Reimbursement Policy:

Diagnostic Testing of Iron Homeostasis and Metabolism - Lab Benefit Program (LBM)

POLICY NUMBER	EFFECTIVE DATE:	APPROVED BY
AHS-G2011	3/01/2023	**RPC (Reimbursement Policy Committee)**

POLICY DESCRIPTION | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES | REVISION HISTORY

Policy Description:

Iron, an essential nutrient with a variety of biological uses, is tightly regulated *in vivo* to maintain homeostasis. Enterocytes absorb iron as Fe^{2+} either in its non-heme form via DMT1 (divalent metal-ion transporter-1) or in heme form presumably through receptor-mediated endocytosis. The enterocytes then release iron through ferroportin where transferrin binds it as biologically inactive Fe^{3+}. Saturated transferrin delivers iron to erythrocyte precursors in bone marrow where it is incorporated into hemoglobin during erythropoiesis. Transferrin may also salvage iron released by the reticuloendothelial system and macrophages (Knutson, 2017).

All cells require iron; consequently, saturated transferrin can also bind to its receptors (TfR1 or TfR2). The bound transferrin receptor (TfR) undergoes receptor-mediated endocytosis followed by export of divalent iron for cellular use (Byrne, Krishnamurthy, & Wessling-Resnick, 2013). Intracellularly, iron is stored within the central cavity of the protein ferritin, a large spherical protein that can store up to 4500 iron atoms per protein. Ferritin has ferroxidase activity required for iron uptake and storage. In conjunction with transferrin and TfR, ferritin is an acute phase reactant that responds to oxidative stress and inflammation (Camaschella, 2020). Moreover, TfR1 and TfR2, upon activation by transferrin, can initiate signaling cascades required for hepcidin expression (Roetto, Mezzanotte, & Pellegrino, 2018). Hepcidin, a small peptide hormone, acts as a modulator of serum iron concentrations by binding to ferroportin, the only iron exporter; ultimately, this results in the degradation of ferroportin and an intracellular accumulation of iron (Pietrangelo, 2015).

Please note that carbohydrate-deficient transferrin is out of the scope for this policy.

Indications and/or Limitations of Coverage:

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request

1) Measurement of serum ferritin levels **MEETS COVERAGE CRITERIA** in any of the following situations:
    a) In the evaluation of an individual with abnormal hemoglobin and/or hematocrit levels.
    b) In the evaluation and monitoring of iron overload disorders.
    c) In individuals with symptoms of hemochromatosis (See Note 1).
    d) In individuals with first-degree relatives with confirmed hereditary hemochromatosis (HH)
    e) In the evaluation of individuals with liver disease.
    f) In the evaluation and monitoring of patients with chronic kidney disease who are being considered for, or are receiving treatment for, anemia at a frequency of every 1 to 3 months.
    g) In the evaluation of hemophagocytic lymphohistiocytosis (HLH) and Still's Disease.
    h) For individuals on iron therapy, at a frequency of every 1 to 3 months.
    i) In males with secondary hypogonadism.
2) Serum transferrin saturation (using serum iron and serum iron binding capacity measurements) **MEETS COVERAGE CRITERIA** in the following:
    a) For the evaluation of iron overload in individuals with symptoms of hemochromatosis (See Note 1).
    b) For the evaluation of iron overload in individuals with first-degree relatives with confirmed hereditary hemochromatosis (HH).
    c) For the evaluation of iron deficiency anemia.
3) The use of ferritin or transferrin measurement, including transferrin saturation, as a screening test in asymptomatic patients **DOES NOT MEET COVERAGE CRITERIA**.

    The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.
4) Serum hepcidin testing, including immunoassays, **DOES NOT MEET COVERAGE CRITERIA.**
5) The use of GlycA testing to measure or monitor transferrin or other glycosylated proteins **DOES NOT MEET COVERAGE CRITERIA.**

a) **NOTE 1**: Symptoms of hemochromatosis, according to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health include the following (NIDDK, 2014):

i) Joint pain

ii) Fatigue

iii) Unexplained weight loss

iv) Abnormal bronze or gray skin color

v) Abdominal pain

vi) Loss of sex drive

Scientific Background:

Iron is necessary for fundamental metabolic processes and acts as the central component in the catalytic sites of numerous essential enzymes and multiprotein complexes, such as mitochondrial respiratory chain complexes and oxygen binding proteins (Hentze, Muckenthaler, & Andrews, 2004; Zhang, Ghosh, & Rouault, 2014). Tight regulation of iron metabolism for maintaining adequate iron levels is achieved by the interaction of a number of iron metabolism-related proteins (Zhang et al., 2014) as well as the hemostatic modulation of iron absorption, utilization, and recycling (Hentze, Muckenthaler, Galy, & Camaschella, 2010). This strict regulation is pertinent due to the potential toxicity of iron from its redox reactivity and the resultant generation of damaging free radicals (Finazzi & Arosio, 2014).

Several mechanisms in the body regulate the dietary absorption of iron and its concentration in other areas, such as plasma and extracellular milieu; this process is known as systemic iron homeostasis (Ganz, 2013). Iron homeostasis is a complex process where the small peptide hormone hepcidin plays a major role by binding the sole mammalian iron exporter, ferroportin. This leads to ferroportin degradation by lysosomes. Furthermore, hepcidin production is sensitive to extracellular iron concentrations by way of the human homeostatic iron regulator (HFE) protein and the transferrin receptors (TfRs). The HFE protein has been shown to interact with both TfR1 and TfR2, initiating the BMP-SMAD signaling pathway upon transferrin binding. This signaling cascade ultimately increases expression of the *HAMP* gene that encodes for hepcidin (Pietrangelo, 2015; Vujić, 2014).

Ferritins are a highly conserved family of proteins that detoxify and store excess iron as less reactive ferrihydrite (Hentze et al., 2004). This intracellular iron storage mechanism allows the cell to maintain and utilize spare iron based on changes in metabolic demand (Finazzi & Arosio, 2014). Mammalian ferritins are heteropolymers comprised of tissue-specific combinations of 24 subunits. These subunits consist of two types: Ferritin L (FTL) and Ferritin H (FTH); a spherical structure is formed from these subunits, facilitating the dynamic storage of iron (Finazzi & Arosio, 2014; Liu & Theil, 2005). The levels and composition of ferritin are regulated by oxidative stress at the transcriptional level (Arosio & Levi, 2010; Bresgen & Eckl, 2015), and by iron responsive proteins (IRP) at the post-transcriptional level (Anderson, Shen, Eisenstein, & Leibold, 2012). Several tissues express a mitochondria-specific ferritin protein that further protect these mitochondria from oxidative damage (Campanella et al., 2009; Paul, Manz, Torti, & Torti, 2017).

Iron is released as needed from ferritin by ferritinophagy, the targeting of ferritin for degradation by lysosomes; this process requires cargo protein nuclear receptor coactivator 4 (NCOA4), as NCOA4-deficient cells cannot degrade ferritin correctly (Mancias, Wang, Gygi, Harper, & Kimmelman, 2014). After release, the iron is transported back to the cytosol by divalent metal transporter 1 (DMT1) (La et al., 2018). This process allows the iron to become available as part of the labile iron pool (Cabantchik, 2014; Kruszewski, 2003).

Degradation of ferritin and resultant accumulation of lethal reactive oxygen species (ROS) has been recognized as a distinct iron-dependent type of regulated, non-apoptotic cell death known as ferroptosis (Hou et al., 2016; Xie et al., 2016). Dysregulated ferroptosis has been implicated in neurotoxicity, neurodegenerative diseases, acute renal failure, drug-induced hepatotoxicity, hepatic and heart ischemia/reperfusion injury, and T-cell immunity (Xie et al., 2016). Abnormal ferroptosis has also been recently found to play a role in drug treatment, particularly in decitabine treatment of myelodysplastic syndrome (MDS). The drug-induced ROS release decreases glutathione (GSH) and glutathione peroxidase 4 (GPX4), features characteristic of this unique cell-death process (Lv et al., 2020).

Ferritin can routinely be detected in serum (Alfrey, 1978) as a result of secretion from macrophages (Cohen et al., 2010) or release during cell death and lysis (Kell & Pretorius, 2014). Serum ferritin (SF) is primarily composed of L subunits, contains relatively little iron, and is partially glycosylated (Santambrogio, Cozzi, Levi, & Arosio, 1987; Wang, Knovich, Coffman, Torti, & Torti, 2010). Causes of elevated SF levels include, but are not limited to, acute or chronic inflammation, chronic alcohol consumption, liver disease, renal failure, metabolic syndrome, or malignancy rather than iron overload (Koperdanova & Cullis, 2015). In healthy adults, levels of SF generally reflect overall iron storage (Costa Matos et al., 2013; Enko et al., 2015; Finch et al., 1986; Jacobs, Miller, Worwood, Beamish, & Wardrop, 1972; Wang et al., 2010; Zanella et al., 1989). This closely correlates with the “gold standards” of measuring iron stores in bone marrow or liver biopsy (Peng & Uprichard, 2017).

Given that iron is an essential component for many metabolic processes, the immune system has developed mechanisms for iron sequestration as part of the inflammatory response in order to prevent invading pathogens and tumors from utilizing iron (Wang et al., 2010). Hence, increased levels of SF during the immune system-based acute phase response do not necessarily correlate with iron availability or stores, but rather are a general indicator of inflammation (Dignass, Farrag, & Stein, 2018). This becomes a critical issue when assessing iron deficiency (ID), as elevations in SF during inflammation can mask the presence of ID (Suchdev et al., 2017). However, this makes the assessment of iron status in the presence of inflammation more complex (Dignass et al., 2018; Knovich, Storey, Coffman, Torti, & Torti, 2009; Munoz, Gomez-Ramirez, et al., 2017). Additionally, the two subunits of ferritin (FTL and FTH) have been reported to differentially locate during periods of inflammation; this complicates the use of these subunits as an inflammatory diagnostic tool (Ahmad et al., 2013). In analyzing data from the Biomarkers Reflecting the Inflammation and Nutritional Determinants of Anemia (BRINDA) project, Suchdev et al. (2017) identified that all their examined indicators of iron status (SF, serum TfR, total body iron) were affected by inflammation, and suggested utilizing C-reactive protein (CRP), a measure of acute inflammation, and α1-acid glycoprotein, a measure of chronic inflammation, in addition to iron indicators to better account for the full range and severity of inflammation.

Extremely elevated SF, in excess of five times the upper limit of normal (Evensen, Swaak, & Nossent, 2007), can indicate adult-onset Still disease. Still disease is a systemic inflammatory disorder that typically affects young women and is characterized by fever, arthritis, and rash (Knovich et al., 2009; Zandman-Goddard & Shoenfeld, 2007). More extremely elevated SF (above 10,000 ug/L), especially in the context of autoimmune disorders, such as Still disease and systemic lupus erythematosus (SLE), and viral infections, indicates the possibility of hemophagocytic syndrome (Emmenegger et al., 2001), which involves the phagocytosis of red blood cells by macrophages (Knovich et al., 2009), along with a final common pathway of elevated triglycerides, ferritin, pancytopenia, and highly fatal multiple organ failure (Sekigawa et al., 2001).

Hepcidin regulates serum iron levels by activating the endocytosis and proteolysis of ferroportin, the sole mammalian iron exporter. In healthy individuals, iron status is monitored by hepatocytes, which regulate hepcidin promoter activity according to iron needs. If iron levels are low, iron is released by ferroportin, allowing hepcidin levels to remain low; if iron overload is detected, hepcidin is activated to sequester the excess iron (Ueda & Takasawa, 2018). Unregulated activity of hepcidin can therefore result in hypoferremia due to iron sequestration (Ganz & Nemeth, 2009). Interleukin-6 (IL-6), an inflammatory cytokine, stimulates hepcidin to decrease erythropoiesis due to a lack of bioavailable iron for hemoglobin (Kroot, Tjalsma, Fleming, & Swinkels, 2011).

No physiologic process is present in the body to excrete excess iron, leaving individuals susceptible to developing iron overload. Iron overload may result from increased absorption, transfusion, or hereditary disease. Excess iron collects within the internal organs, specifically the liver and heart, where it causes chronic free-radical induced injury (Wang et al., 2010). Excess iron may be a symptom or complication of a hereditary disease, such as hereditary hemochromatosis (HH), an autosomal recessive disorder that causes an enhancement in the intestinal absorption of excess iron (Santos, Krieger, & Pereira, 2012). Too much iron in the body can lead to a plethora of problems, including arthritis, skin pigmentation, hypogonadism, cardiomyopathy, and diabetes. The majority of individuals with HH contain mutant hemochromatosis (*HFE*) genotypes, including homozygosity for p.Cys282Tyr or p.Cys282Tyr, and compound heterozygosity for p.His63Asp; based on these results, it is suggested that genetic testing be performed for these mutations in all patients with primary iron overload and an idiopathic increase in transferrin saturation (TSAT) and/or SF values (Santos et al., 2012).

Another genetic disorder characterized by excess iron accumulation is known as neuroferritinopathy (NF). NF was first discovered in 2001 and is a movement disorder identified by excess iron in specific areas of the brain (Lehn, Boyle, Brown, Airey, & Mellick, 2012). NF is the only known autosomal dominant genetic disease of neurodegeneration caused by mutations in the ferritin light polypeptide 1 (*FTL1*) gene (Keogh, Morris, & Chinnery, 2013; Kumar, Rizek, & Jog, 2016). The modification causes mutant L-chain ferritins that negatively alter ferritin function and stability (Kuwata et al., 2019; McNally et al., 2019). Several conditions indicative of NF include brain iron accumulation (NBIA) disorder alongside pantothenate kinase-associated neurodegeneration (PKAN), phospholipase A_2-associated neurodegeneration, mitochondrial membrane protein-associated neurodegeneration (MPAN), and beta-propeller protein-associated neurodegeneration (BPAN) (Hayflick, Kurian, & Hogarth, 2018). NBIAs are typically characterized by dystonia, Parkinsonism, spasticity, and iron accumulation within the basal ganglia. Depending on the NBIA subtype, the condition may also exhibit hyperphosphorylated tau, axonal swelling, and Lewy body formation (Arber, Li, Houlden, & Wray, 2016). NF is typically considered as a diagnosis in patients exhibiting movement disorders, decreased SF, variable phenotypes, negative genetic testing for common movement disorders such as Huntington disease, and imaging showing potential iron deposits in the brain (Kumar et al., 2016).

Iron deficiency (ID), referring to a reduced amount of iron stores, is usually an acquired disorder that affects over one billion people worldwide (Camaschella, 2015; Miller, 2013). Inadequate iron intake is often due to poverty, malnutrition, dietary restriction, and malabsorption; additional causes include menstrual periods, gastrointestinal bleeding, and chronic blood loss (DeLoughery, 2017; Kassebaum et al., 2014; Sankaran & Weiss, 2015). SF analysis is the most efficient test for a diagnosis of ID (DeLoughery, 2017). In children, ID is most commonly caused by insufficient dietary iron intake when compared to a child's rapid growth rate, as well as gastrointestinal issues due to cow's milk (Ozdemir, 2015).

It has been reported that more than one in three pregnant women present with iron-deficiency anemia worldwide (Lewkowitz & Tuuli, 2019). Anemia in pregnant women could affect the fetus' intrauterine growth and may cause neurodevelopmental impairment (Marell et al., 2019). Maternal anemia in early pregnancy is associated with an increased risk of autism spectrum disorder, attention-deficit/hyperactivity disorder, and intellectual disability (Wiegersma, Dalman, Lee, Karlsson, & Gardner, 2019). Efficient vitamin and mineral supplementation are vital during pregnancy for the health of both the mother and of the fetus; however, certain supplements may be more helpful than others. It has been suggested that in pregnant women, intravenous iron administration may be a more effective treatment option than oral iron administration (Lewkowitz & Tuuli, 2019).

Analytical Validity

Low SF (<30ug/L) is a sensitive and specific indicator for ID (Dignass et al., 2018). However, a normal SF level can be misleading in the context of inflammation (Peng & Uprichard, 2017). Dignass et al. (2018) published recommendations which stated that the standard ID level is <30 µg/L and that "A serum ferritin threshold of <100 µg/L or TSAT < 20% can be considered diagnostic for iron deficiency in congestive heart failure (CHF), chronic kidney disease (CKD), and inflammatory bowel disease (IBD). If serum ferritin is 100-300 µg/L, TSAT < 20% is required to confirm iron deficiency. Routine surveillance of serum ferritin and TSAT in these at-risk groups is advisable so that iron deficiency can be detected and managed (Dignass et al., 2018)."

Biomarker glycoprotein acetylation (GlycA) has been associated with chronic inflammation and utilizes nuclear magnetic resonance (NMR) to measure the serum or plasma concentration of the *N*-acetyl methyl functional groups of *N*-acetylglucosamine glycans associated with inflammation; these include transferrin, haptoglobin, α_1-acid glycoprotein, α_1-antitrypsin, and α_1-antichymotrypsin (Ritchie et al., 2015). According to Otvos et al. (2015), the simple integration of the GlycA signal to accurately quantify concentration is not possible due to signal overlap with allylic protons of unsaturated fatty acids in the plasma or serum sample; therefore, a linear least-squares deconvolution determination must be performed. In doing so, Otvos et al. (2015) have shown that GlycA has lower imprecision and variability than high-sensitivity C-reactive protein (hsCRP), cholesterol, and triglyceride testing; however, "because the GlycA signals originating from different plasma glycoproteins are not distinguishable, and the glycan on each is heterogeneous and varies dynamically, only a rough estimate can be made of how much each contributes to measured plasma GlycA concentrations" (Otvos et al., 2015). Consequently, the GlycA test cannot be used to accurately determine concentration of individual proteins, including transferrin.

A study by Dahlfors et al. (2015) measured serum hepcidin in more than 400 patients using a competitive ELISA assay; several types of patients were included in this study including those with liver disorders and iron disorders, as well as healthy individuals. The researchers note that this ELISA assay has a good correlation with light chromatography with tandem mass spectroscopy (LC-MS/MS) (r=0.89), but it does cross-react with forms of hepcidin (hepcidin-20 and -22) that are not associated with iron disorder biomarkers (Dahlfors et al., 2015). Another study by Karlsson (2017) compared the ELISA hepcidin assay to the use of ferritin, C-reactive protein (CRP), and IL-6 to differentiate ID anemia and anemia of inflammation in elder patients. Even though the study was small (n=30), they measured a sensitivity and specificity of the hepcidin assay of 100% and 67%, respectively, as compared to the lower sensitivity but higher specificity of ferritin (91% and 83%, respectively). It was concluded that "Hepcidin shows a strong positive correlation with ferritin, and also correlates positively with C-reactive protein in this patient population (Karlsson, 2017)." Recently, Chen, Liu, and Wright (2019) have developed a high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method, in accordance to CLSI-C62A guidelines, to measure serum hepcidin levels. This method has intra- and inter-day coefficients-of-variation (CVs) of <3% and <6%, respectively, with relative error rates ≤1.2% and ≤4.4% at ambient temperature and 4°C, respectively. The authors also report that the relative error rate after three cycles of freeze-thaw (-70°C) is ≤1.8% (Chen et al., 2019).

A study by da Silva, Silveira, and Fernandes (2019) has showed that both iron deficiency anemia (IDA) and sickle cell disease (SCD) can be detected in whole human blood samples via Raman spectroscopy; this study detected both IDA and SCD, when compared to healthy subject controls, with a sensitivity of 93.8% and a specificity of 95.7%. These results were based on detailed spectra analysis methods such as partial least squares and principal component analysis (da Silva et al., 2019).

Among neonates, Gerday et al. (2020) measured urinary ferritin in neonatal intensive care unit (NICU) patients, and found that in those at risk for iron deficiency (n=49), “a corrected urine ferritin < 12 ng/mL had a sensitivity of 82% (95% CI, 67-93%) and a specificity of 100% (CI, 66-100%) for detecting iron-limited erythropoiesis, with a positive predictive value of 100% (CI, 89-100%).” Though iron deficiency can be confirmed via serum iron, transferrin, SF, among other tests, the volume of blood and costs associated with these tests necessitate a non-invasive and accurate alternative for diagnosing iron deficiency (Gerday et al., 2020).

Jones et al. (2021) investigated the effect of delayed processing on measuring 25 micronutrients and select clinical biomarkers, including iron (ferritin), in human blood samples. Blood from 16 healthy participants was collected and processed within either 2 hours or 24 hours. The concentration difference between the two process delays was compared. All analytes had a 4% or lower change in concentration between the two delays. There was no significant effect of delayed processing on ferritin. The authors concluded that “in blood collected from adult participants, delayed processing of chilled, whole blood for 24 hours did not materially affect the measured concentrations of the majority of micronutrient and selected clinical biomarkers” (Jones et al., 2021).

Bell et al. (2021) performed a meta-analysis to study genes associated with iron homeostasis. Data about blood levels of ferritin, total iron binding capacity, iron saturation, and transferrin saturation was used from three genome-wide association studies from Iceland, the UK, and Demark. The authors identified 56 loci with variants associated with one or more of the biomarkers, 46 of which are novel variants. Specifically, “variants at *DUOX2*, *F5*, *SLC11A2* and *TMPRSS6* associate with iron deficiency anemia, while variants at *TF*, *HFE*, *TFR2* and *TMPRSS6* associate with iron overload” (Bell et al., 2021).

Clinical Validity and Utility

Dysregulated iron metabolism has been implicated in a variety of pathophysiological conditions from mild ID to anemia, iron overload, inflammation, infection, cancer, and cardiovascular and neurodegenerative diseases (Gozzelino & Arosio, 2016). Initial signs and symptoms of iron overload are insensitive and nonspecific, so laboratory studies including ferritin are clinically useful in the identification and treatment of iron overload (Fleming & Ponka, 2012; Knovich et al., 2009; Koperdanova & Cullis, 2015). According to the Hemochromatosis and Iron Overload Screening (HEIRS) study (McLaren et al., 2003), ferritin levels above 200 ng/mL (449 pmol/L) in women or 300 ng/mL (674 pmol/L) in men with no signs of inflammatory disease warrant additional testing. Therapeutic phlebotomy is indicated in patients with hemochromatosis who have high TSAT and SF levels of more than 1000 ng/mL (2247 pmol/L). Therapeutic phlebotomy is also recommended in patients who do not have anemia (Fleming & Ponka, 2012; Salgia & Brown, 2015; van Bokhoven, van Deursen, & Swinkels, 2011). Saeed et al. (2015) used a receiver operating characteristic curve to evaluate the value of ferritin >500 ng/mL for diagnosing hemophagocytic lymphohistiocytosis (HLH) in 344 consecutive patients and found that the optimal maximum SF level for the diagnosis of HLH was 3951 ng/mL.

Abioye et al. (2019) collected data from 2,100 pregnant women in Tanzania to determine how capable hematologic biomarkers such as hemoglobin and hepcidin, were at detecting IDA in pregnant woman; hepcidin administration >1.6 µg/L was found to reduce the risk of anemia at delivery by an estimated 49%. This study suggests that both hemoglobin and hepcidin may be helpful in determining iron supplementation needs in “resource-limited countries” (Abioye et al., 2019).

A study by Ismail, Habib, Talaat, Mostafa, and Elghoroury (2019) studied the role of hepcidin in children with b-thalassemia (n = 88 total). The authors measured both serum hepcidin and SF levels as well as determined the hepcidin:ferritin ratio. As expected, serum hepcidin significantly correlated with the hepcidin:ferritin ratio, but the authors reported that there was no statistically significant difference in serum hepcidin levels between splenectomized and non-splenectomized patients. Serum hepcidin levels were more elevated in individuals with □-thalassemia, especially those with □-thalassemia major (bTM), than in control patients (21.74 ng/mL and 13.01 ng/mL, respectively). The authors conclude, “Knowing that hepcidin in serum has a dynamic and multi-factorial regulation, individual evaluation of serum hepcidin and follow up, e.g. every 6 months could be valuable, and future therapeutic hepcidin agonists could be helpful in management of iron burden in such patient (Ismail et al., 2019).”

Yuniati et al. (2019) studied the association between maternal vitamin D, ferritin, and hemoglobin levels during the first trimester of pregnancy, and how these factors affected birthweight. Data collected from these women included maternal demography, bloodwork to test ferritin levels, 25(OH) vitamin D results in their first trimester, and the final birthweight of the child after delivery. A total of 203 Indonesian women were followed until delivery; it was determined that neither vitamin D, ferritin or hemoglobin levels significantly impacted birthweights in this study. However, the authors suggest that other unknown variables may be at play here and that nutritional supplementation during pregnancy is still important (Yuniati et al., 2019).

Kwiatek-Majkusiak et al. (2020) investigated the connection between hepcidin and chronic neuroinflammation. Serum hepcidin and IL-6 were found to be involved in the progression of Parkinson’s Disease. Dysregulation in immune/inflammatory pathways, wherein levels of serum hepcidin and IL-6 would be elevated, were not only predictive of neurodegeneration, with IL-6- induced hepcidin expression in astrocytes, microglia, and epithelial cells, but also response to deep brain stimulation treatment (Kwiatek-Majkusiak et al., 2020).

Brandtner et al. (2020) found linkages between serum markers of iron metabolism and prognosis of sepsis survival. Positive correlations were found between increased serum iron and SF levels and severity of organ failure (SOFA score) and mortality. High TSAT, elevated ferritin and serum iron levels, and low transferrin concentrations were associated with decreased chances of survival as well. This indicates the utility of iron metabolism in the context of extreme systemic inflammation; from this study, it was also concluded that TSAT can be a stand-alone predictor of sepsis survival (Brandtner et al., 2020).

Nalado et al. (2020) evaluated the diagnostic validity of GDF-15 and hepcidin as biomarkers of IDA in non-dialysis CKD patients. Serum levels of GDF-15 and hepcidin were measured in 312 non-dialysis CKD patients and 184 healthy control participants in Johannesburg, South Africa. For absolute IDA diagnosis among CKD patients, GDF-15 had a predictive value of 74.02%. For functional IDA diagnosis among CKD patients, hepcidin had a predictive value of 70.1%. The authors concluded that “serum GDF-15 is a potential biomarker of absolute IDA, while hepcidin levels can predict functional IDA among CKD patients” (Nalado et al., 2020).

Phillips et al. (2021) studied how the full blood count (FBC) parameters change in older patients. FBC, mean corpuscular volume (MCV), and red cell distribution width (RDW) test results were compiled from male and female patients aged 1-100 years from the National Health Service in England. In males, the mean hemoglobin concentration increased from birth until age 20, then decreased at a steady rate from age 20 to 70, then decreased at a higher rate after age 70. In females, the mean hemoglobin concentration increased from birth until age 14, then decreased slowly from age 14 to 30, then increased again from age 30 to age 60, and then decreased after the age of 60. Overall, “hemoglobin concentrations in males and females begin to converge after age 60 and equalize by approximately 90 years.” The authors concluded that FBC parameters trend throughout life, particularly “a falling hemoglobin level and rising MCV and RDW with older age” (Phillips, Wood, Weaving, & Chevassut, 2021).Mei et al. (2021) performed a cross-sectional study using data from the US National Health and Nutrition Examination Survey to determine physiologically based SF concentration thresholds for iron deficiency in healthy children (12-59 months) and non-pregnant woman (15-49 years). The study analyzed the relationship between SF and hemoglobin, and the relationship between SF and soluble transferrin receptor. The study resulted in SF concentration thresholds for iron deficiency of “about 20 µg/L for children and 25 µg/L for non-pregnant women.” The authors concluded that “physiologically based thresholds for iron deficiency might be more clinically and epidemiologically relevant than those based on expert opinion” (Mei et al., 2021).

Garcia-Casal et al. (2021) performed a meta-analysis studying the diagnostic accuracy of serum and plasma ferritin concentrations for detecting iron deficiency or overload in primary and secondary iron-loading syndromes. The authors used 72 studies, with a total of 6095 participants, that measured serum or plasma ferritin concentrations. The authors compared ferritin blood tests to iron levels in the bone marrow to diagnose iron deficiency, and compared ferritin blood tests to iron levels in the liver to diagnose iron overload. The authors concluded that at a threshold of 30 µg/L, there “is low-certainty evidence that blood ferritin concentration is reasonably sensitive and a very specific test for iron deficiency.” Additionally, there is “very low certainty that high concentrations of ferritin provide a sensitive test for iron overload in people where this condition is suspected.” The authors note that overall confidence in the studies is low because of potential bias, indirectness, and heterogenous evidence, and that there is insufficient evidence to make conclusions about using ferritin concentrations to diagnose iron deficiency or overload in asymptomatic people (Garcia-Casal, Pasricha, Martinez, Lopez-Perez, & Peña-Rosas, 2021).

Auerbach et al. (2021) performed a study to assess the accuracy of diagnosing IDA using the complete blood cell count (CBC) and reticulocyte hemoglobin equivalent (RET-He) analysis. 556 patients referred for the diagnosis and/or treatment of anemia were studied at baseline, and 150 of the participants were later studied after intravenous iron treatment. RET-He identified iron deficiency with a 68.2% sensitivity and 69.7% specificity. RET-He predicted responsiveness to intravenous iron with 84% sensitivity and 78% specificity. The authors concluded that “CBC and RET-He can identify patients with IDA, determine need for and responsiveness to intravenous iron, and reduce time for therapeutic decisions” (Auerbach, Staffa, & Brugnara, 2021).

Guidelines and Recommendations:

Guidelines and recommendations related to the screening of anemia in certain populations are available; however, published recommendations regarding the use of ferritin as a first-line test in asymptomatic individuals have not been identified.

In regard to NF, “At present, no established guidelines or specific management recommendations for patients with NF have been identified. An individualized symptomatic approach to treatment is recommended (Kumar et al., 2016).” To date, the only NBIA guidelines published concerning diagnosis and management of the condition is pantothenate kinase-associated neurodegeneration (PKAN, formerly called Hallervorden-Spatz syndrome) (Hogarth et al., 2017).

American Gastroenterological Association (Ko et al., 2020)

The AGA has published its official recommendations on the gastrointestinal evaluation of iron deficiency anemia (IDA). It has stated:

1. “In patients with anemia, the AGA recommends using a cutoff of 45 ng/mL over 15 ng/mL when using ferritin to diagnose iron deficiency.

In patients with inflammatory conditions or chronic kidney disease, other laboratory tests, such as C-reactive protein, transferrin saturation, or soluble transferrin saturation, may be needed in conjunction with ferritin to diagnose iron deficiency anemia (Ko et al., 2020).

American Society of Clinical Oncology (ASCO) and the American Society of Hematology (ASH) (ASCO & ASH, 2019; Bohlius et al., 2019)

The ASCO and ASH have published guidelines regarding the management of cancer-related anemia with erythropoiesis-stimulating agents (ESAs). It is stated that "With the exception of selected patients with MDS, ESAs should not be offered to most patients with nonchemotherapy-associated anemia. During ESA treatment, hemoglobin may be increased to the lowest concentration needed to avoid transfusions. Iron replacement may be used to improve hemoglobin response and reduce RBC transfusions for patients receiving ESA with or without ID. Baseline and periodic monitoring of iron, total iron-binding capacity, transferrin saturation, or ferritin levels is recommended" (Bohlius et al., 2019).

American Academy of Family Physicians (AAFP) (Lanier, Park, & Callahan, 2018)

The AAPF have recommend the following with "C" evidence ratings (consensus, disease-oriented evidence, usual practice, expert opinion, or case series):

- "A low serum ferritin level is associated with a diagnosis of iron deficiency anemia,"
- "Older patients with suspected iron deficiency anemia should undergo endoscopy to evaluate for occult gastrointestinal malignancy," and
- "Low-dose formulations of iron (15 mg of elemental iron) can be effective for treatment of suspected iron deficiency anemia and have a lower risk of adverse effects than standard preparations" (Lanier et al., 2018).

Also stated is: "Patients with an elevated serum ferritin level or macrocytic anemia should be evaluated for underlying conditions, including vitamin B12 or folate deficiency, myelodysplastic syndrome, and malignancy" (Lanier et al., 2018).

The Endocrine Society (Bhasin et al., 2018)

The Endocrine Society's 2018 guidelines on testosterone therapy in men with hypogonadism state, "In men deemed to have secondary hypogonadism, additional diagnostic evaluations may be needed to exclude hyperprolactinemia, head trauma, iron overload syndromes, hypothalamic or pituitary tumors, and other infiltrative or destructive hypothalamic–pituitary diseases, as well as genetic disorders associated with gonadotropin deficiency. Measuring serum prolactin and iron saturation and/or serum ferritin can help determine the presence of hyperprolactinemia and iron overload syndromes, respectively" (Bhasin et al., 2018).

The American College of Gastroenterology (ACG) (Kwo, Cohen, & Lim, 2017)

ACG practice guidelines regarding the evaluation of abnormal liver chemistries recommend that "All patients with abnormal liver chemistries in the absence of acute hepatitis should undergo testing for hereditary hemochromatosis with an iron level, transferrin saturation, and serum ferritin [Strong recommendation, very low level of evidence]" (Kwo et al., 2017).

World Health Organization (WHO) (WHO, 2020)

The WHO guideline on the use of ferritin concentrations to assess iron status in individuals and populations, published in 2020, updated the previous serum ferritin levels recommendations. The guidelines recommend cut-off serum ferritin levels for iron deficiency in infants (0-23 months) and preschool children (24-59 months) as under 12 µg/L in apparently healthy individuals and under 30 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as under 15 µg/L in apparently healthy individuals and under 70 µg/L in individuals with infections or inflammation. The guidelines recommend cut-off serum ferritin levels for iron deficiency in apparently healthy pregnant women in their first trimester as under 15 µg/L.

The guidelines recommend cut-off serum ferritin levels for risk of iron overload in school age children (5-12 years), adolescents (13-19 years), adults (20-59 years), and older persons (over 60 years) as over 150 µg/L in apparently healthy individuals females, over 200 µg/L in apparently healthy males, and over 500 µg/L in individuals with infections or inflammation.

International Consensus Guideline for Clinical Management of Pantothenate Kinase-Associated Neurodegeneration (PKAN) (Hogarth et al., 2017)

An international group released guidelines concerning the clinical management of the NBIA condition PKAN in 2017. Although no specific recommendation is directly given regarding measurement of iron, Hogarth et al. (2017) state, “The role that iron plays in PKAN pathogenesis is still unclear because iron dyshomeostasis is a secondary phenomenon in this disorder. Nevertheless, high iron levels develop in globus pallidus and probably contribute to cell and tissue damage. The utility of iron chelators has been limited by systemic iron depletion. Newer agents more readily cross the blood-brain barrier yet have a lower affinity for iron, thereby minimizing systemic iron loss.” Concerning diagnosis of PKAN, “People suspected to have PKAN based on clinical features should undergo brain MRI using iron sensitive sequences such as SWI, GRE, T2* as a first line diagnostic investigation to identify the characteristic changes. The MRI abnormality, called the ‘eye-of-the-tiger’ sign, is observed on T2-weighted imaging and consists of hypointense signal in the globus pallidus surrounding a region of hyperintense signal” (Hogarth et al., 2017).

International Consensus Statement on the Peri-operative Management of Anemia and Iron Deficiency (Munoz, Gomez-Ramirez, et al., 2017)

An expert workshop, including a number of experienced researchers and clinicians, was conducted to develop a guidance for the diagnosis and management of anemia in surgical patients. A series of best-practice and evidence-based statements to advise on patient care with respect to anemia have been published via this workshop. It was stated that serum ferritin measurement is the most sensitive and specific test used for the identification of absolute iron deficiency (Munoz, Acheson, et al., 2017).

European Crohn’s and Colitis Organisation (ECCO) (Dignass et al., 2015)

ECCO guidelines published in 2015 concerning iron deficiency and anemia in IBD with an EL-5 recommendation state, “For laboratory screening, complete blood count, serum ferritin, and C-reactive protein [CRP] should be used. For patients in remission or mild disease, measurements should be performed every 6 to 12 months. In outpatients with active disease such measurements should be performed at least every 3 months” (Dignass et al., 2015). Also mentioned in the section concerning the workup for anemia with an EL-4 recommendation is that anemia workups “should be initiated if the hemoglobin is below normal. The minimum workup includes red blood cell indices such as red cell distribution width [RDW] and mean corpuscular volume [MCV], reticulocyte count, differential blood cell count, serum ferritin, transferrin saturation [TfS], and CRP concentration. More extensive workup includes serum concentrations of vitamin

B, folic acid, haptoglobin, the percentage of hypochromic red cells, reticulocyte hemoglobin, lactate dehydrogenase, soluble transferrin receptor, creatinine, and urea" (Dignass et al., 2015).

The U.S. Preventive Services Task Force (USPSTF, 2015a, 2015b)

The USPSTF states, "the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in pregnant women to prevent adverse maternal health and birth outcomes; the current evidence is insufficient to assess the balance of benefits and harms of routine iron supplementation for pregnant women to prevent adverse maternal health and birth outcomes; the current evidence is insufficient to assess the balance of benefits and harms of screening for iron deficiency anemia in children ages 6 to 24 months" (USPSTF, 2015a, 2015b). All recommendations have been given a grade I.

American Society of Hematology (ASH) (Wood, 2014)

In the ASH *Guidelines for Quantifying Iron Overload*, it is stated that "Despite improved availability of advanced imaging techniques, serum ferritin remains the mostly commonly used metric to monitor iron chelation therapy and remains the sole metric in many countries. Serum ferritin measurements are inexpensive and generally correlate with both total body iron stores and clinical outcomes… Given interpatient and temporal variability of serum ferritin values, serum ferritin is best checked frequently (every 3-6 weeks) so that running averages can be calculated; this corrects for many of the transient fluctuations related to inflammation and liver damage." Regarding the use of transferrin, the guidelines also state that "Iron that is bound to transferrin is not redox active, nor does it produce extrahepatic iron overload. However, once transferrin saturations exceed 85%, non-transferrin-bound iron (NTBI) species begin to circulate, creating a risk for endocrine and cardiac iron accumulation. A subset of NTBI can catalyze Fenton reactions and is known as labile plasma iron (LPI). Therefore, transferrin saturation, NTBI, and LPI are potentially attractive serum markers for iron toxicity risk. Transferrin saturation is widely available, but values cannot be interpreted if iron chelator is present in the bloodstream, so patients have to be instructed to withhold iron chelation for at least 1 day before measurement… Although some studies link elevated LPI to cardiac iron accumulation, large validation studies are lacking. Therefore, to date, these metrics remain important and interesting research tools, but are not suitable for routine monitoring" (Wood, 2014). Within the conclusion of the paper, the author notes that "Serum markers of somatic stores (ferritin and transferrin saturation) are useful surrogates for total iron stores and extrahepatic risk, respectively. However, they cannot replace LIC or cardiac T2* assessment for monitoring chelator efficacy or stratifying end organ risk" (Wood, 2014).

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) (Kliger et al., 2013)

The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (KDOQI) published guidelines in 2012. In 2013, the Kidney Disease: Improving Global Outcomes (KDIGO) group reviewed these guidelines in a separate publication. Based on the suggestions made by the KDOQI, the KDIGO "continued to recommend the use of serum ferritin concentration and transferrin saturation (TSAT) to define iron stores and iron availability. For all their imperfections, these metrics remain our best routinely-available tools to assess iron status and manage iron supplementation. In the absence of superior, cost-effective, and easily applicable alternatives, this approach seems reasonable" (Kliger et al., 2013).

Further, the KDOQI stated that ferritin testing along with TSAT as part of the evaluation of iron status in individuals with chronic kidney disease who are being treated for anemia is recommended. Also, in agreement with KDIGO, the KDOQI recommend testing prior to initiation of treatment, once per month during initial treatment, and at least every 3 months after a stable hemoglobin level is reached.

Kidney Disease Improving Global Outcomes (KDIGO, 2012)

In the *2012 KDIGO Clinical Practice Guideline for Anemia in Chronic Kidney Disease* publication, a complete blood count, absolute reticulocyte count, serum ferritin, serum transferrin saturation (TSAT), serum vitamin B_{12}, and serum folate levels are recommended as part of an initial evaluation of anemia for all CKD patients, regardless of age or stage of degree progression. Moreover, for patients undergoing ESA therapy, "including the decision to start or continue iron therapy," both TSAT and ferritin should be tested at least every 3 months; TSAT and ferritin should be tested "more frequently when initiating or increasing ESA dose, when there is blood loss, when monitoring response after a course of IV iron, and in other circumstances where iron stores may become depleted" (KDIGO, 2012).

International Society of Nephrology (ISN) (Madore et al., 2008)

The most recent guidelines from the ISN, released in 2008, state that for CKD patients "who require iron and/or ESA therapy, measurement of serum ferritin and transferrin saturation every 1-3 months is reasonable, depending upon the clinical status of the patient, the hemoglobin response to iron supplementation, the ESA dose, and recent iron status test results; in stable patients with mild anemia (hemoglobin >110 g/l) who are not receiving iron or ESA therapy, assessment of iron status could be performed less frequently, potentially on a yearly basis" (Madore et al., 2008).

Applicable State and Federal Regulations:

A search of the FDA Devices databases on 09/28/2021 for "ferritin" yielded 125 records of approved products.

Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Applicable CPT/HCPCS Procedure Codes:

CPT	Code Description
82728	Ferritin
83540	Iron
83550	Iron binding capacity
84466	Transferrin
0024U	Glycosylated acute phase proteins (GlycA), nuclear magnetic resonance spectroscopy, quantitative

CPT	Code Description
0251U	Hepcidin-25, enzyme-linked immunosorbent assay (ELISA), serum or plasma Proprietary test: Intrinsic Hepcidin IDx™ Test Lab/Manufacturer: IntrinsicDx

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

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Revision History

Company(ies)	DATE	REVISION
EmblemHealth **ConnectiCare**	11/2022	• Reformatted and reorganized policy, transferred content to new template with new Reimbursement Policy Number