ID. Symptoms related to ID, with or without anemia, will be explored in the next section of this thesis.

SIGNS AND SYMPTOMS OF IRON DEFICIENCY WITH OR WITHOUT ANEMIA

Iron is an essential nutrient that is used in many of the systems of the body. While the majority of iron in the body is used for oxygen transport, that role represents a small fraction of the number of pathways in which iron is required. Iron is also required for normal functioning of all the organ systems. Insufficient iron stores may lead to impaired functioning of these systems independent of reduction in oxygen transport ability.

Table 1.1 Recognition Timeline of Iron Deficiency Without Anemia

Year	Study
1845	Blood from some chlorotic individuals appeared normal macroscopically (Becquerel and Rodier 1845)
1883	Microscopic analysis of blood of chlorotic individuals was normal in some cases (Laache 1883)
1908	First experimental trial on individuals with chlorosis (Sahli 1908)
1939	First experimental trial on individuals with dysphagia (Waldenström and Kjellberg 1939)
1960	First placebo controlled double-blind study (Beutler, Larsh and Gurney 1960)
1964	Noted the medical community incorrectly used anemia and ID interchangeably (Stafford and Kemp 1964)

Circulatory System

Anemia

Anemia is the most common sign associated with ID. Insufficient iron availability for erythropoiesis will result in iron deficient anemia (IDA), which is characterized by microcytic, hypochromic red blood cells. IDA has been thought to be responsible for fatigue and other symptoms related to ID (Gomez 2002). The majority of individuals with IDA can be treated effectively with iron supplementation (Massey 1992).

Blood Pressure

Research on rats has indicated that prenatal ID may result in altered neonatal blood pressure (Crowe et al. 1995). These alterations may be sex dependent (Gambling et al. 2003). While the long term consequences of altered fetal blood pressure and associated effects are unclear, some research on humans has indicated a correlation with hypertension later in life (Barker et al. 1990).

Fatigue

ID has been shown to be associated with fatigue (Patterson et al. 2000). Unexplained fatigue was one of the earliest areas of study in ID in the absence of

anemia using a placebo-controlled double-blind study (Beutler, Larsh and Gurney 1960). Beutler, Larsh, and Gurney showed that women with unexplained fatigue responded to iron supplementation even in the absence of anemia. Other studies have confirmed that nonanemic individuals with fatigue respond to iron supplementation prior to changes in Hgb and hematocrit (Hct) (Verdon et al. 2003, Vaucher et al. 2012). It also has been shown that intravenous iron supplementation produces identical results in the treatment of fatigue in nonanemic women (Krayenbuehl et al. 2011). While the exact pathway by which ID can increase fatigue in the absence of anemia has not been shown, it is likely multifactorial and related to the multiple iron dependent enzyme pathways in the brain (Dallman 1986). Metabolic rates, which may be related to fatigue, can be impacted by ID, though the direction of the effect is unclear and may depend on the severity of ID (Martinez-Torres et al. 1984, Beard, Borel and Derr 1990). Fatigue also can be related to neuronal functioning which has been shown to be negatively impacted by ID (Beard et al. 2005, Munoz and Humeres 2012).

Heart Disease

Management of ID in individuals with heart failure has been understood to reduce the morbidity and mortality of the disease (Jankowska et al. 2010, O'Meara and de Denus 2010). Additionally, iron supplementation has been shown to improve exercise tolerance in heart failure patients with IDA in a controlled and blinded study (Okonko et al. 2008).

However, the role ID may play in the pathophysiology of heart failure is not fully understood. Work with rats with IDA showed that ID was associated with increased cytochrome c release from the mitochondria in the heart as well as cardiac hypertrophy and elevated nitric oxide synthase levels compared to non-iron deficient controls (Dong et al. 2005). This confirmed earlier work that showed rats fed an iron-deficient diet developed cardiac hypertrophy compared to the normal cardiac development of rats on an iron-sufficient diet (Medeiros and Beard 1998). There is also evidence that maternal ID may result in cardiac hypertrophy in the fetus and contribute to increased infant mortality (Gambling et al. 2003).

Thrombosis

A potential relationship between ID and thrombosis, venal blood clot formation, has been studied (Huang, Su and Lin 2010). There are a couple of

proposed mechanisms by which ID might cause thrombosis. Iron is involved in the regulation of thrombopoiesis and normal levels of iron are required for proper inhibition of thrombocytosis (Karparkin, Garg and Freedman 1974, Beguin 1999). Iron is also necessary for platelet production. Therefore, moderately iron-deficient individuals may see an increase in thrombosis due to decreased inhibition of thrombopoiesis (Hartfield et al. 1997). Severely iron-deficient individuals may also suffer from thrombocytopenia which could compensate for any reduction in the inhibition of thrombocytosis. Another possible mechanism involves changes in venous flow patterns due to a reduction in red blood cell deformability and increased viscosity (Stehle, Buss and Heene 1991).

Digestive System

Angular Stomatitis

Stomatitis is an inflammation of the mucosal lining of any surfaces in the mouth. Iron-deficient individuals have been known to present with stomatitis (Beard 2001). One possible explanation for this particular manifestation of ID focuses on iron's role in cellular replication (Robbins and Pederson 1970). Insufficient replacement rates of the epithelial cells due to inhibited cellular repair and replication in iron deficient individuals may result in the inflammation associated with stomatitis.

Dysphagia

Dysphagia, or trouble swallowing at the oropharyngoesophageal level, has long been associated with ID. In 1939, Waldenström and Kjellberg (1939) documented cases of dysphagia without anemia that responded to iron treatment. The difficulty swallowing associated with ID may be caused by pain in the mouth, tongue, or pharynx (Edwards 1984) or it may be caused by the presence of an esophageal web as part of Plummer-Vinson syndrome (Rodríguez et al. 2002).

Gallstones

Iron deficient individuals have been found to have a higher incidence of gallstones than those with a normal iron status (Pamuk et al. 2009). ID has been experimentally linked to the formation of both cholesterol and pigment gallstones (Roslyn et al. 1987, Johnston et al. 1997). It is possible the alteration in hepatic enzymes associated with ID could contribute to gallstone formation (Bailey-Wood et al. 1975). ID has been shown to decrease nitric oxide synthase in the gallbladder

which is required for normal gallbladder motility (Goldblatt et al. 2001). It has also been noted that transferrin, which is elevated in ID, is a potent pronucleator of cholesterol crystals, which could contribute to gallstones (Swartz-Basile et al. 2000).

Gastric Achlorhydria

Gastric achlorhydria, a condition where there is reduced production of gastric acid, has been associated with ID since at least 1913 (Faber 1913). Gastric achlorhydria resulting from ID may be caused by at least two separate pathways (Shearman and Finlayson 1968). Gastric acid production may be inhibited through enzymatic inhibition from insufficient iron availability (Delamore and Shearman 1965). Lesions in the gastric mucosa due to insufficient replacement of the parietal cells may result in limited gastric acid secretion producing gastric achlorhydria. The different causes of gastric achlorhydria may explain the varying responses to iron treatment by individuals with gastric achlorhydria (Delamore and Shearman 1965, Desai et al. 1968, Stone 1968). Disruptions in the enzymatic pathways responsible for gastric acid production and secretion may more readily respond to iron treatment while lesions in the gastric mucosa may take substantially longer or may be irreparably damaged (Shearman and Finlayson 1968). Regardless of the pathway, gastric achlorhydria in turn may lead to decreased iron absorption (Goldberg, Lochhead and Dagg 1963, McColl 2009).

Glossitis

Glossitis, a condition in which the tongue is painful and red with atrophy of the lingual papillae, has been associated with IDA and NAID (Osaki et al. 1999, Beard 2001). In an experiment by Osaki et al, (1999) individuals with ID were noted to have experienced a shorter history of glossal pain as well as less severe glossal pain compared to those with anemia. It also was noted that individuals with NAID responded to iron supplementation more readily than anemic individuals and no significant changes in Hgb or Hct were noted in the individuals with NAID, suggesting that glossal pain may occur prior to the development of anemia in iron-deficient individuals and the condition worsens with the severity or duration of the deficiency. While glossal pain also may be caused by vitamin B₁₂ deficiency (Osaki 1979), glossitis has been reported in up to 52% of iron-deficient individuals. The pathophysiology of glossitis in iron-deficient individuals may be similar to that in stomatitis.

Pica

Pica, the compulsive ingestion of unusual foods or non-food substances has been associated with ID and IDA. Initially, pica was thought to cause ID, however, research done by Coltman (1969) and Carlander (1959) indicated that the substances consumed do not replace iron-containing foods, nor do the items inhibit iron absorption.

In 1968, Reynolds et al., (1968) indicated that pagophagia, compulsive ice-chewing, was eliminated in individuals with IDA with iron supplementation (Reynolds et al. 1968). Later research showed that pica was eliminated with iron supplementation in individuals with IDA prior to correction of anemia (Brown and Dyment 1972, Kettaneh et al. 2005). The estimates of the prevalence of pica in individuals with ID or IDA range up to 58% (Rector 1989).

While the exact pathophysiology of pica caused by ID is not clear, there are a few proposed explanations. Some forms of pica may be explained by the disruption of cytochrome oxidase in the buccal membrane. Brown and Dyment (1972) proposed a disruption of an enzyme in the central nervous system that could explain pica. Certain types of pica, especially pagophagia, may alleviate some of the other symptoms of ID such as angular stomatitis and glossitis (Hadjadj, Martin and Fichet 1990).

Plummer Vinson

Plummer-Vinson syndrome (or Paterson-Kelly or Paterson-Brown Kelly syndrome as it is alternatively known) is characterized by ID, anemia, dysphagia, and the presence of esophageal web or webs. The etiology and pathogenesis of the esophageal web remains elusive (Rodríguez et al. 2002). Chisholm (1974) found that 88% of those with Plummer-Vinson were iron deficient or had IDA, but only 10% of those with IDA had Plummer-Vinson Syndrome.

Villous Atrophy

Atrophy of the villi in the intestines has been documented in iron deficient children, with improvement upon iron repletion (Guha et al. 1968). Iron supplementation also has been found to improve villous morphology in an iron-deficient woman (Lizarraga et al. 2009).

Endocrine System

Depression

Iron-deficient individuals have been shown to be at a greater risk for depression than those with normal iron status even in the absence of anemia (Mansson et al. 2005, Vahdat Shariatpanaahi et al. 2007, Yi et al. 2011, Chen et al. 2013). One potential iron-dependent pathway that could explain the association between ID and depression is its role in normal dopamine metabolism (Beard 2003). Studies in rats have shown that ID decreases dopamine receptors in the brain and that changes may be sex-dependent (Youdim et al. 1983, Erikson et al. 2001). It also appears that the consequences of ID on dopaminergic neurotransmission may be irreversible depending upon the stage of development during which ID occurs (Youdim and Ben-Shachar 1987, Lozoff 2011).

Impaired Thermoregulation

The relationship between ID and thermoregulation was explored by Dillman et al. (1979) using the rat model. Their work showed that iron-deficient rats had temperature-dependent elevation of catecholamine levels; at lower temperatures, iron-deficient rats had significantly higher catecholamine levels in the urine and blood. The researchers also noted that correction of anemia through transfusion in iron-deficient rats did not improve the catecholamine response to temperature. With the use of isotopically-labeled norepinephrine, the researchers were able to show that iron-deficient rats suffered from impaired secretion of norepinephrine. The researchers also noted that iron-deficient rats exposed to cold had elevated thyroxine levels but decreased triiodothyronine. Within six days of iron supplementation, iron-deficient rats were found to have identical triiodothyronine levels as controls when subjected to cold. These findings led the researchers to hypothesize that impairment of an iron-dependent enzyme in the conversion of thyroxine to triiodothyronine was responsible for the inhibited thermoregulation.

Obesity

Obesity has been shown to be associated with ID in both children and adults (Pinhas-Hamiel et al. 2003, Nead et al. 2004, Yanoff et al. 2007). Obese individuals have been shown to have elevated hepcidin levels, which may encourage the sequestration of iron, leading to a paucity of available iron (Amato et al. 2010, Tussing-Humphreys et al. 2012). Additionally, the inflammation associated with ID

may elevate serum ferritin (sFer) levels, further reducing iron availability (Cepeda-Lopez, Aeberli and Zimmermann 2010).

Immune System

Impaired Immune Function

The role iron plays in impairing immune function has not been fully elucidated. Work done by Macdougall et al.,(1975) found that children with ID and IDA exhibited decreased bactericidal function of the neutrophils as well as impaired lymphocyte function. It was also noted that the immune responses were normalized after iron supplementation. A study carried out by Andelman and Sered (1966) showed that iron supplementation of infants with IDA corrected the anemia as well as resulted in a reduction in the number of respiratory infections. Iron supplementation of adult males in Indonesia was found to produce a threefold decrease in the prevalence of enteritis and influenza-type infections (Basta et al. 1979). Baggs and Miller (1973) examined the effect the severity of ID has on the immune response of rats. Their research showed that rats with ID but normal Hgb and Hct levels had higher levels of morbidity and mortality than either normal rats or rats with IDA. A study by Chandra (1973) found similar results in bacterial killing by polymorphonuclear leukocytes in that children with moderate ID had a greater reduction in immune function than those with either normal iron status or those with severe ID, however, the results were not significant at the $P < 0.05$ level.

These results indicate that there are at least two different ways iron status can influence immune function. One involves iron's role in many enzymatic pathways related to immune function. Reduced levels of catalase, an iron-containing enzyme, have been found to diminish immune function (Takahara and Miyamoto 1948). Iron also is required for the normal functioning of myeloperoxidase in neutrophils, which may explain the predisposition of some iron-deficient individuals to repeated infections (Krantman et al. 1982). ID also may interrupt interleukin 1 production by the leukocytes, thereby impairing immune function (Helyar and Sherman 1987).

Another mechanism may involve plasma transferrin (Jacobs 1977). Transferrin in the blood compartment has the ability to bind with any free iron, thereby reducing the amount of iron available to bacteria. Those with the greatest degree of ID have the largest amount of transferrin available for iron binding, which might compensate for the impairment of the enzymatic pathways involved in immunity. However, those with moderate ID would also suffer from impaired

enzymatic pathways, but their transferrin saturation levels may not be low enough to compensate for this effect. It also has been proposed that ID may provide some protection against certain types of infections, such as malaria, which further complicates study of the role of ID in the impairment of immune function (Denic and Agarwal 2007).

Gastrointestinal Carcinogenesis

The study of the relationship between ID and gastrointestinal carcinogenesis dates back to at least 1936 when Ahlbom (1936) noted a high rate of carcinoma in the mouths and esophagi of women with Plummer-Vinson syndrome. Work done in the following four decades confirmed this association (Owen 1950, Wynder et al. 1957, Shamma'a and Benedict 1958, Jones 1961, Chisholm 1974). There has been some experimental evidence linking ID with earlier development or higher incidence rates of tumors in rats. Vitale et al. (1977) found iron-deficient rats developed tumors much earlier (126 days versus 245 days) than iron-replete rats. Furthermore, the site of the tumors changed from the colon in iron-replete rats to the liver in iron-deficient rats. Work by Prime, MacDonald and Rennie (1983) showed that rats fed an iron-deficient diet developed oral squamous cell carcinomas earlier than rats fed an iron-replete diet. An iron-deficient diet was found to increase the incidence rates of colonic and duodenal tumors in rats versus a control diet group (Jagadeesan, Rao and Sesikeran 1994).

There are many potential pathways by which ID could contribute to carcinogenesis (Prá et al. 2009). ID may impair DNA synthesis through reduced ribonucleotide reductase activity. Inhibition of ribonucleotide reductase has been found to have a leukemogenic effect (Lofvenberg, Nordenson and Wahlin 1990). Furthermore, offspring of anemic mothers are more likely to develop leukemia (Thompson et al. 2001, Wen et al. 2002, Schuz, Weihkopf and Kaatsch 2007).

Iron is also involved in the repair of DNA. Iron is a component of the enzymatic pathways responsible for mismatch repair, and disruptions in the mismatch repair system have been associated with cancer (Slupphaug, Kavli and Krokan 2003). Iron is essential for proper functioning of DNA glycosylase which is responsible for base excision repair, and impaired base excision repair has been linked to cancer (Fortini et al. 2003).

The role of iron in normal cytochrome functioning also may explain an increased risk of cancer in iron-deficient individuals. Iron is an integral part of the

cytochrome P450 pathway which, among other roles, is responsible for xenobiotic metabolism. Anemic individuals have been found to have lower levels of cytochrome P450 (Harhaji et al. 2004), and decreased cytochrome P450 has been linked to an increased cancer risk (Perentesis 2001). Iron also is involved in cytochrome pathways responsible for oxidative metabolism. Mitochondrial complex IV is inhibited in ID, and inhibition of mitochondrial complex IV is associated with genomic damage (Atamna, Liu and Ames 2001). Additionally, iron is part of normal cytochrome c functioning. ID has been shown to negatively impact cytochrome c (Atamna, Liu and Ames 2001), and a reduction in cytochrome c functioning may be associated with an elevated risk for cancer through diminished apoptosis (Payne et al. 2005).

ID's impairment of peroxidase also may contribute to a potential increased cancer risk. Decreased myeloperoxidase has been associated with increased risk of malignant tumors (Lanza 1998). Reduction in the ability of catalase to neutralize reactive oxygen species has been associated with increased cancer risk (Ahn et al. 2006). Anemic individuals have been known to have reduced catalase activity (Macdougall 1972, Jansson et al. 1985). Catalase also may have a role in apoptosis (Bai and Cederbaum 2000). Children with ID have decreased rates of apoptosis (Berrak et al. 2007).

Iron is part of the nitric oxide synthase pathway and therefore ID may inhibit nitric oxide synthase (Goth and Vitai 1996), which may cause gastric adenocarcinoma (Tatemichi et al. 2005). The role ID may play in other forms of carcinogenesis has not been adequately examined.

Integumentary System

Alopecia

There has been a fair amount of research into the relationship between ID and alopecia, or hair loss, since the relationship was first noted in 1932 (Cunningham 1932). The results of this research are inconclusive as some studies find correlations between iron status and alopecia while other studies do not (Olsen 2006). It is likely that different standards for assessing iron status, different standards for assessment of hair loss or type of hair loss, and/or any additional genetic or environmental factors that are not accounted for may contribute to the conflicting results. Studies, however, have found that people with alopecia, especially chronic telogen effluvium, respond to iron supplementation (Rushton and Ramsay 1992, Rushton et al. 2002). The exact mechanism of this relationship is still not established, but it may be

related to iron's role as a cofactor in ribonucleotide reductase, which is the rate-limiting enzyme in the synthesis of DNA. For this reason, the rapidly dividing cells in the hair follicles may be especially sensitive to reduced iron availability (Kantor et al. 2003).

Blue Sclerae

Blue sclerae is a blue hue of the sclerae of the eyes. It results from an abnormally thin sclerae insufficiently veiling the melanin-rich uveal layer of the eyes (Beghetti, Mermillod and Halperin 1993). Iron is an essential cofactor in collagen formation (Kalra, Hamlyn and Jones 1986), and therefore iron-deficient individuals may have diminished collagen formation resulting in thin sclerae. A study by Kalra, Hamlyn and Jones (1986) indicated blue sclerae may be a good indicator of adults with IDA. Their work estimated the sensitivity at 0.87 (CI 0.74 to 0.95) and the specificity at 0.95 (CI 0.88 to 0.98).

Koilonychias

Koilonychia, the condition in which nails exhibit concavity, is commonly caused by ID. The "spooning" or flattening of the nails results from iron-deficient keratinocytes producing a soft nail substance. When pressure is applied to the fingertip from activities such as writing, a depression or concavity may form in the nail plate. As such, koilonychias resulting from ID is most frequently seen in the thumb and index finger of the dominant hand, but in severe cases can affect other fingers. Hogan and Jones (1970) noted that iron-deficient infants and children that are not anemic may have koilonychias. A study on individuals with hypochromic anemia indicated that about 25% of iron-deficient anemic individuals will have nail abnormalities, but the percent with true koilonychia is somewhat lower (Beveridge et al. 1965).

Muscular System

Decreased Work Capacity and Exercise Performance

Decreased work capacity and exercise performance has long been associated with iron-deficiency anemia. It was assumed for a long time that the decline was due to the effects of anemia. However, a growing body of evidence suggests that iron-deficient individuals without anemia respond to iron treatment in the areas of work capacity and exercise performance (Friedmann et al. 2001, Hinton and Sinclair

2007). An experiment by Finch et al., (1976) noted that rats fed an iron-deficient diet had decreased exercise performance that responded to iron treatment before any change in circulating levels of Hgb or Hct. In a separate experiment, the researchers induced anemia in rats and fed different groups diets of varying iron levels. After giving all the groups transfusions to normalize the Hgb concentrations, it was noted that rats fed an iron-adequate diet saw dramatic improvements in fatigue resistance within three days but rats fed an iron-deficient diet did not see any improvement even after nine days with normal Hgb levels. The authors also noted decreased levels of cytochrome pigments and myoglobin, as well as decreased rates of oxidative phosphorylation of pyruvate-maleate, succinate, and α -glycerophosphate in mitochondrial preparations from the skeletal muscle of the rats on iron-deficient diets. Only the rate of phosphorylation of α -glycerophosphate responded in a similar rate to iron repletion as fatigue resistance. The authors then concluded that α -glycerophosphate oxidase is responsible for the decreased fatigue resistance of rats with NAID. This work has been supported by other research indicating that ID affects endurance activities that rely predominantly on muscle oxidative capacity whereas anemia affects fast-acting muscles through a reduction in the ability of oxygen to be transported to the muscles (Dallman 1982, Davies et al. 1982, Davies et al. 1984, Perkkio et al. 1985, Beard 2001).

The above studies done on animal models were confirmed in humans by later work by Brownlie et al., (2002), who found significant improvement in endurance of women with NAID in a randomized placebo-controlled double-blind study. A randomized placebo-controlled double-blind experiment by Brutsaert et al., (2003) found that women with NAID had greater improvement in fatigue resistance with iron supplementation than without.

Restless Legs Syndrome

Restless legs syndrome, a disorder characterized by an unbearable need or urge to move the legs (Walters 1995), afflicts 2-5% of the population (Strang 1967, Lavigne and Montplaisir 1994). Experimental evidence linking ID to restless legs syndrome dates back to at least 1953 when Nordlander (1953) found a complete relief of symptoms with intravenous iron supplementation. Later studies found individuals with restless legs syndrome responded to oral iron supplementation (Lavigne and Montplaisir 1994, O'Keeffe, Gavin and Lavan 1994). Randomized, placebo-controlled, double-blind studies have confirmed the efficacy of intravenous

iron (Sloand et al. 2004, Grote et al. 2009) and oral iron supplementation (Wang et al. 2009) in providing relief of the symptoms of restless legs syndrome. However, a study by Earley et al., (2000) documented normal blood levels of sFer and transferrin, but found low levels of ferritin and transferrin in the cerebrospinal fluid of individuals with restless legs syndrome. The theory that individuals with restless legs syndrome may suffer from the impaired ability of the brain to store or transport iron was further supported with work by Allen et al., (2001) who found decreased levels of iron concentrations in the substantia nigra and putamen using magnetic resonance imaging (MRI).

The mechanism by which ID can induce restless legs syndrome has not been fully established, but it may be due to iron's role as a cofactor in tyrosine hydroxylase which is the rate-limiting step in dopamine metabolism (Cooper, Bloom and Roth 2003). Dopamine agonists have been found to improve symptoms (Chesson et al. 1999, Hening et al. 1999), while antagonists may worsen the symptoms of restless legs syndrome (Ekblom 1960). Research has shown that iron supplementation will normalize dopamine activity in iron-deficient animals (Ashkenazi, Ben-Shachar and Youdim 1982, Ben-Shachar, Finberg and Youdim 1985). However, Ben-Shachar, Finberg, and Youdim (1985) noted that normalization of iron status does not reverse the effects of ID occurring in rat pups before and during nursing. These findings raise the possibility that irreversible neural damage may result from ID during the fetal and neonatal period in life.

Nervous System

Alzheimer's Disease

Alzheimer's disease is a progressive form of dementia. While the cause of Alzheimer's disease is currently unknown, research has indicated a potential role for ID in the development of the disease.

One possible pathway involves the role ID plays in tissue aluminum accumulation. Research on rats has indicated that ID may lead to accumulation of aluminum in certain tissues (Brown and Schwartz 1992). While aluminum is a known neurotoxin, its potential role in Alzheimer's disease is likely, but unproven (Campbell 2002, Gupta et al. 2005, Shcherbatykh and Carpenter 2007, Tomljenovic 2011).

Another potential pathway is the alteration of Alzheimer's related genes during neonatal ID. Research on iron-deficient rats suggests an upregulation of genes associated with Alzheimer's disease that may have lasting effects (Carlson et al. 2008).

A third potential mechanism by which ID may influence Alzheimer's disease pathogenesis is the role iron plays in copper homeostasis in the brain. ID has been shown to cause copper accumulation in the rat brain (Monnot et al. 2011). Elevated copper levels may be linked to Alzheimer's disease, however the exact mechanism is unknown (Skjorringe, Moller and Moos 2012).

Febrile Seizure

Febrile seizure is one of the most common neurological pathologies in childhood (Sadeghzadeh, Khoshnevis Asl and Mahboubi 2012). While the etiology of febrile seizures remains unclear, research indicates that individuals with IDA may be more prone to febrile seizures (Daoud et al. 2002, Naveed-ur-Rehman and Billoo 2005, Fallah et al. 2013).

Neurobehavioral Abnormalities (i.e. attention deficit hyperactivity disorder, ADHD)

Children with Attention Deficit Disorder (ADD) and ADHD have been shown to be more likely iron deficient than children without these disorders. (Chen et al. 2013). Konofal et al., (2004) found a correlation between sFer and ADD/ADHD symptoms even in the absence of anemia. A double-blind placebo-controlled study found that nonanemic children with ADD and ADHD responded to iron supplementation (Konofal et al. 2008). The mechanism by which ID contributes to ADD and ADHD remains unknown.

Poor Motor Function

ID has been found to be associated with poor motor function with and without anemia in both humans and rats (Unger et al. 2007, Shafir et al. 2008). The effects of ID during development on motor function have been shown to remain even after correction of ID (Shafir et al. 2006).

Reduced Learning Capacity and Cognitive Function

Research on nutritionally iron-deficient rats indicates that ID reduces learning capacity. It was noted that the longer the duration of ID, the greater the deficit. These differences were noted before changes in Hgb were observed. Furthermore, correction of iron status did not correct the decreased learning capacity, suggesting irreversible developmental damage (Yehuda, Youdim and Mostofsky 1986). Iron is

required for cell proliferation and differentiation and the high rate of growth during brain development leads to high iron demands (Le and Richardson 2002). The developing hippocampus is highly susceptible to the effects of ID (de Deungria et al. 2000, Rao et al. 2003). The role of iron in energy metabolism likely explains most of the vulnerability to ID, however, iron also is involved in myelination and dopamine neurotransmission which could exacerbate the developmental deficiencies (Fretham, Carlson and Georgieff 2011).

Studies on humans have found that ID during early life can have lasting cognitive effects even after iron repletion (Burden et al. 2007, Riggins et al. 2009, Lukowski et al. 2010).

Individuals with IDA also have been shown to have higher blood lead levels than those with normal iron status (Choi and Kim 2003). Elevated blood levels have been shown to be correlated with decreased cognitive performance (Kordas 2010). Experiments involving rats have shown that rats with ID have increased lead absorption compared to rats with normal iron status (Ragan 1977). Furthermore, iron supplementation of developing rats has been shown to prevent some disturbances in the blood-brain barrier associated with lead exposure (Wang et al. 2007).

Schizophrenia

Epidemiologic studies have indicated that maternal prepartum ID may increase the likelihood of developing schizophrenia later in life (Brown and Susser 2008, Insel et al. 2008, Sorensen et al. 2010). Furthermore, individuals with schizophrenia may have lower than average iron levels (Yanik et al. 2004, Arinola et al. 2010).

Reproductive System

Iron supplementation has been associated with decreased risk of ovulatory infertility (Chavarro et al. 2006). Additionally, those with celiac disease, a condition which may lower iron stores, have been found to be at greater risk for infertility (Sher and Mayberry 1994).

ID anemia also has been associated with a two-fold increase in the risk of preterm delivery (Scholl 2005). Iron supplementation has been shown to increase birth weight (Haider et al. 2013). It has been proposed that ID may increase serum norepinephrine concentrations thereby inducing both fetal and maternal stress

leading to corticotropin-releasing hormone production that may induce preterm labor as well as raise fetal cortisol levels and thus inhibit fetal growth (Allen 2001).

Respiratory System

Breath-Holding Attacks

Breath-holding attacks are phenomena in young children wherein they stop breathing for up to one minute. While the pathophysiology is unclear, it appears that iron supplementation is effective at reducing both the severity and frequency of breath-holding attacks (Zehetner et al. 2010). Furthermore, in one case study of a single individual, the alleviation of breath-holding attacks occurred prior to changes in Hgb and Hct (Tam and Rash 1997).

Urinary System

Urinary Tract Infections

There is some evidence that iron-deficient individuals may be more prone to urinary tract infections (Myers, O'Grady and Dolan 2004). This may be due to the increased infection susceptibility of those with ID (Tansarli et al. 2013).

ASSESSMENT OF IRON STATUS

There currently is not a method for assessing functionally available iron in the body. However, there are many different assays that may be used to indirectly evaluate iron status. These indices vary in cost, availability, confounding factors, and both intra-assay variation and inter-assay variation. Clinically, the complete blood count (CBC) is the most common blood test performed to evaluate iron status. This automated set of tests has multiple factors that can cause false results (See Table 1.2).

Hemoglobin

Hgb is an iron-containing protein found in red blood cells that transports oxygen throughout the body. Hgb has a quaternary structure composed of four porphyrin subunits, however, Hgb composition changes during embryonic, fetal, and adult life (Manning et al. 2007).

Hgb disorders can be inherited or nutritional in origin. Inherited Hgb disorders include structural Hgb variants, of which over 700 have been identified, as well as thalassemias that are characterized by the ineffective production of particular globin

Table 1.2 Tests Included in a Complete Blood Count (CBC)

CBC Indices	Definition	Interfering Factors
Red blood cell (RBC)	Count of a the number of circulating red blood cells in one mL of peripheral venous blood	Pregnancy, living at high altitudes, hydration status, gentamicin, methyldopa, chloramphenicol, hydantoins, and quinidine
Hemoglobin (Hgb)	Measure of the total amount of Hgb in the peripheral blood	Pregnancy, living at high altitudes, drugs such as gentamicin, methyldopa, antibiotics, antineoplastic drugs, aspirin, indomethacin, rifampin, and sulfonamides
Hematocrit (Hct)	Measure of the percentage of the blood volume that is made up of red blood cells	Abnormalities in RBC size, elevated white blood cell counts, hemodilution, hemoconcentration, pregnancy, living at high altitudes, certain drugs such as chloramphenicol and penicillin
Mean corpuscular volume (MCV)	$\frac{\text{hematocrit (\%)} \times 10}{RBC \text{ (million/mL)}}$	Abnormal RBC size, elevated white blood cell counts, the presence of cold agglutinins, large red blood cell precursors
Mean corpuscular hemoglobin (MCH)	$\frac{\text{hemoglobin (g/dL)} \times 10}{RBC \text{ (million/mL)}}$	Abnormal RBC size, elevated white blood cell counts, the presence of cold agglutinins
Mean corpuscular hemoglobin concentration (MCHC)	$\frac{\text{hemoglobin (g/dL)} \times 100}{\text{hematocrit (\%)}}$	Abnormal RBC size, elevated white blood cell counts, the presence of cold agglutinins
Red blood cell distribution width (RDW)	$\frac{\text{standard deviation } (\sigma) \text{ of MCV} \times 100}{\text{mean MCV}}$	Extremely elevated white blood cell counts, large red blood cell precursors, presence of cold agglutinins

(Source: Pagana and Pagana, 2009)

chains (Weatherall and Clegg 2001). Nutritional causes of Hgb disorders include insufficient availability of iron, vitamin B₁₂, and/or folic acid.

While Hgb is primarily involved in oxygen transport to tissues of the body, Hgb may play a role in other functions. Research by Biagioli et al., (2009) indicates

that Hgb may provide a source of stored oxygen in the brain for periods of anoxic conditions and could be related to Parkinson's disease. Hgb may also function as an antioxidant and regulator of iron metabolism in macrophages, alveolar cells, and in the kidney (Liu, Zeng and Stamler 1999, Newton et al. 2006, Nishi et al. 2008). The measurement of Hgb levels in the blood can be influenced by many factors. The specific assay method may result in considerable variation in Hgb levels (Agarwal and Heinz 2001, Gomez-Simon et al. 2007, Patel et al. 2007, Van de Louw et al. 2007). The source of the blood sample may impact measured Hgb. Capillary blood may have a different concentration than venous blood that may, in turn, be different from arterial blood (Mokken et al. 1996, Yang et al. 2001, Gehring et al. 2002). There also may be variation in sample sites for capillary blood (Boulton, Nightingale and Reynolds 1994, Morris et al. 1999). Hgb concentration may vary over time (Looker et al. 1990). Body position during blood draw may impact measured Hgb concentration with changing from a standing position to seated resulting in up to a 5% decrease in measured Hgb levels (Ekelund, Eklund and Kaijser 1971). Other factors such as improper blood draw technique, incorrect handling of the sample, and hydration status can impact Hgb readings (Pallares et al. 1996, Pagana and Pagana 1999).

The many causes of variation in Hgb concentration include genetic, nutritional, and methodological. These variations complicate and limit the utility of using Hgb to determine iron status.

Hematocrit

Hct is the percentage of blood volume composed of red blood cells. Hct can be measured directly via centrifugation or calculated via an automatic analyzer that multiplies the red blood cell count by the mean cell volume. A study by Han, Serrano, and Devine (2010) found that the automated analyzer method may calculate Hct values on average 11% greater than the centrifugation method. Mayer (1965) studied the effect of position during blood draw, exercise, and meals on Hct concentration. He found that blood drawn from people in a sitting position had significantly higher Hct than those lying down. It was also noted that recent physical activity may cause a short term elevation in Hct whereas consumption of a meal may decrease Hct for up to six hours. Repeated fasting during Ramadan was shown to result in lowered Hct (Attarzadeh Hosseini and Hejazi 2013).

Based on 12 studies, Thirup (2003) noted that within-subject biological variation and the variation within one analytical technique may result in up to a 12% change in Hct when measured between 1 day and 1-2 months, in a normal healthy adult population. In addition, there appears to be a seasonal variation in Hct in certain populations. Furthermore Hct may be influenced by hydration status with it being elevated in hemoconcentration resulting from dehydration. As with Hgb, Hct is impacted by impaired red blood cell production due to other non-iron nutritional deficiencies or genetic conditions and therefore may be of limited usefulness as an iron-specific indicator.

MCV

MCV is the average volume of a red blood cell. It can be calculated by multiplying the volume of blood collected by the Hct and dividing that product by the red blood cell count in that volume. Alternatively, it can be measured by automated counters.

MCV is primarily used to classify those with anemia. Individuals with a MCV below the normal range are classified as microcytic, individuals with an MCV in the normal range are normocytic, and those with an MCV exceeding the normal range are macrocytic. Microcytosis, normocytosis, and macrocytosis may all exist with or without anemia.

Microcytosis may be caused by ID, thalassemias, anemia of chronic disease, lead toxicity, and sideroblastic anemia (Van Vranken 2010). Normocytosis is only a concern in those with anemia. Normocytic anemia may be caused by a multitude of factors including blood loss, hypersplenism, sickle cell disease, hemolysis, autoimmune hemolytic anemia, marrow hypoplasia, renal failure, liver disease, endocrine deficiency, anemia of chronic disease, sideroblastic anemia, pregnancy, overhydration, initial stages of any anemia, or a combination of conditions that cause microcytic and macrocytic anemia (Brill and Baumgardner 2000). Macrocytosis may be caused by alcohol use, vitamin B₁₂ deficiency, folate deficiency, medications, liver disease, hematologic disease, reticulocytosis, and hypothyroidism (Oh et al. 2008). While ID may result in microcytosis, the use of MCV as an indicator of iron status is quite limited due to other possible causes of microcytosis as well as the possibility of being confounded by the causes of macrocytosis.

RDW

RDW is a calculated indicator of the variation in red blood cell volume. RDW is calculated by dividing the standard deviation of the MCV by the MCV and is therefore the coefficient of variation of the MCV. Higher than normal RDW, or anisocytosis, is indicative of greater variation in cell size. It may be caused by ID, hemolytic anemia, folate deficiency and vitamin B12 deficiency, thalassemias, chronic liver disease, and sickle cell anemia (Park and Kim 1987). As with Hgb, Hct, and MCV, an abnormal RDW value may result from iron-deficient erythropoiesis or other causes and therefore is neither specific to ID nor sensitive to those with ID without iron-deficient erythropoiesis. Additional blood tests related to assessment of iron status that are not part of the CBC test are summarized in Table 1.3 and discussed below.

Total Iron-Binding Capacity

Total Iron-Binding Capacity (TIBC) is measurement of the total amount of iron that can be bound in the blood. Transferrin is the primary iron-transporting protein in the blood and the TIBC is generally assumed to be reflective of transferrin levels (Short et al. 1984). However, other serum proteins such as albumin that are not involved in iron transport can also bind with iron resulting in a falsely high TIBC level (Gabbe, Heinrich and Icacigic 1982). Furthermore, the interassay variation may be as high as 35% making it difficult to establish generic reference standards (Kasvosve and Delanghe 2002).

An elevated TIBC is usually indicative of low iron stores and could be a sign of ID. Lower than normal TIBC values could result from cirrhosis, hemolytic anemia, hypoproteinemia, pernicious anemia, and sickle cell anemia (Pagana and Pagana 1999). Research by Peter and Wang (1981) indicates that TIBC may not be as sensitive to early stages of ID as other indicators, as TIBC is only reflective of the iron-transport capacity and not functional iron availability in cells.

Serum Ferritin

Serum ferritin is a measurement of the amount of ferritin in the blood. Ferritin is an iron-binding protein responsible for the controlled storage and release of iron both in the intracellular and extracellular compartments. Ferritin that contains iron can be referred to as either ferritin or holoferritin. Ferritin that is devoid of iron is referred to as apoferritin. Ferritin is composed of 24 subunits of two types, heavy (H) and light (L). The ratio of the two subunits varies widely between tissues and

Table 1.3 Additional Iron Status Tests

Indicator	Definition	Factors causing interpretation issues
Total Iron Binding Capacity (TIBC)	TIBC is a measurement of all proteins available for binding mobile iron	Recent blood transfusions, recent high iron consumption, hemolytic diseases, fluorides, adrenocorticotrophic hormone (ACTH), cholestyramine, chloramphenicol, colchicine, deferoxamine, methicillin, testosterone, dextran, estrogens, ethanol, methyldopa, and oral contraceptives
Serum Ferritin	Measurement of the amount of ferritin in the serum	Recent blood transfusions, recent high iron consumption, inflammation, hemolytic diseases, and ethanol consumption
Transferrin Saturation	$\frac{\text{Serum Iron}}{\text{TIBC}} \times 100\%$	Recent blood transfusions, recent high iron consumption, hemolytic diseases, fluorides, oral contraceptives, ACTH, chloramphenicol
Serum Transferrin Receptor	Measurement of circulating transferrin receptors in the blood.	Megaloblastic anemia, thalassemias, sickle cell anemia, and auto immune hemolytic anemia
Hepcidin	Measurement of the hepcidin level in the blood	Recent high iron consumption, hemochromatosis, inflammation, and variation in reference standards.
Serum Iron	Measurement of the quantity of iron bound to transferrin	Recent blood transfusions, recent high iron consumption, hemolytic diseases, chloramphenicol, dextran, estrogens, ethanol, iron preparations, methyldopa, oral contraceptives, ACTH, cholestyramine, colchicine, deferoxamine, methicillin, and testosterone
Stainable Bone Marrow	Percent of marrow normoblasts that contain stainable iron granules	Pernicious anemia, collagen diseases, chronic infection, chronic pancreatic insufficiency, uremia, and iron dextran

Sources: (Mast et al. 1998, Pagana and Pagana 1999, Ganz et al. 2008, Fischbach and Dunning III 2009, Pasricha et al. 2011)

impacts both iron storage and availability (Harrison and Arosio 1996). The two subunits may also have separate functions with the H subunit involved in cellular iron utilization and the L subunit associated with iron storage (Bomford, Conlon-

Hollingshead and Munro 1981, Arosio et al. 1991). Regulation of expression of the H and L subunits occurs at both transcription and translation (Hentze, Muckenthaler and Andrews 2004, Iwasaki, Hailemariam and Tsuji 2007). Cells may be able to regulate the ratio of H:L subunits through differential rates of secretion and degradation (Goralska, Holley and McGahan 2003, Goralska et al. 2005). Additionally, the subunits themselves may be significantly altered with age which could affect the ability of ferritin to control the storage and release of iron (Goralska, Fleisher and McGahan 2007). The maintenance of a specific H:L ferritin subunit ratio is important for proper cellular homeostasis (Harned et al. 2010).

Ferritin functions primarily as a means to sequester and buffer the intracellular iron pool. To do this ferritin converts ferrous iron (Fe^{2+}) to ferric (Fe^{3+}) which is stored in the mineral core of the ferritin protein and is available for release as necessary (Knovich et al. 2009).

Research has indicated that ferritin may have functions unrelated to iron storage. Work by Brailsford et al., (1985) indicates that apoferritin may function as a stimulator of superoxide production in neutrophils as a means of defense against bacteremia. Ferritin also may have a role in the formation of new blood vessels (Coffman et al. 2009). The ferritin H subunit may be involved in reducing mesothelioma cell susceptibility to apoptosis (Aung et al. 2007). Research on the L subunit indicates a potential role in cellular proliferation unrelated to iron (Cozzi et al. 2004). The source of ferritin in the serum is not clear, however, research by Cohen et al., (2010) indicates it may be secreted by a nonclassical lysosomal pathway.

Serum ferritin may be elevated in iron overload disorders such as hemochromatosis and thalassemia (Fleming and Ponka 2012). Hemochromatosis is a condition in which the body accumulated excessive amounts of iron. Primary hemochromatosis, also referred to as hereditary hemochromatosis, is genetic in origin with certain ethnic groups having higher prevalence levels (Niederau, Strohmeyer and Stremmel 1994).

Factors unrelated to iron status can elevate sFer. sFer is an acute-phase protein and is elevated in periods of inflammation (Herbert et al. 1997). Research by Guyatt et al., (1992) as well as Bermejo and Garcia-Lopez (2009) indicates that individuals with inflammation may have sFer values well within the normal range yet be iron deficient. Inflammation associated with obesity may elevate sFer levels, which could lead to decreased availability of iron (Greenberg and Obin 2006, Yanoff

et al. 2007). Inflammation due to exercise, including strenuous exercise and endurance exercise, has been associated with elevated sFer (Gropper et al. 2006, Peeling et al. 2009).

Other external factors may elevate sFer. Alcohol consumption may elevate sFer, potentially as a means of counteracting the oxidative stress associated with alcohol (Lee and Jacobs 2004, Alatalo et al. 2009). Smoking may cause an increase in sFer (Tamura et al. 1995).

Low sFer is primarily indicative of ID. However, there are other conditions that may falsely depress sFer values, namely hypothyroidism and ascorbic acid deficiency (Roeser et al. 1980, Knovich et al. 2009).

Hypothyroidism has been shown to reduce hepatic ferritin by 36% in rats (Deshpande and Nadkarni 1992). Ferritin may be regulated by thyroid function both during transcription of at least the H subunit and post-transcription (Jump, Mariash and Oppenheimer 1982, Chazenbalk, Wadsworth and Rapoport 1990, Iwasa et al. 1990, Leedman et al. 1996). L subunit expression levels also are impacted by hypothyroidism in rats, however the alteration in expression may be tissue dependent (Levenson and Fitch 2000).

Work by Fineberg, Kasbekar, and Lewus (1959) found that ascorbic acid mediated the uptake of Fe^{3+} by apoferritin. Miller and Perkins (1969) found that ascorbic acid was effective at transferring transferrin-bound iron to ferritin. It has been shown that the incorporation of iron into ferritin is ascorbic acid dependent (Mazur, Green and Carleton 1960, Mazur 1961, Mazur, Carleton and Carlsen 1961). Their work suggested Fe^{3+} , ATP, and ascorbic acid form a complex to allow Fe^{3+} to be reduced prior to uptake by ferritin. Research on ascorbic acid-deficient guinea pigs found that there was reduced uptake of radioactively-labeled iron when administered intravenously when compared to control guinea pigs (Mazur 1961).

Serum ferritin has been shown to vary 14.5% in healthy individuals (Pilon et al. 1981). Substantial interassay variation also has been shown (CDC 2006). However, sFer is generally considered to be the best single indicator of iron status due to its specificity and in spite of its limited sensitivity (Grondin et al. 2008).

Transferrin

Transferrin is an iron-binding glycoprotein responsible for iron transport in the body. It is primarily synthesized in the liver and is an acute phase reactant protein with a negative response, meaning circulating levels in the blood will drop during

inflammation (Ritchie et al. 1999). Transferrin levels are indirectly assessed in the evaluation of serum iron and total iron-binding capacity.

Serum Transferrin Receptor

Transferrin receptors are the main pathway by which cells acquire iron from transferrin (Skikne 2008). The amount of transferrin receptors in the blood has been found to be correlated with body levels (Flowers et al. 1989). However, 80% of the transferrin receptors are found in erythroid marrow (Cook 1999). Therefore changes in serum transferrin receptor levels in the blood are primarily driven by erythropoietic activity (Kohgo et al. 1986, Beguin et al. 1988, Huebers et al. 1990).

Hepcidin

Hepcidin is a peptide mediator of iron absorption and mobilization produced by the liver. High levels of hepcidin reduce iron absorption by the gastrointestinal tract and inhibit the release of iron by the reticuloendothelial system to circulating transferrin (Wish 2006). However, it is also an acute phase reactant with elevated levels occurring during inflammation (Dallalio, Fleury and Means 2003).

Serum Iron

Serum iron is a measurement of the amount of iron bound to transport proteins in the blood. Serum iron has been shown to vary substantially in an individual throughout the day and on a day-to-day basis making it an unreliable indicator of iron status (Statland, Winkel and Bokelund 1976, Statland and Winkel 1977, Pilon et al. 1981, Dale, Burritt and Zinsmeister 2002). Serum iron may be elevated due to the use of birth control medications as some birth control pills contain iron. It also may be elevated due to hemolytic anemia, hepatitis, vitamin B-12 deficiency and vitamin B-6 deficiency. Due to these limitations, the Centers for Disease Control and Prevention (CDC) has determined that, "...serum iron is not, however a good indicator of iron stores and is not a sensitive measure of iron deficiency" (CDC 2008).

Stainable Bone Marrow

Stainable bone marrow is a visual quantification of the non-heme iron content of bone marrow stained by potassium ferrocyanide (Prussian Blue) (Wulfhekel and Dullmann 1990). The presence or absence of stainable iron granules has long been

considered the gold standard in determining iron status (Burns et al. 1990, Intragumtornchai et al. 1998). Many other iron status indicators are evaluated for specificity and sensitivity for their ability to predict stainable bone marrow iron (Kis and Carnes 1998, Means et al. 1999). The method of acquiring the sample, whether via aspiration or needle biopsy, has been shown to cause significant variation in the estimation of iron stores (Fong et al. 1977, Krause, Brubaker and Kaplan 1979). Additionally, it has been noted that there may be interobserver variation of 31%, complicating its utility as a gold standard for iron status (Barron, Hoyer and Tefferi 2001). Furthermore, case studies have indicated that stainable bone marrow may not correlate with functionally-available iron (Thomason and Almiski 2009).

Other Indicators of Iron Status

Serum ferritin iron is a measure of the amount of iron contained within the ferritin in the blood. It is thought that this assay is not confounded by inflammation, however, more research is warranted (Herbert et al. 1997).

Quantification of tissue iron stores may be possible with the use of an MRI. This technique may allow for non-invasive measurement of iron in specific regions of the body (Bartzokis et al. 1993).

Salivary ferritin represents another possible indicator of iron status. It has been found to be elevated in iron-deficient children and may provide a less invasive measurement of iron status (Jagannathan et al. 2012).

PROGRESSION OF IRON DEFICIENCY

Guiang et al., (1997) noted that ID causes both intra- and inter-organ prioritization of iron which can be influenced by the age, species, and rate of induction of ID. An example of intra-organ prioritization can be found in myocardial ID which appears to induce a prioritization of iron distribution to myoglobin at the expense of cytochrome c (Guiang et al. 1998). This finding also was noted in rat muscle tissue (Siimes, Refino and Dallman 1980).

Research indicates that effects of iron depletion may have lasting effects on the concentration of iron in specific tissues well after iron repletion. Work by Felt and Lozoff (1996) found that rats with maternal prenatal ID had altered brain iron concentrations later in life even when that ID was corrected during pregnancy. Research by Piñero et al., (2000) noted that the regions of the rat brain responded differently to iron depletion. Additionally, the repletion of iron stores in iron-deficient rats resulted in altered brain iron distribution compared to controls and iron-deficient

rats. They also noted that the duration of the ID and the stage of development could impact the response to iron repletion. Kwik-Urbe, Golub and Keen (2000) noted that these lasting changes could have functional effects in rats. However, some of the lasting impacts may not be due to lasting changes in iron status, but changes in gene expression (Georgieff 2011).

The interaction of iron with other nutrients also appears to impact the progression of ID; however more work is needed to elucidate the interactions. For example, rats that experienced maternal copper deficiency induced during pregnancy exhibited ID in brain tissues (Prohaska and Gybina 2005). ID in the brain may continue well after copper repletion with functional effects (Penland and Prohaska 2004). However, copper-deficient rats tend to accumulate iron in the liver (Williams, Kennedy and Green 1983). Copper deficiency may also prevent the symptoms of ID from being remedied by iron supplementation or injection (Reeves and DeMars 2006).

Other nutrients may impact the progression of ID, however, the research is limited. Zinc deficiency may lead to iron accumulation in various tissues. Iron levels in the livers of zinc-deficient monkeys were found to be elevated (Keen et al. 1988). Zinc deficiency may lead to iron accumulation in the testes and other tissues in the rat (Rogers, Keen and Hurley 1985, Oteiza et al. 1995, Niles et al. 2008). Manganese toxicity has been shown to elevate iron levels in rat brain tissues (Lai et al. 1999). Magnesium deficiency in rats fed an iron-overloaded diet altered iron accumulation in the liver and spleen (Kimura and Yokoi 1996).

SUMMARY

ID has long been known to be distinct from anemia. The diverse array of functions iron is involved with in maintaining homeostasis explains the variety of symptoms associated with ID. Screening for ID currently involves multiple indices with varying levels of specificity for iron status.

CHAPTER 2 – DO CURRENT MEDICAL PROCEDURES SUFFICIENTLY DIAGNOSE IRON DEFICIENCY?

INTRODUCTION

For over 50 years, iron deficiency anemia (IDA) and iron deficiency (ID) have been frequently and incorrectly used interchangeably (Stafford and Kemp 1964, Yip 1989). In common current medical practice, IDA detection often is based on the values provided in the complete blood count (CBC), one of the most frequently used blood assays (Sultana et al. 2011). However, not all values in the CBC are equally utilized in making a clinical diagnosis. Research by Sandhaus and Meyer (2002) indicates that hemoglobin (Hgb) and hematocrit (Hct) are the two indices most frequently used by physicians to consider the possibility of IDA. Mean corpuscular volume (MCV) and red blood cell distribution width (RDW) also are used to lesser degrees. None of these four CBC indices (Hgb, Hct, MCV, and RDW) solely confirms ID or IDA. These CBC indices can only be used to indicate the possibility of disturbed iron status.

Diagnosis of ID relies on a variety of iron-status indicators. Serum ferritin (sFer), Total iron-binding capacity (TIBC), transferrin saturation, serum transferrin receptor, hepcidin, serum iron, and stainable bone marrow are used in the assessment of iron status. Serum ferritin is regarded as the best single indicator of iron status when not complicated by inflammation (Chua et al. 1999, WHO 2001). Serum ferritin levels of less than 15 ng/mL are generally accepted as indicative of ID (Pfeiffer et al. 2009). However, varying cutoffs have been used to examine the role of ID in various pathologies. Select sFer cutoffs of clinically significant symptoms and medical conditions are presented in Table 2.1. Since these more specific iron status indicators are generally only measured when one or more CBC value indicates the possibility of IDA, this study explores the adequacy of using CBC values to screen for the possibility of ID, and nonanemic iron deficiency (NAID) in particular, in the general population.

THESIS OBJECTIVES

The objectives of this study were to 1) determine whether the use of common CBC blood indices as the initial screening for ID provides adequate results for identification of ID and 2) to identify the severity of ID that might occur in the absence of anemia.

Table 2.1 Select Ferritin Levels of Clinically Significant Symptoms of Iron Deficiency

sFer (ng/mL)*	Symptom	Author	Notes
≤12	Decreased learning capacity and memory	(Bruner et al. 1996)	Placebo-controlled double-blind study indicated response to iron treatment in individuals without anemia
≤15	Depression	(Vahdat Shariatpanaahi et al. 2007)	Case-controlled study in nonanemic individuals found correlation between sFer and depression
<20	Impaired exercise performance	(Rowland et al. 1988)	Placebo-controlled double-blind study indicated response to iron treatment in individuals without anemia
<30	ADHD	(Konofal et al. 2008)	Placebo-controlled double-blind study indicated response to iron treatment
≤45	Restless legs syndrome	(Grote et al. 2009)	Placebo-controlled double-blind study indicated response to iron treatment
≤50	Fatigue	(Verdon et al. 2003)	Placebo-controlled double-blind study indicated response to iron treatment in individuals without anemia
<70	Hair loss	(Rushton 2002)	Responded to iron supplementation

*sFer is as reported in the literature and has not been standardized to account for variation in assay technique

METHODS

Data Collection

Data from eight periodic National Health and Nutrition Examination Surveys (NHANES) were downloaded from the Internet and converted into data files using

SAS 9.2 and JMP 10 Pro application software. The data sets can be found at http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm. Detailed instructions for accessing NHANES II and NHANES III data can be found in Appendices A and B respectively.

These studies included NHANES II (1976-1980), NHANES III (1988-1994), and six contiguous NHANES studies (1999-2010). A national sample was recruited for each of these CDC studies using a multistage, stratified sampling design. These surveys included an interview covering a wide array of topics as well as a health examination. The health examination included a series of questionnaires, a physical examination and collection and analysis of various blood and urine indices.

Participants or their guardians signed informed consent forms for the interview and the health examination. With the exception of the NHANES II study, each study received formal approval from the NCHS Research Ethics Review Board (previously the NHANES Institutional Review Board). Further information is provided on the Centers for Disease Control and Prevention website (<http://www.cdc.gov/nchs/nhanes/irba98.htm> Accessed 20 April 2014).

Detailed descriptions regarding blood collection and processing can be found in the laboratory and procedure manuals provided for each of the NHANES study data sets on the CDC website (http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm Accessed on 20 April 2014).

Identification of Anemia

CBC parameters were determined using the Beckman Coulter MAXM (Beckman Coulter, Brea, CA, USA) device in all studies. Normal Hgb, Hct, MCV, and RDW width were defined using the CDC guideline as shown in Table 2.2 and Table 2.3.

Iron Status Assessment

Three sFer assay methods were used throughout these eight studies. See Table 2.4 for assay details and regression equations used to standardize sFer values for comparison. Calculated sFer values were rounded to the nearest integer. The Bio-Rad assay is a single-incubation two-site immunoradiometric assay. The assay uses highly purified ¹²⁵I-labeled ferritin antibody as a tracer as well as ferritin antibodies immobilized on polyacrylamide beads for the solid phase. Serum is mixed with the

Table 2.2 Anemia Cutoffs for Hemoglobin (Hgb) and Hematocrit (Hct)

Anemia Cutoffs			
	Age (in years)	Males	Females
Hgb (< g/dL)	1	11.0	11.0
	2 - 4	11.1	11.1
	5 - 7	11.5	11.5
	8 - 11	11.9	11.9
	12 - 14	12.5	11.8
	15 - 17	13.3	12.0
	18 and older	13.5	12.0
Anemia Cutoffs			
	Age (in years)	Males	Females
Hct (< %)	1	32.9	32.9
	2 - 4	33.0	33.0
	5 - 7	34.5	34.5
	8 - 11	35.4	35.4
	12 - 14	37.3	35.7
	15 - 17	39.7	35.9
	18 and older	39.9	35.7

<http://www.cdc.gov/mmwr/preview/mmwrhtml/00051880.htm> Accessed 4/20/2014

Table 2.3 Anemia Cutoffs for Mean Corpuscular Volume (MCV) and Red Cell Distribution Width (RDW)

Anemia Cutoffs		
Age (in years)	MCV (<fL)	RDW (>%)
1 - 2	77	14
3 - 5	79	14
6 - 11	80	14
12 - 15	82	14
16 and older	85	14

<http://www.cdc.gov/mmwr/preview/mmwrhtml/00051880.htm> Accessed 4/20/2014

Table 2.4 Ferritin Standardization Using CDC Supplied Regression Equations

NHANES STUDIES	Ferritin BioAssays	Regression Equations
NHANES II, NHANES III, NHANES 1999-2000, 2001-2002	Bio-Rad Laboratories "QuantImmune Ferritin IRMA" kit	sFer≤25 : 1.2534 x sFer + 1.4683; 25<sFer≤65: 1.2001 x sFer + 1.4693; sFer >65: 1.0791 x sFer + 4.8183
NHANES 2003-2004* through NHANES 2007-2008	Roche/Hitachi assay with the Roche/Hitachi 912*	Ferritin values standardized to this assay
NHANES 2009-2010	Roche Elecsys-170 clinical analyzer	$10^{\frac{\text{Log}_{10}(\text{sFer}) - 0.049}{.989}}$

*sFer analysis during the NHANES 2003-2004 used the Bio-Rad assay for the first half of the study and the Roche/Hitachi assay for the second half. All NHANES sFer data for this 2003-2004 were adjusted to the Roche/Hitachi assay by the CDC.

tracer and solid-phase antibody mixture and incubated during which time the ferritin binds with one of each type of antibody creating a "sandwich."

The Roche/Hitachi assay is an immunoturbimetric technique using latex-bound ferritin antibodies. The serum sample is combined with the latex-bound ferritin antibodies and turbidimetrically measured at 700 nm following agglutination.

A crossover study was performed by the CDC to compare the results of the Bio-Rad assay with the Roche/Hitachi assay. Because a poor correlation was noted at low sFer levels (<15 ng/mL), the CDC developed three equations based on a piecewise linear regression model using percentile values to standardize sFer values in 2003 and 2004. These equations were used in this analysis to adjust the Bio-Rad derived sFer values prior to 2003 to comparable Roche/Hitachi values (See Table 2.4).

The Roche Elecsys-170 is a chemiluminescent assay technique using labeled antibodies to bind with sFer and then, upon the induction of a magnetic field, to be attracted to an electrode. Upon application of voltage to the electrode, chemiluminescent emission is induced and measured by a photomultiplier. The CDC performed a crossover study with NHANES 2007-2008 data and provided a regression equation to allow comparison of data with previous studies (See Table 2.4).

Table 2.5 Participants Meeting Inclusion Criteria from NHANES Studies (1976-2010)

(n)	Inclusion Criteria
121,440	Total Possible Participants
59,618	Also with sFer data available
59,008	Also with Hgb and Hct data available
57,271	Also not known to be pregnant at the time of the study
56,714	Also not known to have given birth the year prior to the study
32,241	Females
24,473	Males

Both genders and ages from toddlers to elderly were included in our data analysis. The minimum data required was Hgb, Hct and sFer. See Table 2.5 for NHANES participants meeting inclusion criteria. We excluded pregnant individuals and those known to have given birth within the 12 months prior to the sample collection. Also excluded were individuals whose Hgb or Hct were calculated rather than measured.

Individuals were placed into age groups based on the recommendations from the CDC (Johnson, Paulose-Ram and Ogden 2013). The specific variable names from studies used in this analysis can be found in Appendix C.

Analyses were conducted using SAS (SAS Institute, Cary, NC, USA), JMP 10 Pro (SAS Institute, Cary, NC, USA), and Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA). Sample weighting was not included in our analyses. The 95% confidence intervals (CI) were calculated using the normal approximation.

For this analysis, ID was defined only based on adjusted sFer levels. In individuals ages one to five years, a sFer value of <18ng/mL was used. In individuals older than 5 years, ID is defined as sFer <21 ng/mL. These values were chosen as they are equivalent to sFer of <12 ng/mL and <15ng/mL respectively when using the Bio-Rad assay technique.

RESULTS

Of the 121,440 participants in the NHANES studies between 1976 and 2010 involved in this analysis, 46.7% met inclusion criteria. The gender distribution of those included in this study was 56.8% female and 43.2% male.

The majority of females with sFer <21ng/mL had normal Hgb and Hct values. As can be seen in Figure 2.1a, females, ages 6-11 years were missed at the highest rate with 89.2% (95% CI, 85.6 to 92.8) of iron-deficient individuals having normal Hgb and Hct. Hemoglobin and Hct were more effective at screening for ID in females, ages 40-59 years, however the combined usage of both indicators still missed 59.4% (95% CI, 56.5 to 62.3). Males, as shown in Figure 2.1b, had a similar level of percent missed when using Hgb and Hct to screen for ID. Those over 59 years old were missed least often at 38.9% (95% CI, 30.9 to 46.9) of the time. Males ages six through eleven years old were the male age group most frequently missed with 90.6% (95% CI, 86.9 to 94.3) of individuals having normal Hgb and Hct values. The incorporation of MCV and RDW improved detection of ID in all age groups, but still missed a substantial proportion of individuals with ID.

CBC indices also were evaluated for their ability to predict varying levels of clinically significant ID (see Table 2.1). As can be seen in Figure 2.2, Hgb and Hct fail to detect the majority of females ages 20-39 with ID even when the cutoff for ID is less than 20 ng/mL.

Serum ferritin levels above 20 ng/mL have been associated with symptomatic iron deficiency (see table 2.1) The combined use of Hgb, Hct, MCV, and RDW still fail to detect the majority of females ages 20-39 with ID with a cutoff of sFer above 30 ng/mL. Additional graphs can be found in Appendix B for all gender and age groups.

Figure 2.3 shows the percent of individuals with ID undiagnosed by commonly used CBC indices in relation to the sFer cutoff used to define ID. The varying clinical sFer cutoffs used in Table 2.1 would have varying percentages of individuals undiagnosed.

Table 2.6 shows the failure of Hgb and Hct to detect ID at varying levels. With the exception of males over 59 years old, solely using Hgb and Hct will under diagnose more than 75% of individuals in any range of sFer over 21 ng/mL.

DISCUSSION

The results from this study clearly show that Hgb, Hct, MCV, and RDW do not reliably predict ID even at the most conservative sFer cutoff with over 50% being missed in most age groups. Regardless of the sFer level used to define ID, the majority of individuals with ID will remain undiagnosed when screened using the commonly used CBC indicators. Considering the high prevalence of ID, as well as the

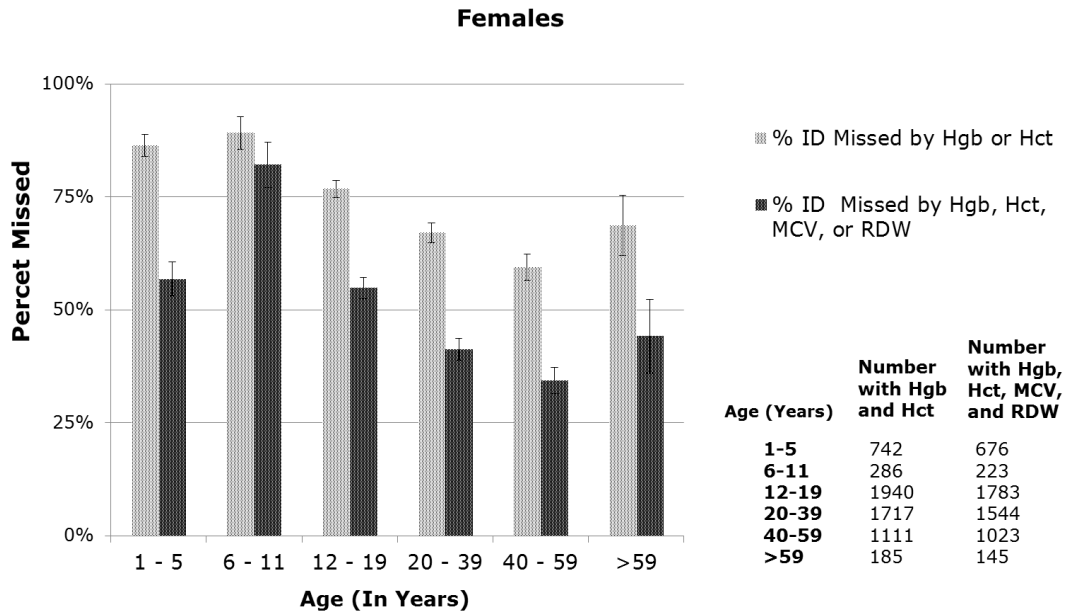


Figure 2.1a Failure of CBC Indices (Hgb, Hct, MCV, and RDW) to Detect Iron Deficiency in Females (NHANES 1976-2010).

Iron deficiency was defined as having sFer <18 ng/mL for individuals aged 1-5 and <21 ng/ml for individuals ages >5 years.

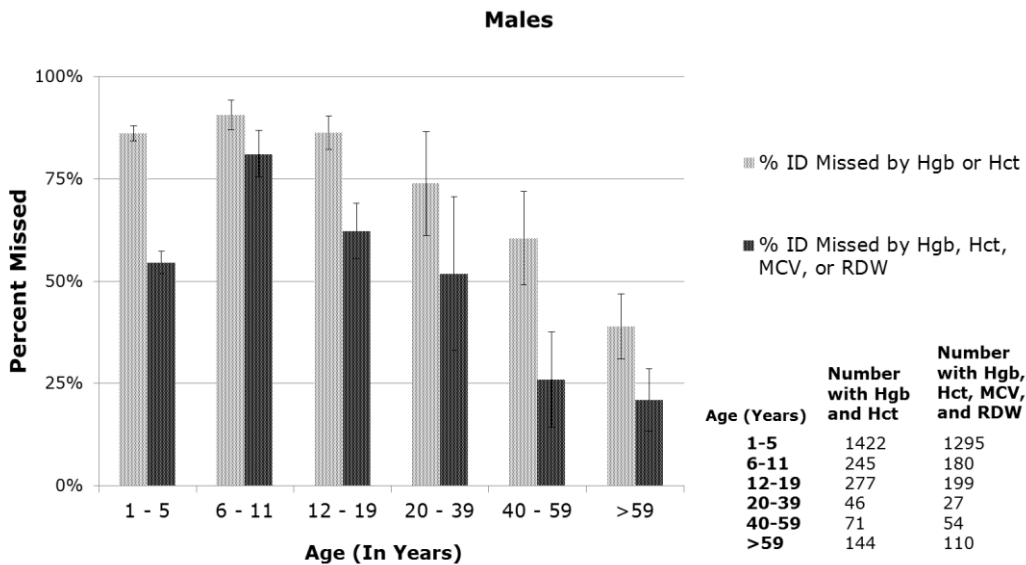


Figure 2.1b Failure of CBC Indices (Hgb, Hct, MCV, and RDW) to Detect Iron Deficiency in Males (NHANES 1976-2010).

Iron deficiency was defined as having sFer <18 ng/mL for individuals aged 1-5 and <21 ng/ml for individuals ages >5 years.

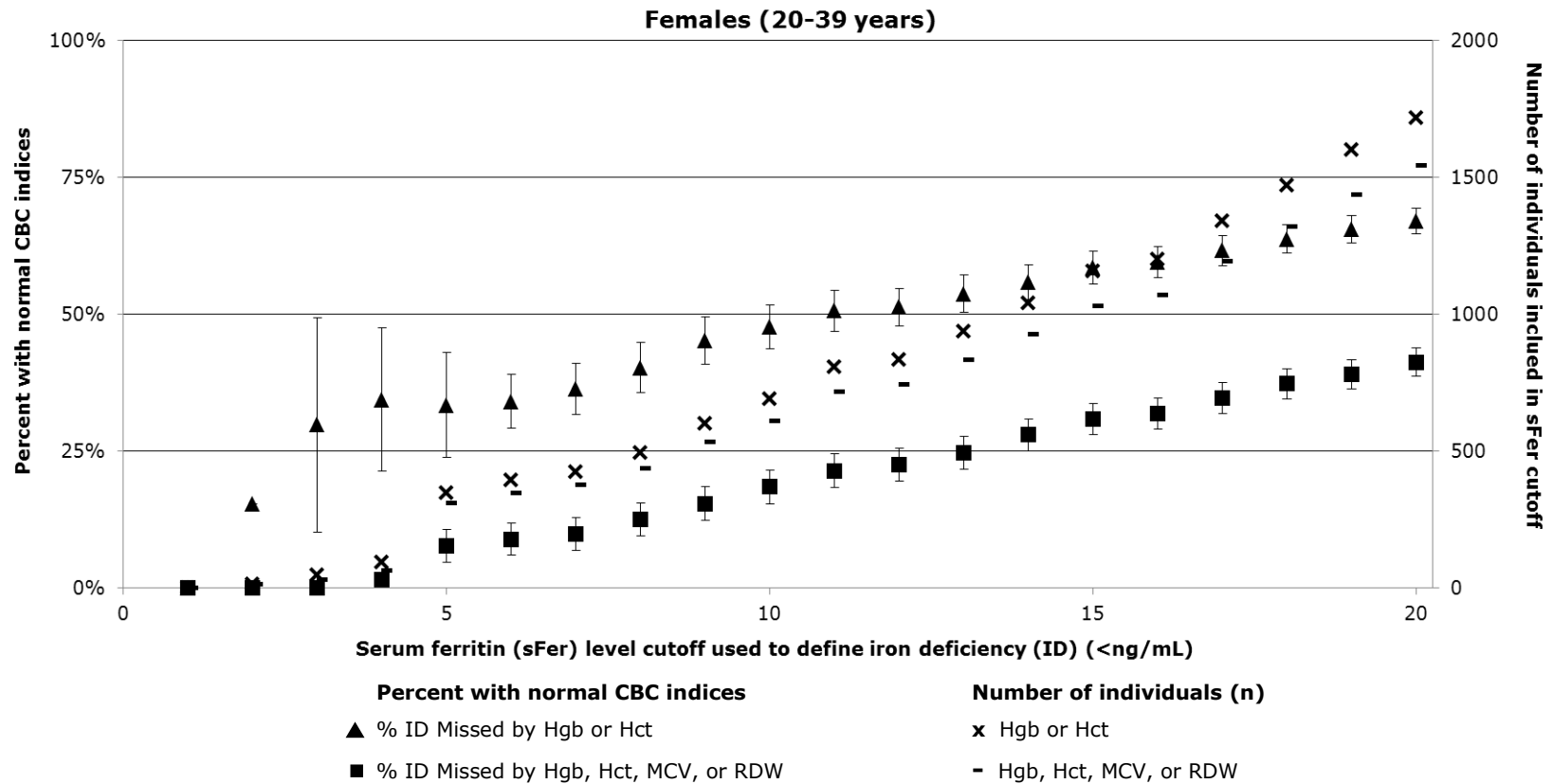


Figure 2.2 Failure of CBC Indices (Hgb, Hct, MCV, and RDW) to Detect Varying Levels of Iron Deficiency (sFer) in Females Ages 20-39.

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

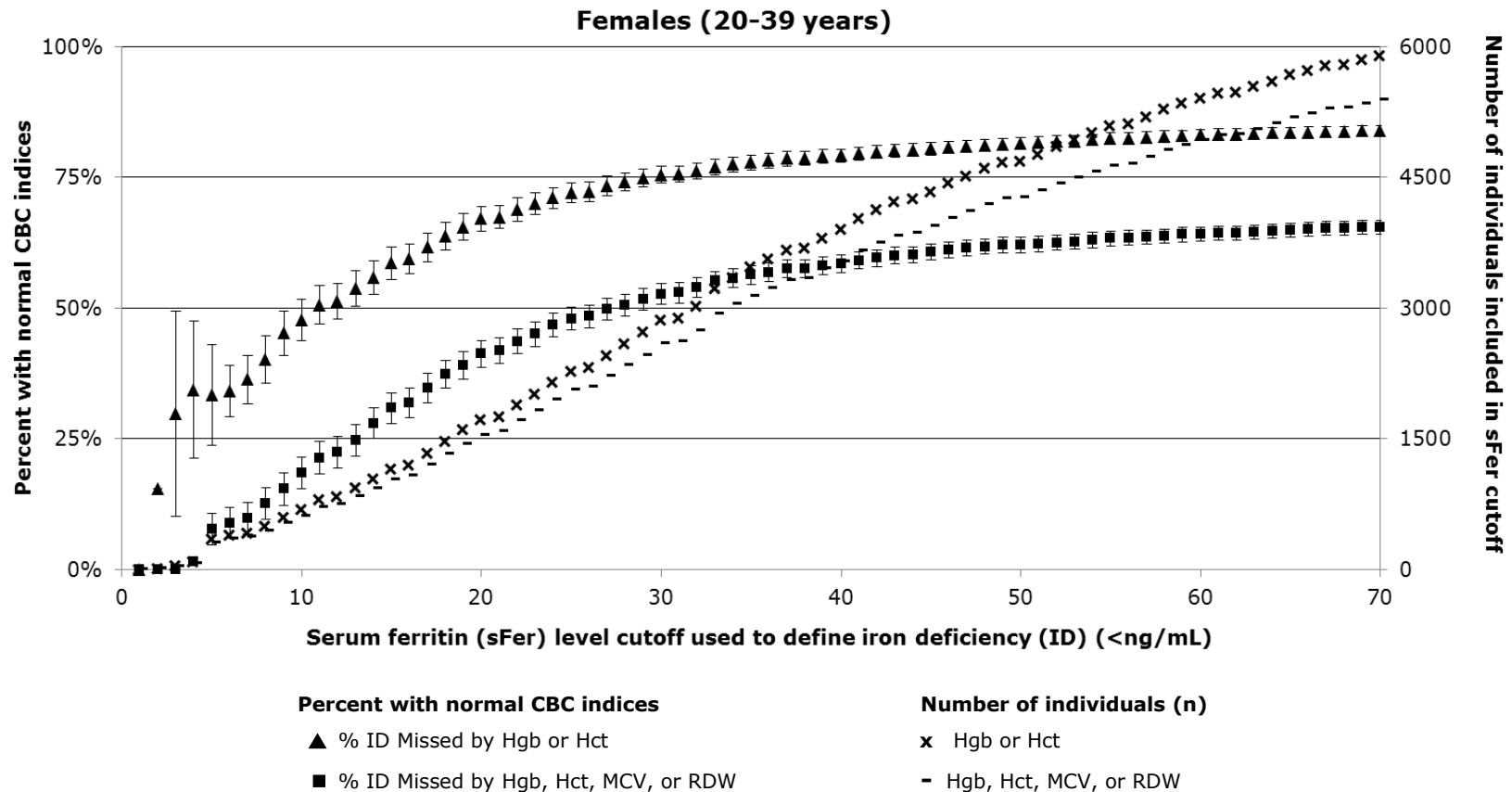


Figure 2.3 Cumulative Percentage of Females (Ages 20-39) with normal CBC indices in relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

Table 2.6 Failure of Hemoglobin or Hematocrit to Detect Iron Deficiency at Various Ranges of sFer (NHANES 1976-2010)

Females			Males		
Serum Ferritin ng/mL	Fail to Identify No. /Total	Failure Rate % (95% CI)	Serum Ferritin ng/mL	Fail to Identify No. /Total	Failure Rate % (95% CI)
Ages 1-5					
1-5	78/110	70.9% +/- 8.5%	1-5	99/155	63.9% +/- 7.6%
6-10	146/165	88.5% +/- 4.9%	6-10	192/229	83.8% +/- 4.8%
11-15	301/322	93.5% +/- 2.7%	11-15	349/396	88.1% +/- 3.2%
16-20	457/484	94.4% +/- 2%	16-20	585/642	91.1% +/- 2.2%
21-35	1553/1650	94.1% +/- 1.1%	21-35	1632/1771	92.2% +/- 1.3%
36-50	868/907	95.7% +/- 1.3%	36-50	738/821	89.9% +/- 2.1%
51-70	443/481	92.1% +/- 2.4%	51-70	372/418	89% +/- 3%
Ages 6-11					
1-5	8/10	80% +/- 24.8%	1-5	7/8	87.5% +/- 22.9%
6-10	25/26	96.2% +/- 7.4%	6-10	15/18	83.3% +/- 17.2%
11-15	65/74	87.8% +/- 7.4%	11-15	61/73	83.6% +/- 8.5%
16-20	157/176	89.2% +/- 4.6%	16-20	139/146	95.2% +/- 3.5%
21-35	743/830	89.5% +/- 2.1%	21-35	839/920	91.2% +/- 1.8%
36-50	562/607	92.6% +/- 2.1%	36-50	600/657	91.3% +/- 2.2%
51-70	381/431	88.4% +/- 3%	51-70	424/464	91.4% +/- 2.6%
Ages 12-19					
1-5	123/260	47.3% +/- 6.1%	1-5	11/15	73.3% +/- 22.4%
6-10	312/434	71.9% +/- 4.2%	6-10	21/28	75% +/- 16%
11-15	427/526	81.2% +/- 3.3%	11-15	78/93	83.9% +/- 7.5%
16-20	628/720	87.2% +/- 2.4%	16-20	129/141	91.5% +/- 4.6%
21-35	1837/2026	90.7% +/- 1.3%	21-35	742/812	91.4% +/- 1.9%
36-50	1162/1271	91.4% +/- 1.5%	36-50	659/721	91.4% +/- 2%
51-70	777/842	92.3% +/- 1.8%	51-70	684/747	91.6% +/- 2%

Table 2.6 (Continued) Failure of Hemoglobin or Hematocrit to Detect Iron Deficiency at Various Ranges of sFer (NHANES 1976-2010)

Females			Males		
Serum Ferritin ng/mL	Fail to Identify No. /Total	Failure Rate % (95% CI)	Serum Ferritin ng/mL	Fail to Identify No. /Total	Failure Rate % (95% CI)
Ages 20-39					
1-5	116/347	33.4% +/- 5%	1-5	1/3	33.3% +/- 53.3%
6-10	213/342	62.3% +/- 5.1%	6-10	4/10	40% +/- 30.4%
11-15	349/469	74.4% +/- 3.9%	11-15	14/17	82.4% +/- 18.1%
16-20	371/443	83.7% +/- 3.4%	16-20	15/16	93.8% +/- 11.9%
21-35	1652/1868	88.4% +/- 1.5%	21-35	98/104	94.2% +/- 4.5%
36-50	1115/1220	91.4% +/- 1.6%	36-50	155/165	93.9% +/- 3.6%
51-70	1125/1202	93.6% +/- 1.4%	51-70	339/362	93.6% +/- 2.5%
Ages 40-59					
1-5	54/239	22.6% +/- 5.3%	1-5	4/10	40% +/- 30.4%
6-10	143/283	50.5% +/- 5.8%	6-10	5/14	35.7% +/- 25.1%
11-15	222/301	73.8% +/- 5%	11-15	14/20	70% +/- 20.1%
16-20	241/288	83.7% +/- 4.3%	16-20	20/27	74.1% +/- 16.5%
21-35	753/849	88.7% +/- 2.1%	21-35	76/98	77.6% +/- 8.3%
36-50	600/659	91% +/- 2.2%	36-50	95/106	89.6% +/- 5.8%
51-70	620/676	91.7% +/- 2.1%	51-70	157/176	89.2% +/- 4.6%
Ages >59					
1-5	7/18	38.9% +/- 22.5%	1-5	1/11	9.1% +/- 17%
6-10	9/23	39.1% +/- 19.9%	6-10	3/24	12.5% +/- 13.2%
11-15	45/66	68.2% +/- 11.2%	11-15	28/55	50.9% +/- 13.2%
16-20	66/78	84.6% +/- 8%	16-20	24/54	44.4% +/- 13.3%
21-35	260/313	83.1% +/- 4.2%	21-35	126/209	60.3% +/- 6.6%
36-50	351/404	86.9% +/- 3.3%	36-50	153/209	73.2% +/- 6%
51-70	516/581	88.8% +/- 2.6%	51-70	246/319	77.1% +/- 4.6%








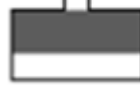
	Normal	Negative iron balance	Iron deficient erythropoiesis	Iron-deficiency anemia
Iron Stores				
Erythron Iron				
Marrow Iron Stores	1-3+	0-1+	0	0
Serum Ferritin (ng/mL)	50-200	<20	<15	<15
TIBC (µg/dL)	300-360	>360	>380	>400
Serum Iron (µg/dL)	50-150	Normal	<50	<50
Transferrin Saturation (%)	30-50	Normal	<20	<10
RBC Morphology	Normal	Normal	Normal	Hypochromic Microcytic

Figure 2.4 Stages of Iron Deficiency. (Adapted from Fauci et al. 2008)

many symptoms associated with it (see Chapter 1), it is paramount that standard medical practice and other screening methods adequately detect individuals with ID. The standard progression of ID, as shown in Figure 2.4 indicates that IDA occurs only after iron stores have been depleted and sFer is less than 15 ng/mL.

Serum iron and transferrin saturation do not differ from a normal state until ID impacts erythropoiesis. Serum ferritin and TIBC may indicate a negative iron balance prior to iron-deficient erythropoiesis. The results of this analysis are largely in agreement with the classic view of the progression of ID. It is important to note that there has been extensive research on the symptoms and treatment of those symptoms in NAID, some of which are discussed in Table 2.1. Individuals with NAID would, by definition, be included in those undiagnosed by commonly used CBC indices. However, these data show that even individuals with a sFer of less than 15 ng/mL may not have iron-deficient erythropoiesis. This may be explained by available iron allocation and/or hemoconcentration.

The prioritization of available iron allocation has been noted by Guiang et al. (1997), with red blood cell production being spared at the expense of other tissues. This nutrient triage may be influenced by species, age, gender and degree of iron deficit. Anemia would not be induced until the body was unable to meet the iron demands of erythropoiesis, which could result in prolonged ID in other tissues in the case of only a slight, but chronic negative iron balance.

Hemoconcentration, the increase in cellular concentration in the blood, may result from acute dehydration or a chronic reduction in plasma. Acutely dehydrated individuals with ID could have normal levels of Hct and Hgb resulting in a misdiagnosis if those parameters were used to screen for ID. Another possible cause of hemoconcentration is a decrease in blood volume (McGee, Abernethy and Simel 1999). Protein deficiency has been shown to decrease blood volume by decreasing the amount of solute in the plasma (Metcoff, Favour and Stare 1945). Their work indicated that both chronic and acute protein deficiency may result in hemoconcentration in rats. Rats with chronic protein deficiency did not have alterations in Hgb concentration while rats with acute protein deficiency had elevated Hgb concentrations. Protein deficiency may also result in the preferential allocation of protein to erythropoiesis over other tissues, illustrating another pathway that favors sustained erythropoiesis over deficiency in other tissues (Bethard et al. 1958).

Studies on protein-deficient humans are limited, however studies on individuals with anorexia nervosa may provide insight into the effects of protein deficiency on iron status in humans. It has been found that individuals with anorexia nervosa frequently have sufficient hemoconcentration to mask anemia (Caregaro et al. 2005).

Individuals with anorexia nervosa may represent an extreme case of protein deficiency which indicates that even under those conditions, the body is able to maintain normal Hgb and Hct concentrations. The iron status of individuals with anorexia nervosa is dynamic. Many studies incorporate treatment for anorexia nervosa in their design. This results in a shift from catabolism to anabolism, which can have inverse effects on iron balance and may explain the conflicting results reported in anorexia nervosa (Kennedy et al. 2004). It has also been speculated that protein deficiency may also protect against ID by limiting growth and thus iron requirements (Beard, Huebers and Finch 1984).

It is important to note that both iron allocation prioritization and hemoconcentration may mask ID in individuals, regardless of current iron balance.

Maintenance of normal CBC values in those with a negative iron balance is dependent upon the severity of the imbalance and the degree of hemoconcentration that can be induced. Those with depleted iron stores that are being restored through a positive iron balance may still have normal CBC values as the additional iron is preferentially used to build red blood cell mass and correct hemoconcentration.

The results from NHANES data are in support of the decades of research indicating that symptoms of iron deficiency can exist in the absence of anemia (Dallman, Beutler and Finch 1978). These results cross age groups and gender and are in agreement with other work, such as that by White (2005), who found that most toddlers with ID are not anemic.

Limitations

The greatest limitation to this study is that not all iron-deficient individuals were included. In this study, iron deficiency was solely defined by sFer levels. Serum ferritin can be elevated in inflammation, liver disease, strenuous exercise, infection, alcohol consumption and obesity (Tamura et al. 1995, Lee and Jacobs 2004, Greenberg and Obin 2006, Gropper et al. 2006, Yanoff et al. 2007, Alatalo et al. 2009, Peeling et al. 2009). Therefore, individuals with ID but normal or elevated sFer would not be identified as having ID in this study. It would be necessary to use a better estimate of iron status to provide a more accurate idea of the percent of those with ID that are missed by commonly used CBC assays. The incorporation of multiple indicators of iron status would allow for increased discrimination between those with ID and those without.

Additionally, individuals with anemia due to other causes besides ID may have been counted among those being detected for ID. While this is only a minor limitation in this study, it could be a cause for inaccuracy in studies examining the symptoms of iron deficiency that can occur in the absence of anemia.

CONCLUSION

Commonly used CBC indices fail to detect ID adequately. Inadequate detection of ID using CBC measures may result in misdiagnosis or delayed diagnosis leading to unnecessary medical expenses in addition to decreased quality of life for individuals with ID. The high percentage of individuals with ID that are left undiagnosed by the CBC indicators could have a significant impact on the medical system. The disproportionate impact of ID on females raises the issue of gender

equity in terms of the percent of the population missed by current standards of medical practice.

FUTURE STUDIES

This preliminary study estimating the percent of individuals with ID that are undiagnosed by CBC values is based on the NHANES population and may not reflect the population as a whole. Sample weighting would need to be taken into account to provide an estimate of the percent undiagnosed in the general population. However, a better estimate of iron status than just sFer also would be necessary to get a more accurate estimate.

Future work should be focused on three areas, 1) assessment or reassessment of iron status indicators and assay techniques, 2) research into the effects of NAID, and 3) estimation of ID prevalence including both IDA and NAID.

Iron status indicators have traditionally been evaluated by their ability to predict stainable bone marrow, however, that may not be the standard that should be used. Stainable bone marrow iron is primarily and imperfectly reflective of available iron for erythropoiesis, not functionally available iron for all processes (Barron, Hoyer and Tefferi 2001). Iron status indicators used in the detection of ID should therefore focus on the ability to detect an iron-deficient pathology.

Additional work is required on the evaluation of intra- and inter-assay variation for specific iron status indicators. In the case of sFer, additional research may be necessary to evaluate the accuracy of different antibodies to detect sFer of different subunit composition. Published research should also state the assay technique used to enable comparison of results as the various techniques have significant variation. Additionally, it is important to distinguish between IDA and NAID in published literature and in the clinical setting.

The evaluation of iron status indicators for detecting ID will likely need to occur concurrently with the studies on the effects ID in the absence of anemia. Studies on the mechanism by which ID inhibits or promotes various pathologies may provide insight into the utility of specific iron status indicators. Studies looking at the response of various symptoms to iron treatment will need to take into account other confounding factors such as the interaction of different nutrients in deficiency and overload and iron status.

Additionally, more reliable iron status indicators are needed to establish the prevalence of ID in certain populations and allow for resources to be appropriately

applied in detecting and treating ID. Presently, all relatively non-invasive methods to determine ID have numerous confounding issues and may not allow for adequate estimate of prevalence (see Chapter 1).

Another phenomenon that warrants additional work is research into the lasting effects of ID. Research has indicated that, depending on developmental stage, the effects of ID may extend well beyond the correction of iron status. It is likely that changes in iron allocation, accumulation, and efficiency of utilization may be impacted by prior low iron status. Therefore, research not incorporating temporal changes in iron status may arrive at inaccurate conclusions. There is also the possibility that genetics could influence iron allocation and efficiency of utilization beyond the traditional role it plays in iron overload.

Appendix A. NHANES II to JMP Importation Instructions

1. Go to <http://www.cdc.gov/nchs/nhanes/nhanesii.htm>
2. Right click on the data file of interest and using "save link as", save it to "C:\Users\nhanes\Desktop\Brian\NHANES II\Raw Data"
Note: You can either create this directory or use another of your choosing but these instructions assume you have created this directory.
3. On the CDC page, right click on the formatted SAS code link associated with the data file of interest, using "save link as", save it to C:\Users\nhanes\Desktop\Brian\NHANES II\Formatted SAS code
Note: You can either create this directory or use another of your choosing but these instructions assume you have created this directory.
4. Open SAS 9.3
5. Click on "File", and then "Open Program" and then find the program you just saved in the "Formatted SAS code" folder
6. When SAS opens, the DU####.sass tab should be open. You may want to maximize the SAS window as well as the DU####.sas tab. Scroll through the file about 3/5's of the way and you will see two lines that read (the 3/5's is variable, but I would say a decent rule of thumb for most files in NHANES II)

DATA D_5411;

INFILE 'c:\temp\DU5411.txt' LRECL = 2000 MISSOEVER;

Change the 'c:\temp\DU5411.txt' to the location of your DU####.txt file

Ex


' C:\Users\nhanes\Desktop\Brian\NHANES II\Raw Data\DU5411.txt'

Note: Basically you are changing the directory to one of your choosing. NHANES assumes you have saved all the data to the "temp" folder on your C drive. Rather than create that directory, I created another one, so you need to change the reference. The filename itself, in this instance "DU5411.txt" doesn't change, only the location changes.

If you have trouble locating the line in the sas file, it is immediately after a horizontal line in the code, and is followed by a long series of values in a column similar to this one

```
N2LB0006 8
N2LB0010 $1
N2LB0011 4
N2LB0012 4
N2LB0013 $11
N2LB0024 4
```

...

Click the Submit Icon which looks like this:  You can also click on the "Run" menu, and then click on "Submit".

Appendix A. (Continued) NHANES II to JMP Importation Instructions

A couple of pointers:

Make sure that you don't have any lines of code highlighted as it may only run those lines.

Make sure your cursor is placed after the last line of the program, otherwise it may only run the code from the cursor on down.

Example:

```
N2AL0691 = "DILUENT-FIRST READING-NUMBER WITH VA ..."  
N2AL0692 = "DILUENT-SECOND READING-NUMBER WITH V ..."  
N2AL0693 = "HISTAMINE-FIRST READING-NUMBER WITH ..."  
N2AL0694 = "HISTAMINE-SECOND READING-NUMBER WITH ..."  
N2AL0695 = "UNUSED POSITION"
```


;


RUN;

You need to copy all of the code in the DU_####.sas window. This can be done by hitting Ctrl + A, to select all, and then Ctrl + C to copy.

7. Open JMP Pro 10

Click on "File", then the "SAS" menu, then the "New SAS Program". Once in the SAS program window, press Ctrl + V to paste the copied code. Click on the

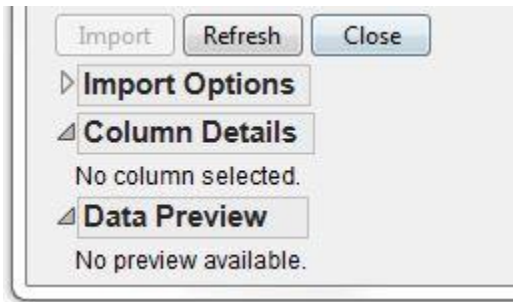
"Submit to SAS" icon which looks like this . In the "Connect to SAS Server" window that pops up, select "Connect to SAS on this machine" and then select "OK".

Click on the "Browse Data" icon which looks like , alternatively, you can select "File", "SAS", and then "Browse Data". Select "No" in the "JMP Alert" window that opens up and asks "... Do you want to configure a connection now?"

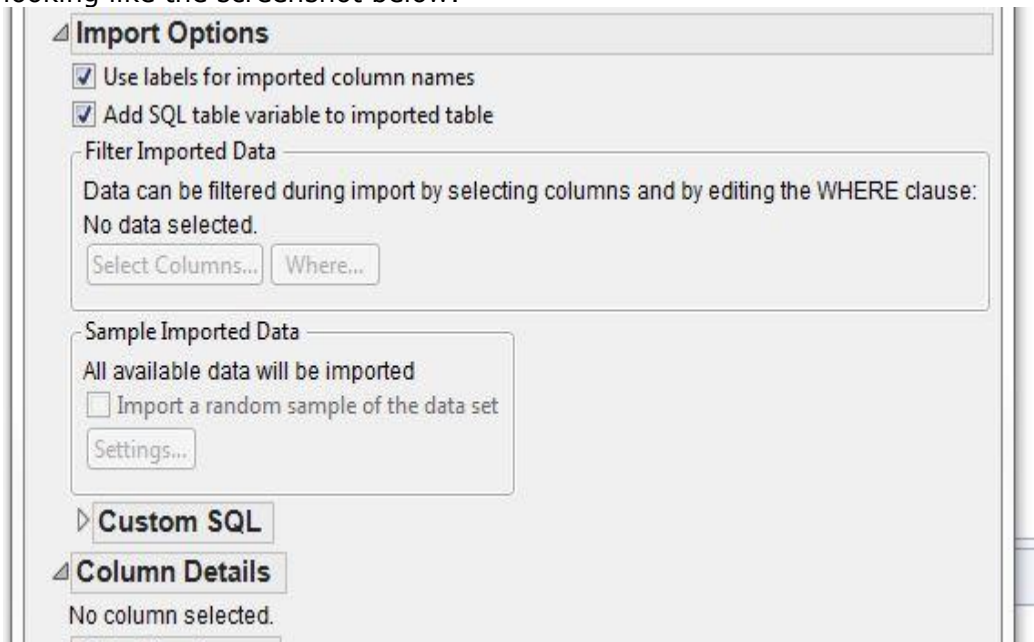
In the "Browse SAS Data" window that is opened, select the "Local" option from the "SAS Server" menu at the top. Under the "Libraries:" box, select "Work". You should then select your SAS program of interest, saved in the D_#### format such as D_5411 in the "Data:" box.

Open up the "Import Options" menu by clicking on the arrow icon to its left. You can see the little triangle to the left of "Import Options" in the screenshot below. You want to click on that triangle to open up the "Import Options" menu.

Appendix A. (Continued) NHANES II to JMP Importation Instructions



Make sure both the "Use labels for imported column names" and the "Add SQL table variable to imported table" boxes are checked. It should end up looking like the screenshot below:



Click the "Import" button. Once the process is completed, it should automatically open a window with all the data with both the columns and variables labeled. Save the file to "C:\Users\nhanes\Desktop\Brian\NHANES II\Raw JMP Files" with the appropriate file name, such as "Hematology and Biochemistry, Ages 6 months to 74 years, 5411.jmp".

Appendix A. (Continued) NHANES II to JMP Importation Instructions

8. Do this for all files of interest. Special notes on specific files below.

"Chest X-Ray Examination 5252" can't be imported using this method as it does not have formatted SAS code as of September 4, 2013.

"Electrocardiogram" can't be imported using this method as it does not have formatted SAS code as of September 4, 2013.

"24-Hour Recall, Specific Food Item, 5704" can't be imported using this method as it does not have formatted SAS code as of September 4, 2013.

"Medical History Questionnaire, Ages 12 -74 years, 5020", names the saved data file as "DU5411.txt". This is an error; the filename should be changed to "DU5020.txt". The formatted SAS code will not run unless the filename reference in the code matches the actual filename of the data.

"Model Gram, 5702" can't be imported using this method as it does not have formatted SAS code as of September 4, 2013.

"Nutrient Composition, 5703" does not contain SEQN numbers and should be handled separately

"Growth Charts (November 2012)" has data from five different NHANES

9. "Hepatitis A and B", "Herpes I and II", and "Syphilis Serological Markers" have data in the .xpt format. This data can be exported into JMP using the following method:

Right click on the data file of interest select "save link as". Save the file to C:\Users\nhanes\Desktop\Brian\NHANES II\Raw Data

Navigate to the file you just saved and right click on it and select "Open with SAS 9.3"

You can then select the "Tools" tab, then "Table Editor".

Once in Table Editor, select the "File" at the top of the screen, then "Open". In the window that opens up, double click on the folder labeled "work". Next, select the "Nh2hepat", "Nh2herpe", or "Nh2syphi" that corresponds to your table of interest.

You then need to save it to the Sasuser library. To do this, click "File" then "Save As". Double click on the "Sasuser" library in the box on the left in the window that opens up. In the field labeled "Member Name", enter the corresponding filename such as "Nh2hepat". Then click "Save".

Appendix A. (Continued) NHANES II to JMP Importation Instructions

Open JMP pro 10. Click on "File" then "SAS", then "Browse Data". In the JMP Alert window that asks if you want to configure the SAS Metadata Server connection, select "No".

In the window that opens up, select the "Local" option from the "SAS Server" dropdown menu. Double-click on Sasuser in the box in the left then click on the file of interest, such as "Nh2hepat". You need to configure the import options as before. In the dropdown menu for the "Import Options" check the "Use labels for imported column name" box and leave the "Add SQL table variable to imported table". Then click "Import".

You then need to save it as before. Save the file to "C:\Users\nhanes\Desktop\Brian\NHANES II\Raw JMP Files" with the appropriate file name, such as "Hepatitis A and B".

10. Open all the JMP files you have created for NHANES II.

Go to the "Allergy and Skin Testing, 5309" window, select "Tables" then "Join"

In the "Join" window, in the "Join Allergy and Skin Testing, 5309" box select "Anthropometry, 5301".

In the "Source Columns" region, select "SEQN" in both the "Allergy and Skin Testing, 5309" and "Anthropometry, 5301" boxes.

In the "Matching Specifications" region, make sure "By Matching Columns" is selected from the dropdown menu. Check both the "include non-matches" in "main table" and "with table boxes, and then click match.

In the options tab make sure the only check boxes are: "Merge Same Name Columns", "Copy Formula", and "Suppress Formula Evaluation".

In the "Output table name:" box, type "NH2 Merge 1"

Open the "NH2 Merge 1" window, and merge it with "Audiometric Air Conduction Test Ages 4-19 Years, 5306" using the preceding steps, except change the Output table name to "NH2 Merge 2".

Continue following the same pattern, incrementally changing the merge number until all files have been merged together.

Appendix B. NHANES III to JMP Importation Instructions

1. Go to <http://www.cdc.gov/nchs/nhanes/nh3data.htm>
2. Right click on the data file of interest and using "save link as", save it to
C:\Users\nhanes\Desktop\Brian\NHANES III\Raw Data
Note: You can either create this directory or use another of your choosing but these instructions assume you have created this directory.
3. On the CDC page, right click on the SAS code link associated with the data file of interest, save to
C:\Users\nhanes\Desktop\Brian\NHANES III\ SAS code
Note: You can either create this directory or use another of your choosing but these instructions assume you have created this directory.
4. Open SAS 9.3
5. Click on "File", and then "Open Program" and then the find the program you just saved in the "SAS code" folder
6. This should open a _____.sas window. The first lines of code should look something like this:
7. `FILENAME ADULT "D:\Questionnaire\DAT\ADULT.DAT" LRECL=3348;`
`*** LRECL includes 2 positions for CRLF, assuming use of PC SAS;`

DATA WORK;

`INFILE ADULT MISSOVER;`

`LENGTH`

`SEQN 7`

`DMPFSEQ 5`

Change the "D:\Questionnaire\DAT\ADULT.DAT" to the location of the corresponding data file

Ex `"C:\Users\nhanes\Desktop\Brian\NHANES III\Raw Data\ ADULT.DAT"`


Appendix B. (Continued) NHANES III to JMP Importation Instructions

Note: Basically you are changing the directory to one of your choosing. NHANES assumes you have a "DAT" folder in a "Questionnaire" folder in your "D" drive. Rather than create that directory, I created another one, so you need to change the reference. The filename itself, in this instance "ADULT.DAT" doesn't change, just its location.

Scroll down to the last line of code after the last semicolon and delete the "→". Next, type "Run;" and hit enter. It should end up looking something like this:

```
HAZNOK5R = "Number of BP's used for average K5";
```

```
Run;
```

Click the Submit Icon which looks like this:  You can also click on the "Run" menu, and then click on "Submit".

A couple of pointers:

Make sure you don't have any lines of code highlighted as it may only run those lines.

Make sure your cursor is after the last line of the program, otherwise it may only run the code from the cursor on down.

Example:


```
HAZMNK1R = "Average K1 BP from household and MEC"  
HAZNOK1R = "Number of BP's used for average K1"  
HAZMNK5R = "Average K5 BP from household and MEC"  
HAZNOK5R = "Number of BP's used for average K5";
```

```
Run;
```

Appendix B. (Continued) NHANES III to JMP Importation Instructions

Open the table editor window by click on the "Tools" menu, then "Table Editor" at the top.

Once in the table editor, click "File" then "Open". In the window that opens up, you want to double click on the "work" library. Either "work" library should work. It will then open up that library and you should find a file labeled "Work". You then want to open that file.

Once your table is opened, you want to save it to a convenient location. Click "File", then "Save As". In the window that opens up, you want to create a new SAS library in a more convenient location. To do this, click on the "create new library" icon that looks like this . In the new window that opens up, click on the "Browse" button along the path line.

Navigate to C:\Users\nhanes\Desktop\Brian\NHANES III
And create a new folder titled "SAS Library", open it, and click ok.

In the name box, type "ADULT", "YOUTH", "LAB", or "EXAM", whichever corresponds to your file. *Note: SAS is picky about the names, keep them short, no spaces etc.* You can then click "Ok".

This should take you back to a window listing the libraries; you want to open the library that corresponds to your library of interest, such as "ADULT". Once there, type "Adult" or whatever the appropriate filename is in the "member name" box and click save.

Appendix B. (Continued) NHANES III to JMP Importation Instructions

8. Open JMP Pro 10

Click "File" then "Open". Navigate to the "SAS Library" folder at this location: C:\Users\nhanes\Desktop\Brian\NHANES III. Select your file of interest; note the filenames are slightly different such as "adult.sas7bdat". Select the "SAS variable labels" option where it asks "Set JMP column names from". Then hit open. Save it to "Raw JMP Files" in the NHANES III folder with the corresponding title.

9. This method will work for the following files:

Household Adult File
Household Youth File
Laboratory File
Examination File
Second Laboratory File
Household Adult Update File
Household Youth Update File
Electrocardiogram
Vitamin and Mineral File
Combination Food File
Individual Food File
Variable Ingredient File
Total Nutrient Intake File
Volatile Toxicant
Healthy Eating Index
Natality
Hepatitis C Virus Genotype
Knee Osteoarthritis X-ray
Leptin Data
Antibody to HPV- note change the filename in the sas code from hv.dat to hpv.dat
Antibody to Measles Data
Diphtheria Antibody Data
Hip Bone Geometry data

Appendix B. (Continued) NHANES III to JMP Importation Instructions

You may have issues with the following files:

Supplement Concentration File- Does not contain SEQN, only has info on supplements

Supplement Product Information File- Does not contain SEQN, only has info on supplements

Food Look Up Tables- Including:

IDCODE

Codebook

Brands and Prepd

-Do not contain SEQN data

Second Exam Files. Including:

Combination Foods File- Second Exam

Total Nutrient Intake-Second Exam

Examination-Second Exam

Individual Food File-Second Exam

Second Lab-Second Exam

Lab-Second Exam

Variable Ingredients File-Second Exam

It looks like these files contain replicates of certain individuals for statistical studies. The replicates would need to be analyzed appropriately

Supplemental Nutrition Survey-Including the following files

Supplemental Nutrition Survey Individual Foods File 1

Supplemental Nutrition Survey Individual Foods File 2

Supplemental Nutrition Survey Total Nutrition Intake

It looks like these files contain data on certain individuals, however there are multiple entries for each individual. The data would need to be handled differently than the rest of the data, so the files were not included.

Multiple Imputation-Including the following files:

Core

IMP1

IMP2

IMP3

IMP4

IMP5

These files are for complex statistical analysis.

The SAS code formatting is substantially different from the other files and I have not worked through the importation process.

Appendix B. (Continued) NHANES III to JMP Importation Instructions

10. Many of the NHANES III files have data in the .xpt format. This data can be exported into JMP using the following method:

Right click on the data file of interest select "save link as". Save the file to C:\Users\nhanes\Desktop\Brian\NHANES III\Raw Data

Navigate to the file you just saved and right click on it and select "Open with SAS 9.3"

You can then select the "Tools" tab, then "Table Editor".

Once in Table Editor, select the "File" at the top of the screen, then "Open". In the window that opens up, double click on the folder labeled "work". Next, select the file that that corresponds to your table of interest such as "depp".

You then need to save it to the Sasuser library. To do this, click "File" then "Save As". Double click on the "Sasuser" library in the box on the left in the window that opens up. In the field labeled "Member Name", enter the corresponding filename such as "depp". Then click "Save".

Open JMP pro 10. Click on "File" then "SAS", then "Browse Data". In the JMP Alert window that asks if you want to configure the SAS Metadata Server connection, select "No".

In the window that opens up, select the "Local" option from the "SAS Server" dropdown menu. Double-click on Sasuser in the box in the left then click on the file of interest, such as "depp". You need to configure the import options as before. In the dropdown menu for the "Import Options" check the "Use labels for imported column name" box and leave the "Add SQL table variable to imported table". Then click "Import".

Appendix B. (Continued) NHANES III to JMP Importation Instructions

You then need to save it as before. Save the file to "C:\Users\nhanes\Desktop\Brian\NHANES III\Raw JMP Files" with the appropriate file name, such as "Hepatitis A and B". Open all the JMP files you have created for NHANES III.

This will work for the following files.

Periodontal Pathogen Antibody
Pertussis
Antibody to Cytomegalovirus IgG and IgM
Vitamin K
Surplus Sera Optical Density to Cytomegalovirus
Surplus Sera Antibody to Human Herpes Virus 8
Surplus Sera Laboratory Component: Insulin Like Growth Factor
Surplus Sera Laboratory Component: Antibody to GAD65
Surplus Sera Laboratory Component: Racial/Ethnic Variation in Sex Steroid
Hor.
Surplus Sera Laboratory Component: Antibody to Toxocara Larva Migrans
Surplus Sera Laboratory Component: Cystatin C
Surplus Sera Laboratory Component: Antibody to MUMPS
Surplus Sera Laboratory Component: Antibody to Hepatitis E Virus IgG
Surplus Sera Laboratory Component: Antibody to Periodontal Antigens
Dietary Interview- Vitamin K and 3-Methylshistidine Total Nutrients Primary
Re.
Dietary Interview- Vitamin K and 3-Methylshistidine Total Nutrients Second
Rec.
Surplus Sera Laboratory Component: Dialkylphosphates
Surplus Sera Laboratory Component Inhibin B, Luteinizing Hormone and
Testost
Hepatic/Gallbladder Ultrasound and Hepatic Steatosis
Hepatic/Gallbladder Ultrasound and Hepatic Steatosis-Second Exam
Surplus Sera Laboratory Component: Racial/Ethnic Variation in Sex Steroid
Hor.
Surplus Sera Laboratory Component: β -trace Protein & β 2 Microglobulin
Surplus Sera Laboratory Component: Cystatin C, β -trace Protein & β 2
Microglo
Surplus Sera Laboratory Component: Monoclonal Gammopathy of Unknown
Sig...
Surplus Sera Laboratory Component: IgG and IgM Antibody to Hepatits E
Virus

Appendix B. (Continued) NHANES III to JMP Importation Instructions

The following files may be difficult to import:

Dietary Interview- Vitamin K and 3-Methylshistidine Individual Foods Primary Recall and Dietary Interview- Vitamin K and 3-Methylshistidine Primary Foods, Second Recall contain replicates of individuals and would need to be handled separately

Surplus Sera Laboratory Component: Partial Hepatitis C Virus RNA sequences isolated from individuals- Three of these files contain replicates and would need to be handled separately; the fourth doesn't appear to contain replicates but would need to be imported along with the other files to be of use.

Growth Charts- contains replicates and would need to be handled separately

Appendix C. Codes and Counts for Variables Used in Analysis

Table C.1 Number with Data Available and Age Data Codes and Counts

		NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Number with Data available		25,286	33,994	62,160
Number with Examination Data Available		20,322	33,994	61,160
Age	Age column name used in analysis	AGE IN YEARS (AT EXAMINATION)	Age at interview (screener) - qty	Age at Screening Adjudicated - Recode
	Age SAS column name	N2AL0190	HSAGEIR	RIDAGEYR
	Number missing age data	4,964	0	0
	Number less than 1 year of age	299	n/a	2,955
	Number with age data	20,322	33,994	62,160

Table C.2 Ferritin Codes and Counts

	NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Ferritin column used in analysis	SERUM FERRITIN NANOGRAM PER MILLILIT ...	Serum ferritin (ng/mL)	Ferritin (ng/mL) Notes: In 1999 and 2001 it was in Nutritional Biochemistries, in 2003 it was in Ferritin and Transferrin Receptor, in 2005 and later it was in Ferritin
Ferritin SAS column name	N2LB0475	FRP	LBXFER in all except 2003 when it was LBDFER
Blank but applicable code	8,888	8888	n/a
Number blank but applicable	676	2,893	n/a
Not Applicable Code	9,999	n/a	n/a
Number Not Applicable	14,189	n/a	n/a
Null value	.		
Number Null	1	4,708	34,092
Number Also with Ferritin Data	5,157	26,393	28,068

Table C.3 Hemoglobin and Hematocrit Codes and Counts

	NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Hemoglobin column name used in analysis	HEMOGLOBIN (GRAMS PER DECILITER) (G/ ...	Hemoglobin (g/dL)	Hemoglobin (g/dL)
Hemoglobin SAS column name	N2LB0361	HGP	LBXHGB
Hemoglobin Blank but applicable code	n/a	88888	n/a
Number blank but applicable	n/a	458	n/a
Number with hemoglobin data available	5157	25,935	28,030
Hemoglobin measured column name	HEMOGLOBIN IMPUTATION CODE	n/a	n/a
Hemoglobin measured SAS column name	N2LB0364	n/a	n/a
Hemoglobin measurement label	Not Imputed	n/a	n/a
Hemoglobin measurement SAS code	1	n/a	n/a
Number with hemoglobin measured	5,050	25,935	n/a
Hemoglobin calculation label	Imputed	n/a	n/a
Hemoglobin calculated SAS code	2	n/a	n/a
Number with hemoglobin calculated	107	n/a	n/a

Hgb & Hct

Table C.3 (Continued) Hemoglobin and Hematocrit Codes and Counts

Hgb &Hct (Continued)	Hematocrit column name used in analysis	HEMATOCRIT (PERCENT)	Hematocrit (%)	Hematocrit (%)
	Hematocrit SAS column name	N2LB0357	HTP	LBXHCT
	Hematocrit blank but applicable code	n/a	88888	n/a
	Number blank but applicable	n/a	460	n/a
	Number with hematocrit data available	5,157	25,933	28,030
	Hematocrit measured column name	HEMATOCRIT IMPUTATION CODE	n/a	n/a
	Hematocrit measured SAS column name	N2LB0360	n/a	n/a
	Hematocrit measurement label	Not Imputed	n/a	n/a
	Hematocrit measurement SAS code	1	n/a	n/a
	Number with hematocrit measured	5,152	25,933	n/a
	Hematocrit calculation label	Imputed	n/a	n/a
	Hematocrit calculated SAS code	2	n/a	n/a
	Number with hematocrit calculated	5	n/a	n/a
	Number Also with H&H Measured	5045	25,933	28,030

Table C.4 Pregnancy Status Codes and Counts

		NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Pregnancy Status	Currently Pregnant Column Name	ARE YOU PREGNANT NOW?	Pregnancy status recode	NHANES 1999-2010 used a variety of different pregnancy markers. I only included those that are not known to be pregnant by any of the markers to the next page.
	Currently Pregnant SAS column name	N2SH0785	MAPF12R	
	Currently Pregnant label	yes	1	
	Currently Pregnant SAS code	1	1	
	Number Currently Pregnant	76	306	
	Currently Not Pregnant label	No	2	
	Currently Not Pregnant SAS Code	2	2	
	Number Not Pregnant	1,409	5,552	
	Blank but applicable code	8	8	
	Number blank but applicable	4	22	
	Don't know code	9	9	
	Number don't know	11	9	
	Number with a null value	3,545	20,044	
	Labels for those not known to be pregnant	Null, No, Blank But Applicable, Don't Know	Null, 2, 8, 9	
	Number Also Not known to be pregnant	4,969	25,627	

Table C.4 (Continued) Pregnancy Status Codes and Counts

Currently Pregnant Column Name	Pregnancy Status at Exam - Recode	Pregnancy Status - Recode (old version)	Think that you are pregnant now?	Are you currently pregnant?	Pregnancy test result	Are you pregnant now
Currently Pregnant SAS column name	RIDEXPRG	RIDPREG	RHQ140	SEQ060	URXPREG	RHD143
Currently Pregnant label	Pregnant	1	1	1	1	1
Currently Pregnant SAS code	1	1	1	1	1	1
Number Currently Pregnant	1302	613	506	267	1291	
Currently Not Pregnant label	Not pregnant	2	2	2	2	2
Currently Not Pregnant SAS Code	2	2	2	2	2	2
Number Not Pregnant	9953	3937	1131	2156	10364	
Blank but applicable code	n/a	n/a	n/a	n/a	3	
Number blank but applicable	n/a	n/a	n/a	n/a	80	
Don't know code	9	9	9	9	4	
Number don't know	542	31	20	15	1	
Number with a null value	16233	23499	26373	25592	16293	
Labels for those not known to be pregnant	Null, 2, 9	Null, 2, 9	Null, 2, 9	Null, 2, 9	Null, 2, 3, 4	
Counts of those not pregnant	26728	27467	27524	27748	26738	
Number Also Not known to be pregnant		Number not known by this measure and those to the left - 26,702	Number not known by this measure and those to the left - 26,681	Number not known by this measure and those to the left - 26,679	Number not known by this measure and those to the left - 26,678	Number not known by this measure and those to the left - 26,675

Table C.5 Recently Pregnant Codes and Counts

	NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Not known to have given birth in the last 12 months Column Name	HOW MANY MONTHS AGO DID THAT PREGNAN	Months since last pregnancy ended	How many months ago have baby?
Not known to have given birth in the last 12 months SAS column name	N2SH0789	MYPC20	RHQ197
0-3 Months ago SAS code	4	n/a	I also removed 295 individuals where current age was equal to Age at last live birth (SAS CODE RHQ190)
Number 0-3 months ago	25	n/a	
4-6 months ago SAS code	3	n/a	
Number 4-6 months ago	23	n/a	
7-9 months ago SAS code	2	n/a	
Number 7-9 months ago	13	n/a	
10-12 months ago SAS code	1	n/a	
Number 10-12 months ago	17	n/a	
Blank but applicable SAS code	8	n/a	
Number blank but applicable	2	n/a	
Less than 4 months ago SAS code	n/a	1	
Number less than 4 months ago	n/a	13	
4 to less than 7 months ago SAS code	n/a	2	
Number 4 to less than 7 months ago	n/a	11	
7 to less than 10 months ago SAS code	n/a	3	
Number 7 to less than 10 months ago	n/a	6	
10-12 months ago SAS code	n/a	4	
Number 10-12 months ago	n/a	3	
13-24 months ago SAS code	n/a	5	
Number 13-24 months ago	n/a	5	
Number Null	4,889	25,589	26,434
Labels for those not known to have given birth in the last 12 months	Null	Null, 5	Null, >12
Number 12 months and less			149
Also not known to have given birth in the last 12 months	4,889	25,594	26,231

Recently Pregnant

Table C.6 Gender Codes and Counts

		NHANES II	NHANES III	NHANES 1999-2000 Through NHANES 2009-2010
Gender	Gender column label	SEX (QUES.4)	Sex	Gender
	Gender SAS column label	N2AL0055	HSSEX	RIAGENDR
	Female SAS code	2	2	2
	Number that are female	2,472	13,192	16,577
	Male SAS code	1	1	1
	Number that are male	2,417	12,402	9,654

Appendix D. Cumulative percentage of individuals with normal CBC indices in relation to sFer in NHANES (1976-2010)

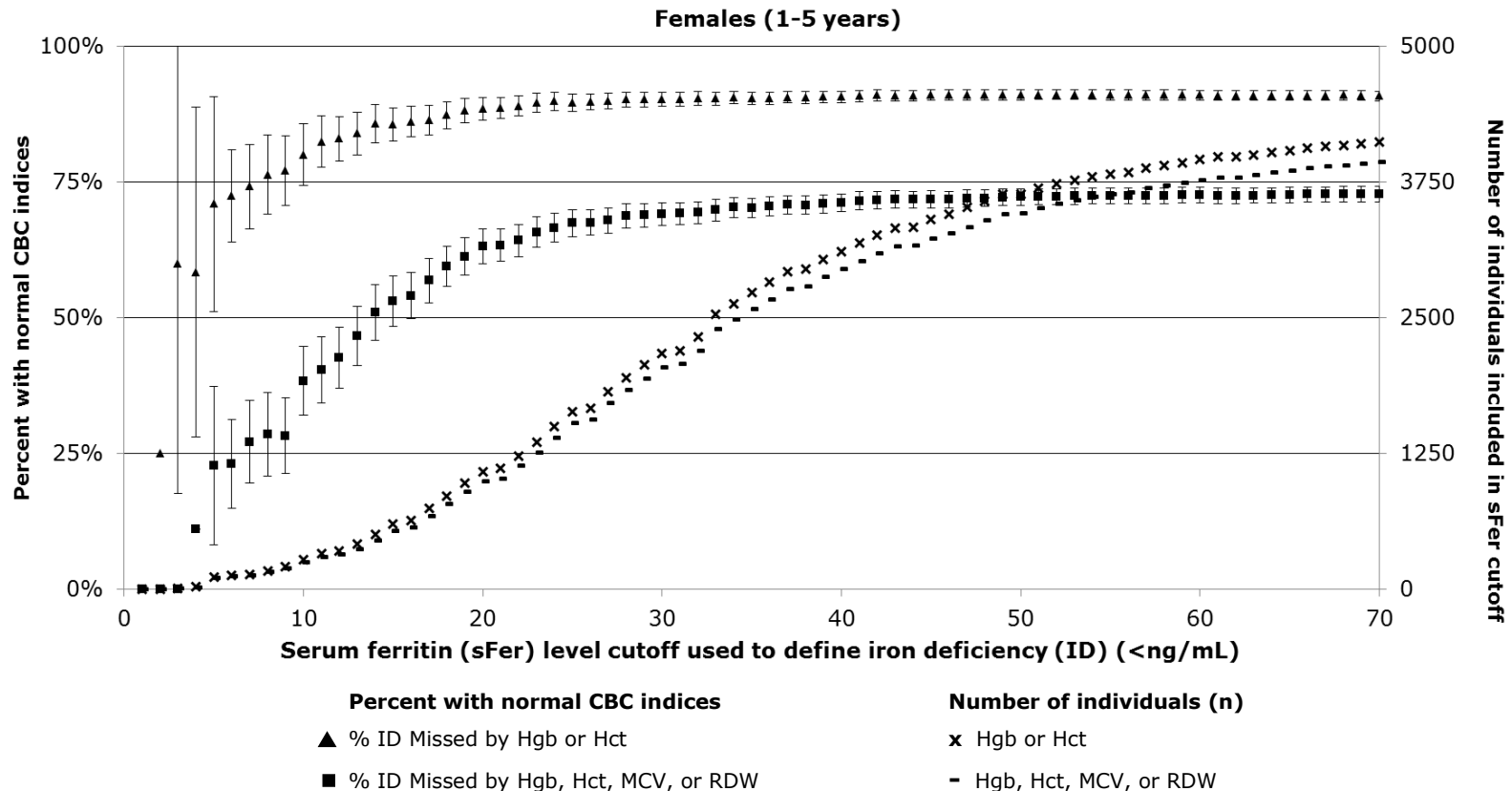


Figure D.1 Cumulative Percentage of Females (Ages 1-5) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

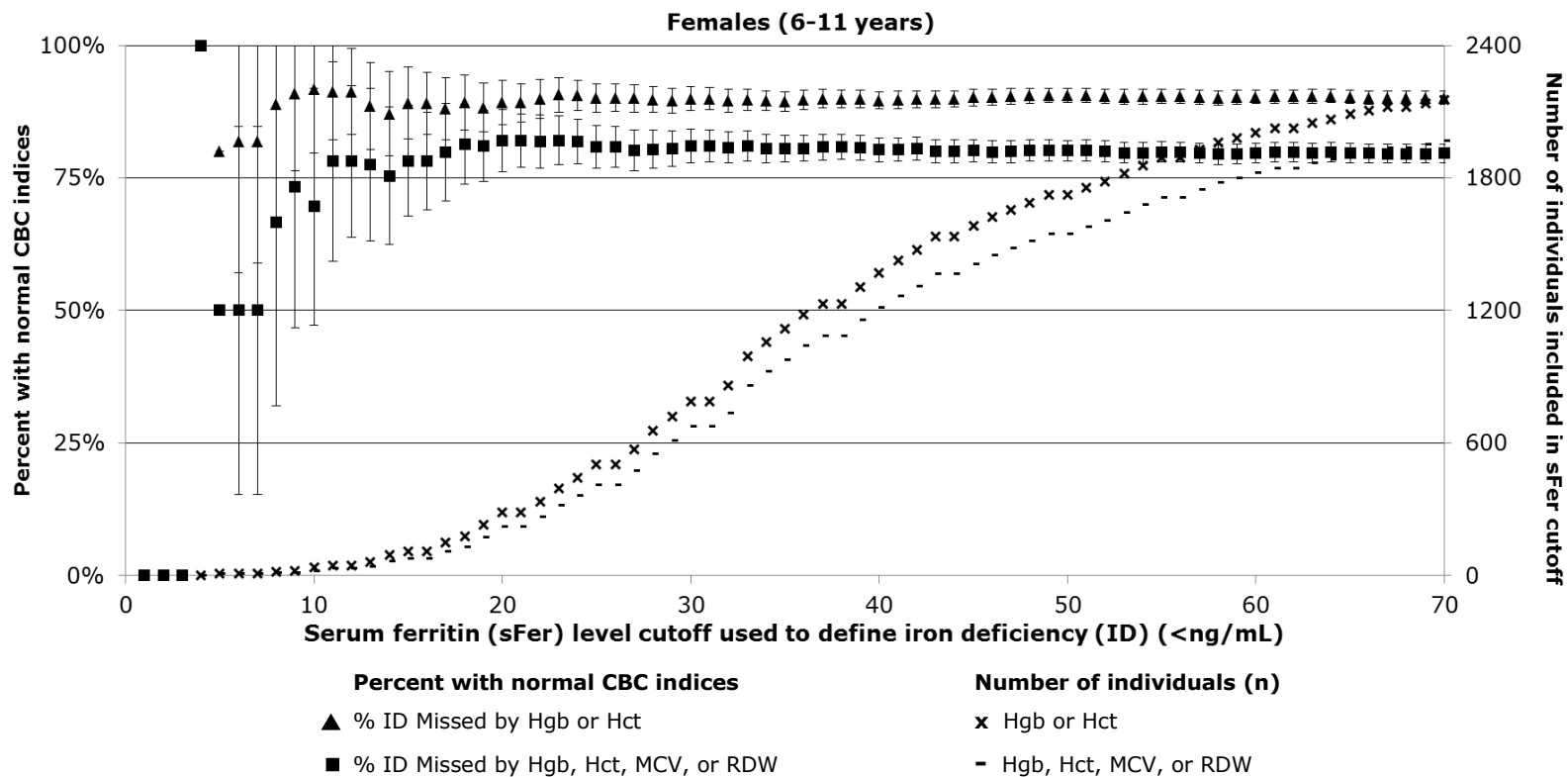


Figure D.2 Cumulative Percentage of Females (ages 6-11) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

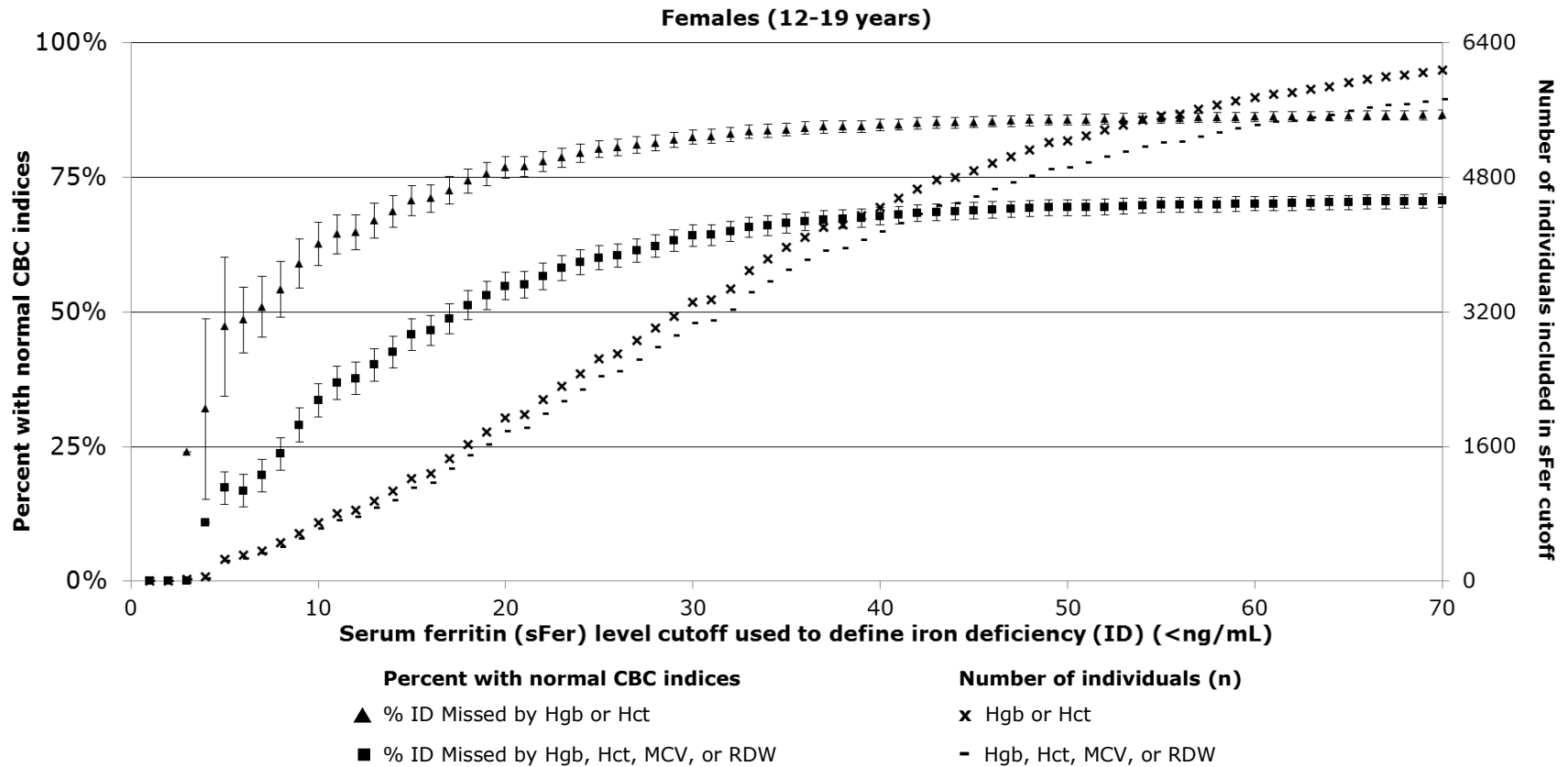


Figure D.3 Cumulative Percentage of Females (ages 12-19) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

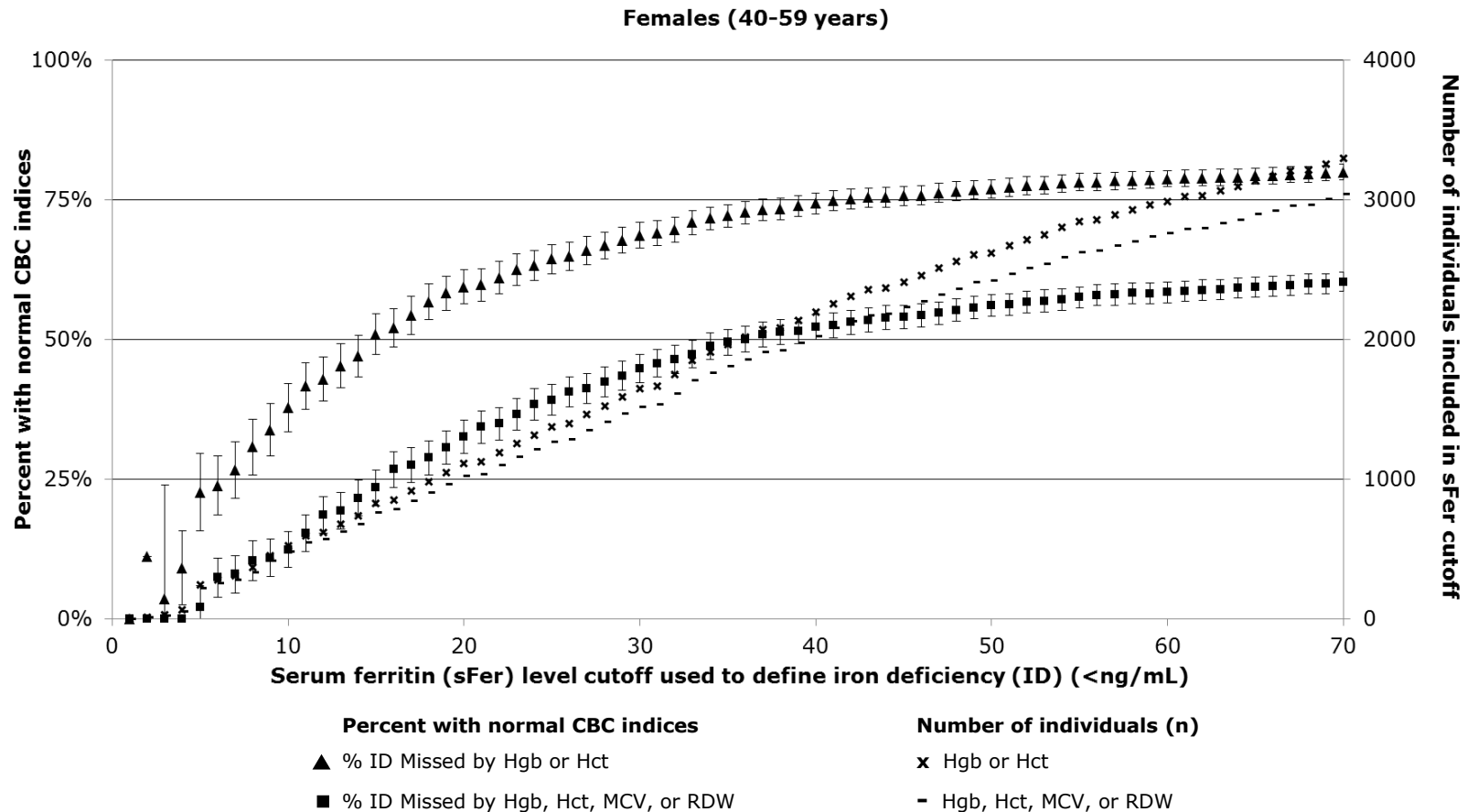


Figure D.4 Cumulative Percentage of Females (ages 40-59) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

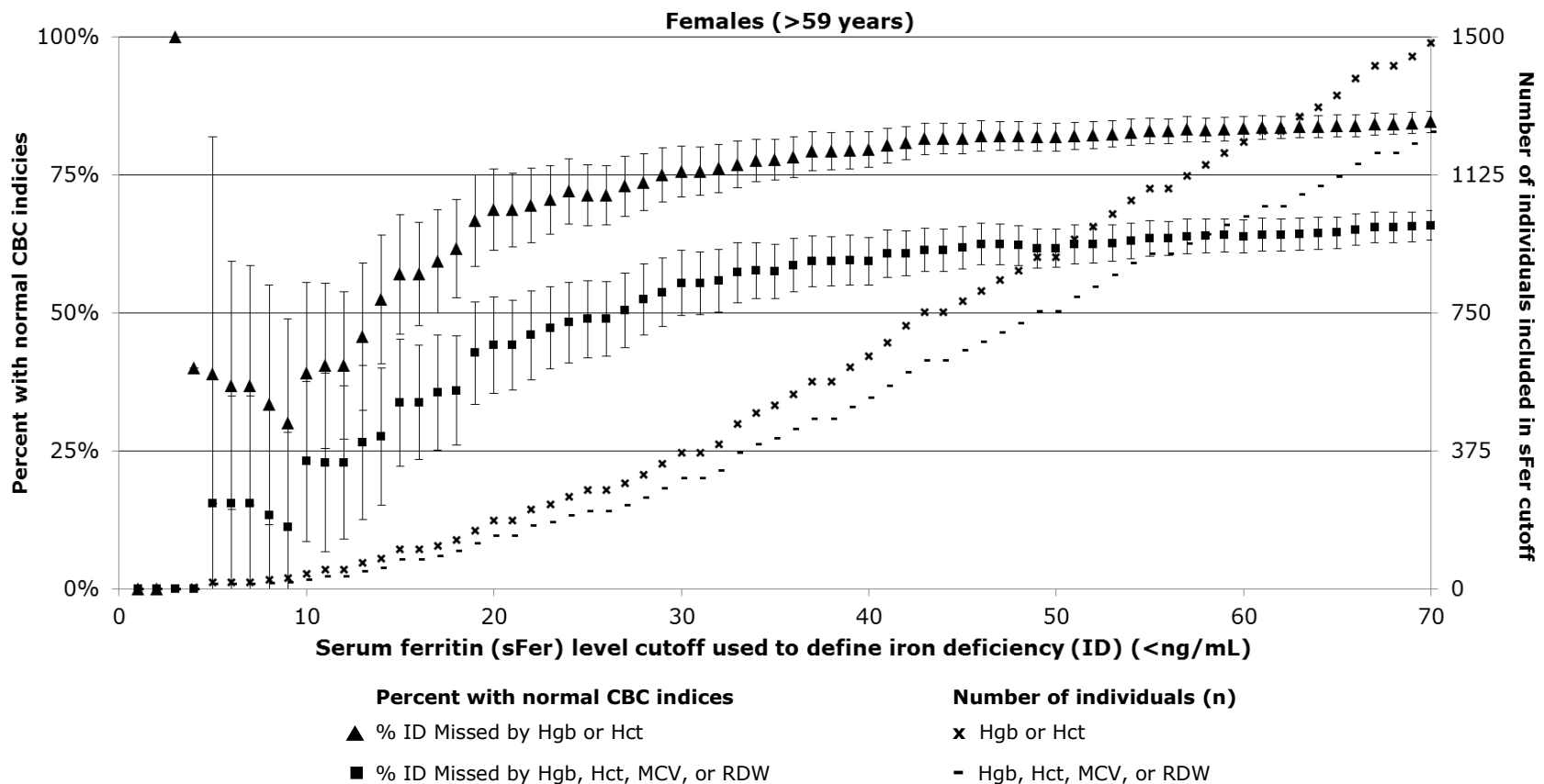


Figure D.5 Cumulative Percentage of Females (ages >59) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

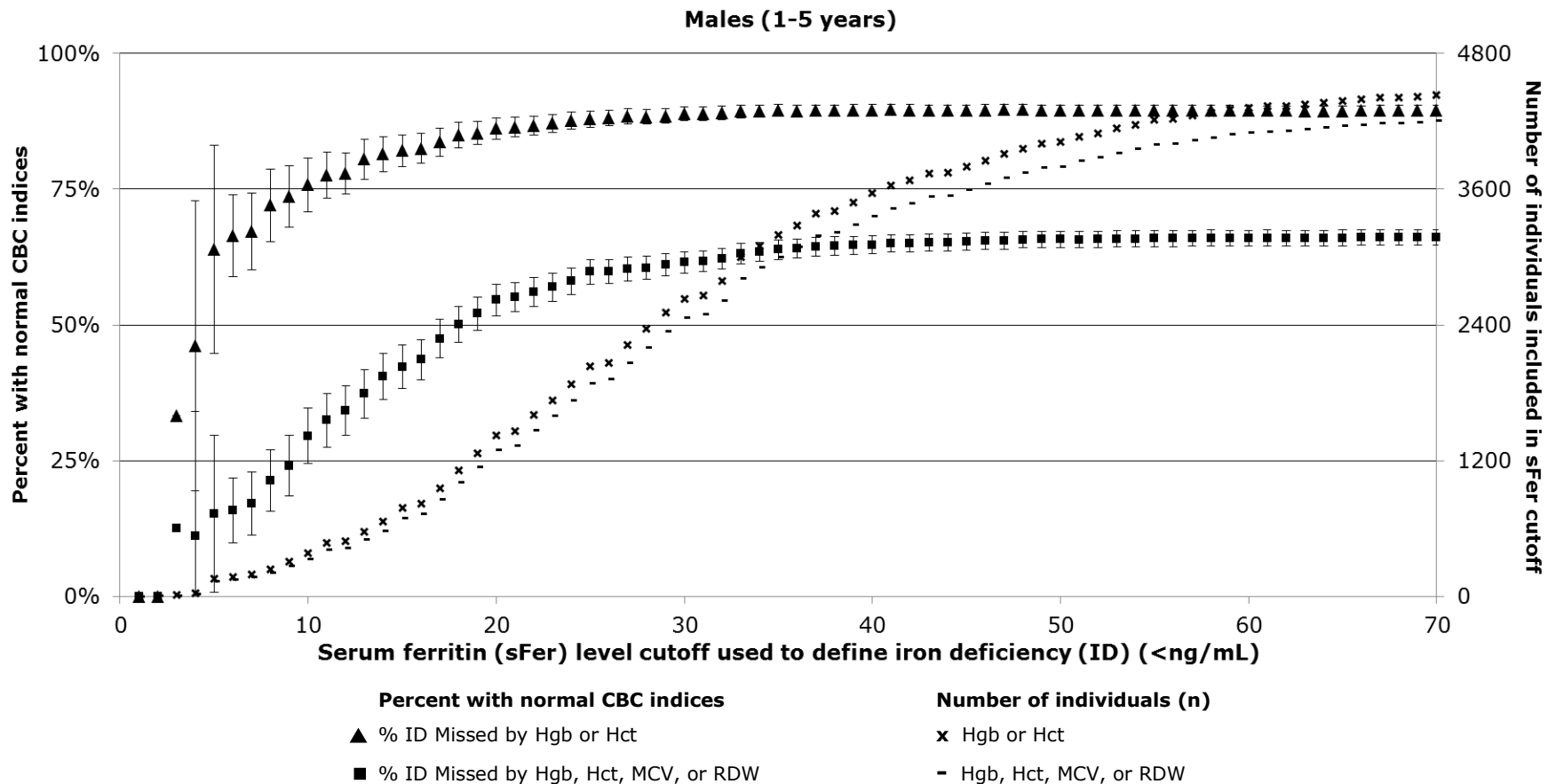


Figure D.6 Cumulative Percentage of Males (ages 1-5) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

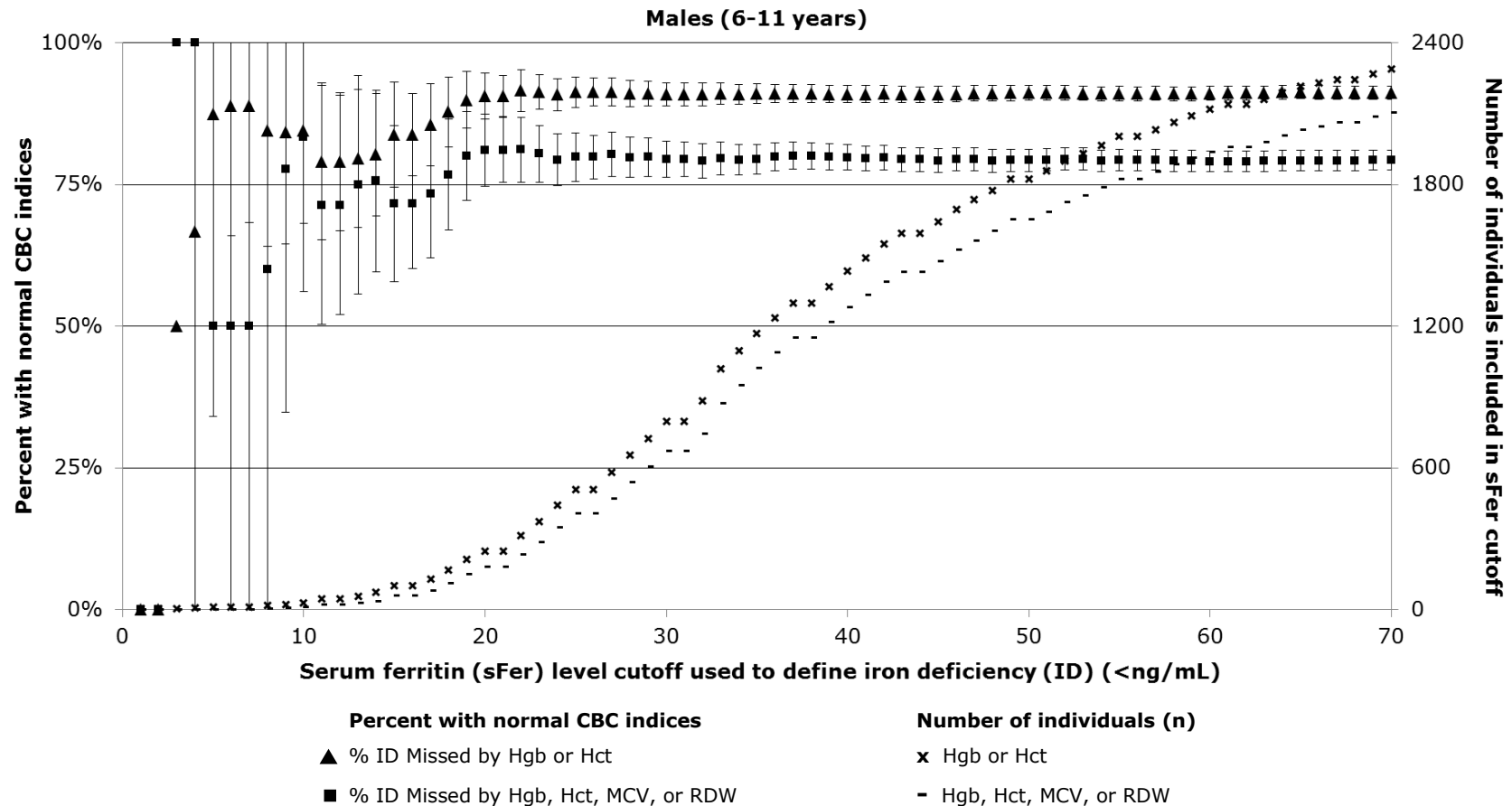


Figure D.7 Cumulative Percentage of Males (ages 6-11) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

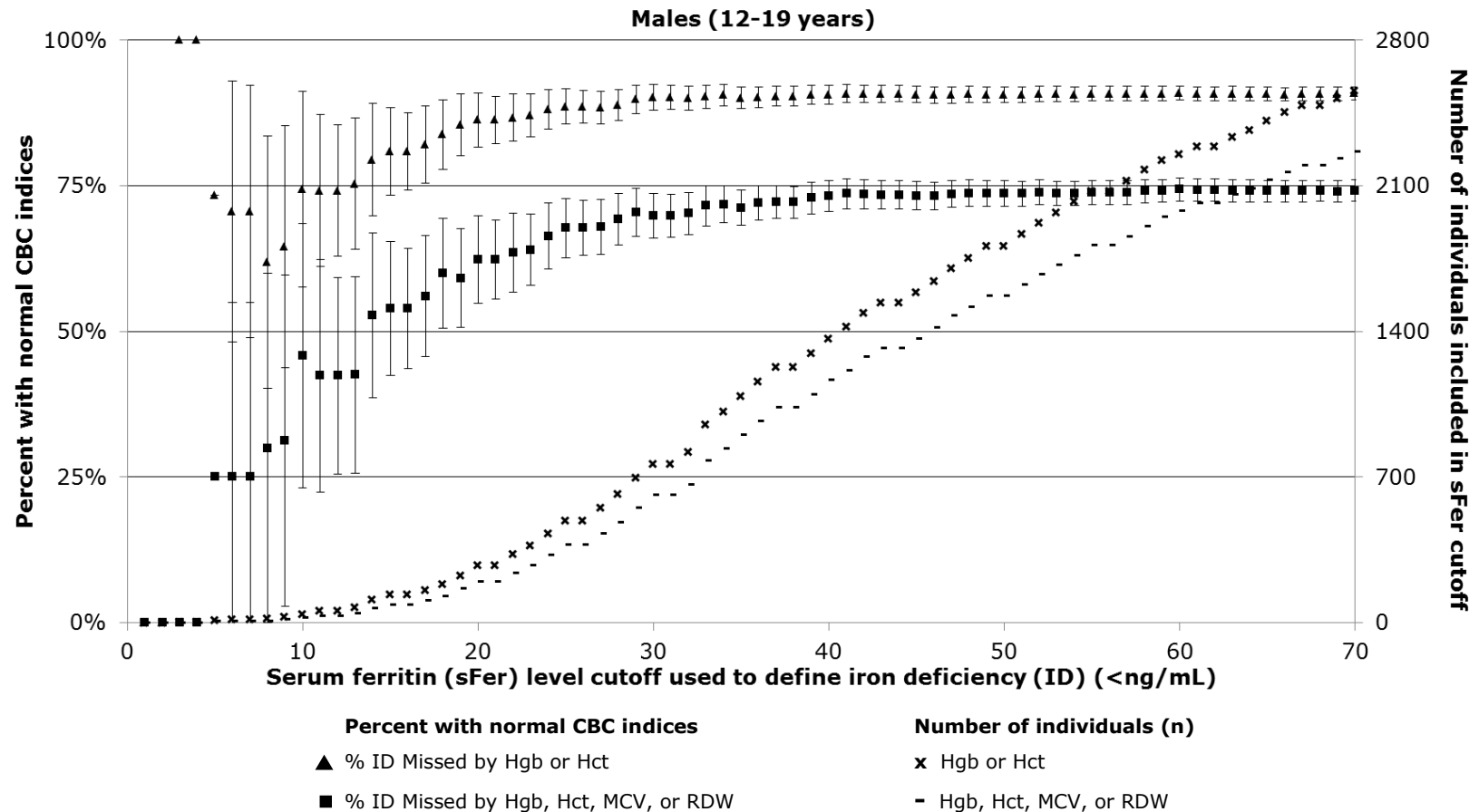


Figure D.8 Cumulative Percentage of Males (ages 12-19) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

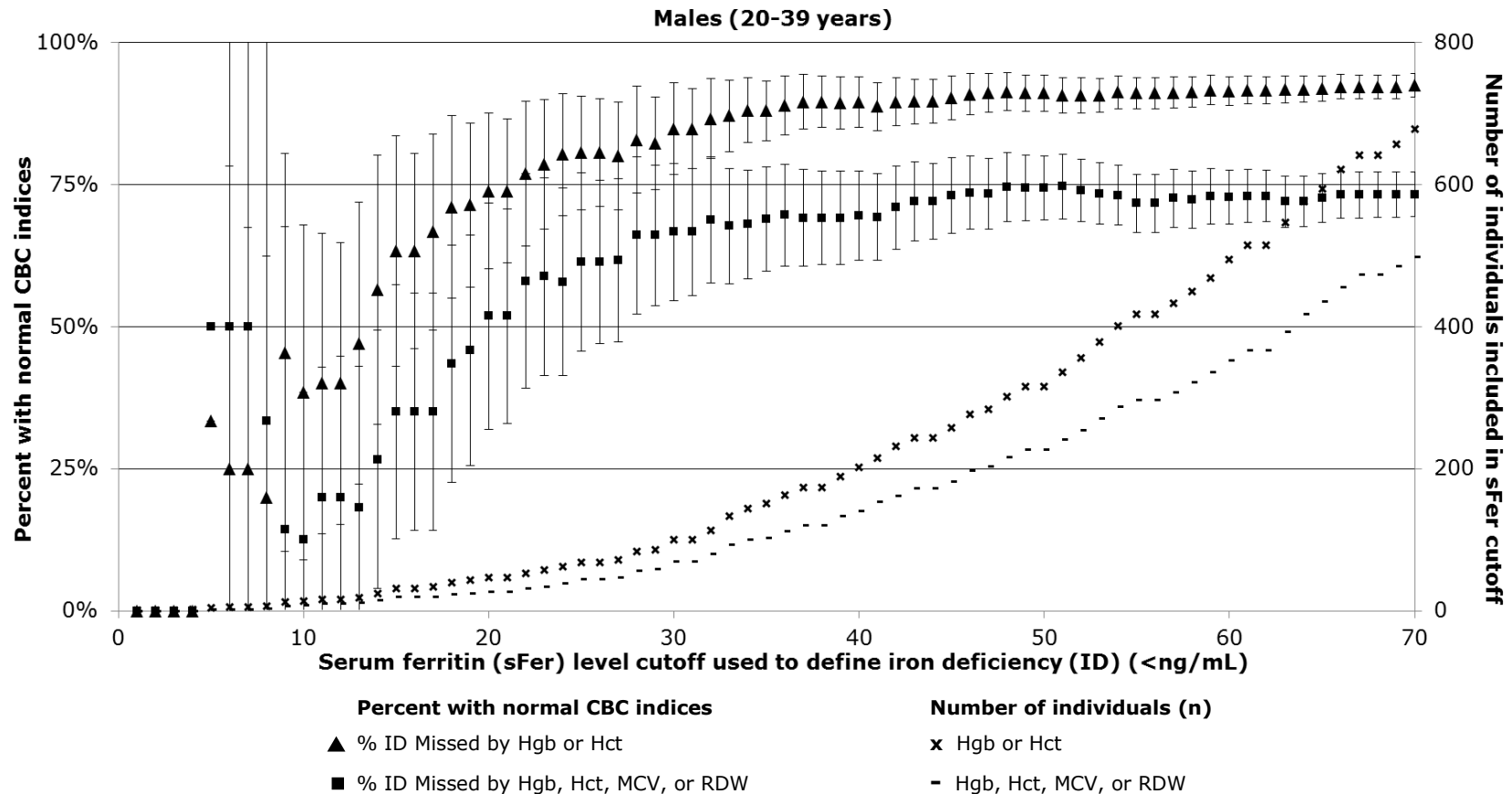


Figure D.9 Cumulative Percentage of Males (ages 20-39) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

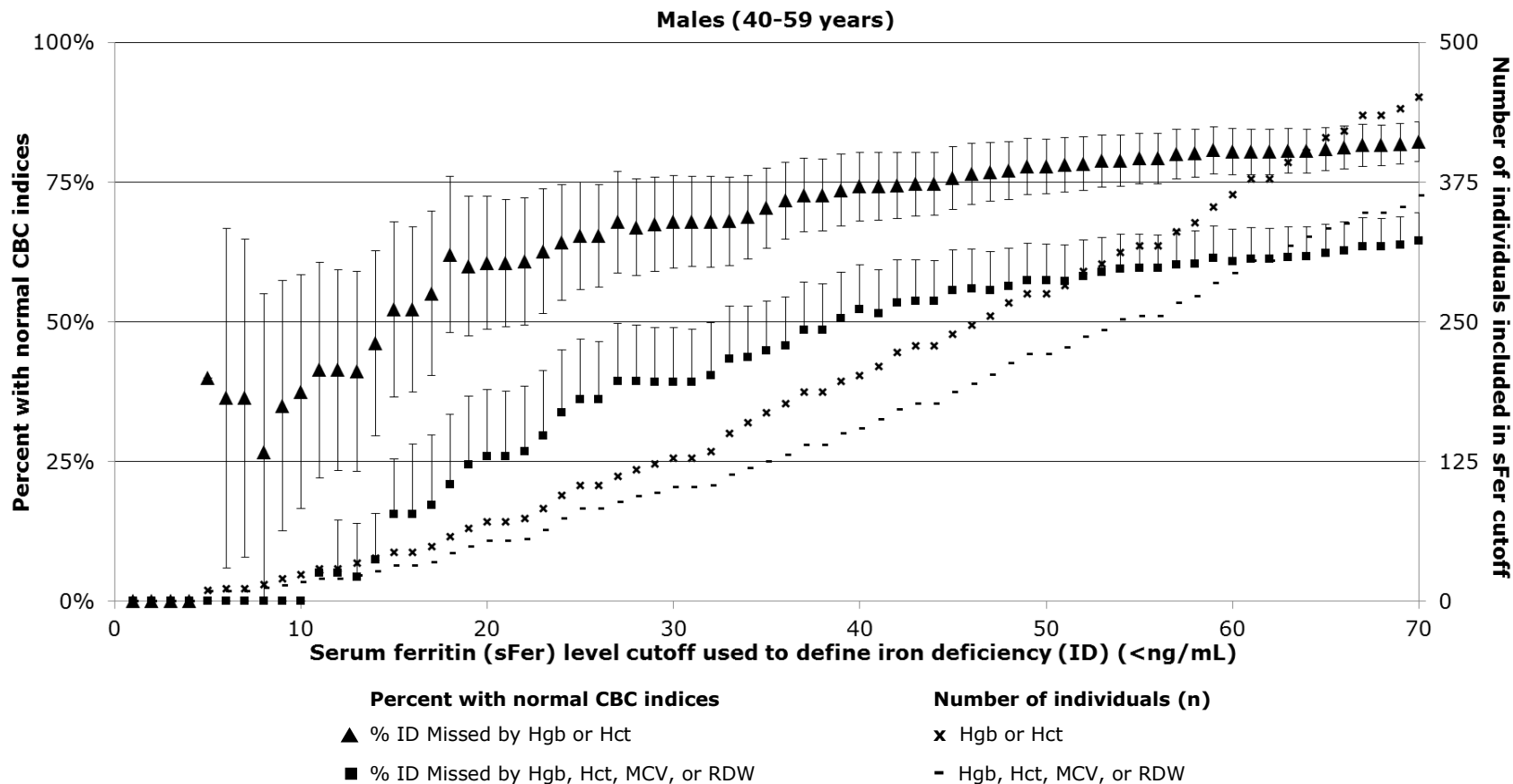


Figure D.10 Cumulative Percentage of Males (ages 40-59) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

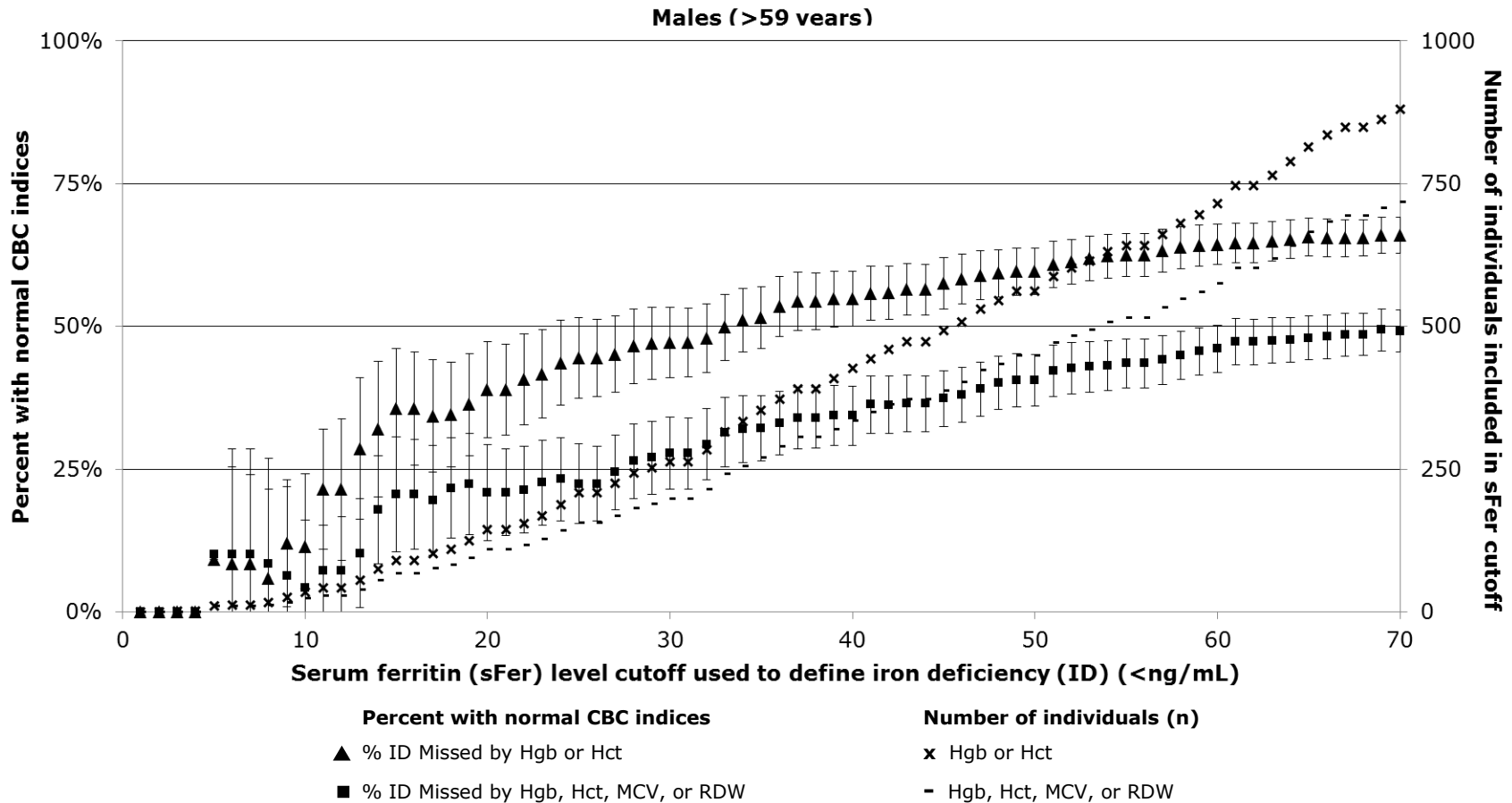


Figure D.11 Cumulative Percentage of Males (ages >59) with Normal CBC Indices in Relation to sFer in NHANES (1976-2010).

The left Y-axis represents the percent of females with normal CBC indices for either Hgb and Hct (▲) or Hgb, Hct, MCV, and RDW (■). The X-axis represents the range of sFer levels that have been used to define iron deficiency. The Y-axis on the right is the number of individuals below the various sFer cutoff values. Confidence intervals were calculated using the normal approximation.

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