# Iron Homeostasis and the Pathophysiology and Management of Iron Deficiency

Gordon D. McLaren, Roman L. Kleynberg, and Gregory J. Anderson

## Introduction

Iron is an essential element and is required for the synthesis of hemoglobin as well as multiple other proteins in all body cells. Iron in excess of needs is stored in reticuloendothelial cells in the liver, spleen, and bone marrow and in hepatocytes.

Under normal conditions, body iron stores remain relatively constant. In humans, there is no mechanism for active iron excretion, so the regulation of iron balance depends on the control of intestinal iron absorption. Most dietary iron absorption takes place through duodenal enterocytes. Ferrous iron ($Fe^{2+}$) crosses the enterocyte brush border via divalent metal-ion transporter 1 (DMT 1). It is subsequently exported across the basolateral membrane through the transporter ferroportin. The iron oxidase hephaestin increases the efficiency of this process and converts ferrous iron to the ferric ($Fe^{3+}$) form. Plasma ferric iron is transported bound to transferrin and delivered to erythroid precursors in the bone marrow and to other cells throughout the body. The delivery of enterocyte iron to the systemic circulation is controlled by hepcidin, a liver-derived peptide that binds ferroportin causing it to be internalized and degraded. When iron stores are depleted, hepcidin expression is

G.D. McLaren, MD (✉)
Hematology/Oncology Section (11/111-H), Department of Veterans Affairs, Long Beach Healthcare System, 5901 East Seventh Street, Long Beach, CA 90822, USA

Division of Hematology/Oncology, Department of Medicine, University of California, Irvine, Orange, CA, USA
e-mail: gordon.mclaren@va.gov

R.L. Kleynberg, MD
Department of Veterans Affairs, Long Beach Healthcare System, 5901 East Seventh Street, Long Beach, CA 90822, USA

Division of Hematology/Oncology, Department of Medicine, University of California, Irvine, Orange, CA, USA
e-mail: rkleynbe@uci.edu

G.J. Anderson, PhD
Iron Metabolism Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia

School of Medicine and School of Chemistry and Molecular Bioscience, University of Queensland, Brisbane, Australia
e-mail: greg.anderson@qimrberghofer.edu.au


S.A. Abutalib et al. (eds.), *Nonmalignant Hematology*, DOI 10.1007/978-3-319-30352-9_2

decreased. This in turn increases the ferroportin concentration on the basolateral membrane and thereby dietary iron absorption.

Hepcidin also controls systemic iron exchange, as ferroportin is expressed on the surface of macrophages and hepatocytes. Iron removed from senescent erythrocytes within the reticuloendothelial system is released via ferroportin to the plasma and recycled to developing erythrocytes in the bone marrow and to other tissues. The decreased hepcidin level in iron deficiency allows increased ferroportin expression and rapid release of iron to the plasma.

Iron deficiency is the most common nutritional disorder worldwide (World Health Organization 2000 [WHO/NHD/00.7]). In developed countries, iron deficiency is most often the result of blood loss, although some cases result from iron malabsorption.

## Case 1: Clinical Presentation, Diagnosis, and Treatment of Microcytic Anemia

A 47-year-old man complains of weakness and occasional dizziness beginning several days previously. Physical examination shows pallor and pale conjunctivae.

| | |
|---|---|
| *Hematology laboratory report:* | |
| Hemoglobin (Hb) = 6.3 g/dL | MCV = 70 fL |
| Hct = 0.21 | MCH = 21 pg |
| RBC = $3.0 \times 10^{12}$/L | MCHC = 30 g/dL |
| Reticulocytes = 4.0 % | RDW = 19.7 % |
| WBC = $7.0 \times 10^9$/L (normal: 4.5–10.0 × $10^9$/L) | |
| Platelets = $400 \times 10^9$/L (normal: 140–440 × $10^9$/L) | |
| *Peripheral blood smear:* | |
| RBC | Marked microcytosis and hypochromia with moderate variation in size (anisocytosis) and shape (poikilocytosis). No basophilic stippling. No increase in polychromatophilia |
| WBC | Normal number and morphology |
| Platelets | Normal |

**Question 1. What is the condition most likely to be associated with these findings?**

A. Beta-thalassemia minor
B. Iron deficiency anemia
C. Anemia of inflammation
D. Refractory anemia with ringed sideroblasts (RARS)

**Expert Perspective** The recent onset of symptoms suggests a relatively acute process such as blood loss resulting in iron deficiency. Beta-thalassemia minor and RARS are more chronic. Beta-thalassemia minor and the anemia of inflammation (anemia of chronic disease) are associated with less severe anemia. Iron deficiency anemia (IDA) is suggested by the combination of a low mean cell volume (MCV) and an elevated red cell distribution width (RDW), the latter being the earliest indicator in the CBC of the onset of IDA. Some automated cell counters measure mean reticulocyte cellular hemoglobin content (CHr), which can indicate early iron deficiency, before the development of anemia (Ullrich et al. 2005). Changes in the complete blood count (CBC) in patients with iron deficiency anemia are contrasted with those seen in the anemia of inflammation in Table 1. In beta-thalassemia minor, the RDW is normal despite a low MCV. Similarly, in the anemia of inflammation, the RDW is typically normal, and the MCV is normal or slightly decreased (Gangat and Wolanskyj 2013). RARS is a myelodysplastic syndrome associated with a normal to increased MCV.

**Question 2. Which of the following would not be an appropriate test or combination of tests to confirm a diagnosis of iron deficiency?**

A. Serum ferritin
B. Serum ferritin, serum iron, and total iron-binding capacity (TIBC)
C. Serum ferritin, serum iron, TIBC, and serum hepcidin
D. Serum ferritin, serum iron, TIBC, and serum transferrin receptors

**Expert Perspective** A low serum ferritin alone is diagnostic of iron deficiency. Iron studies, i.e.,

**Table 1** Typical changes in the complete blood count with iron deficiency anemia and the anemia of inflammation

| Condition | Degree of anemia | Mean corpuscular volume | Red cell distribution width | White blood cells | Platelets |
|---|---|---|---|---|---|
| Iron deficiency anemia | Mild to severe | Decreased | Increased | Normal | Normal to increased |
| Inflammation | Mild | Normal to decreased | Normal | Normal to increased | Normal to increased |

From: Rakel and Bope (2002), with permission

**Table 2** Typical changes in measures of iron status in iron deficiency and inflammation

| Condition | Serum iron | Total iron-binding capacity | Transferrin saturation | Serum ferritin | Serum transferrin receptors |
|---|---|---|---|---|---|
| Iron deficiency | Decreased | Increased | Decreased | Decreased | Increased |
| Inflammation | Decreased | Decreased | Decreased | Normal to increased | Normal |

From: Rakel and Bope (2002), with permission

serum iron and TIBC, can also be helpful in the diagnosis of iron deficiency, as the combination of a low serum iron, elevated TIBC, and low transferrin saturation (usually <15 %) is characteristic. Use of the combination of serum ferritin and iron studies can also be helpful when both iron deficiency and inflammation are present, as signaled by a low or low-normal TIBC. In this situation, serum ferritin may be normal, but a concentration >200 μg/L, even in the presence of inflammation, is unusual in patients with iron deficiency (Cook 1982). The measurement of serum transferrin receptors (TfR) is also helpful in this situation because the level typically is not affected in inflammation, and an elevated level is consistent with iron deficiency (Punnonen et al. 1997; Lok and Loh 1998; Skikne et al. 2011). These changes in tests of iron status in iron deficiency anemia and the anemia of inflammation are summarized in Table 2. Measurement of CHr, where available, is also useful, as it has equivalent sensitivity to TfR in detecting iron deficiency anemia (Markovic et al. 2007). Although methods are available for measuring serum hepcidin levels (Thomas et al. 2011), this test has not yet become generally available in most clinical settings, and there is a need for better standardization (Kroot et al. 2009). Expression of hepcidin in the liver is regulated by body iron requirements that, at least in part, reflect the degree of iron saturation of circulating transferrin (Wilkins et al. 2006). When iron stores are depleted, liver hepcidin production is low, resulting in low circulating levels of the hormone (Ganz 2013). The pathways of hepcidin regulation in the liver are depicted in Fig. 1. With greater availability of standardized hepcidin assays anticipated in the near future, measurement of serum hepcidin likely will become an important addition to the armamentarium of tests for assessment of iron stores. A definitive diagnosis of iron deficiency can be made on the basis of an absence of stainable iron in the bone marrow, but this is rarely necessary given the noninvasive tests available. A retrospective confirmation of the diagnosis of iron deficiency can be made on the basis of an increase in hemoglobin with iron replacement therapy.

| *Report of serum biochemical tests:* |
|---|
| Serum ferritin = 5 μg/L |
| Transferrin saturation = 3.3 %, with decreased serum iron (15 μg/dL) and increased TIBC (450 μg/dL) |

**Question 3.** The diagnosis of iron deficiency anemia is now established. The patient denies any symptoms of peptic ulcer disease, change in bowel habits, or rectal bleeding. He is given six stool cards for fecal occult blood testing (FOBT), and three of them are positive.

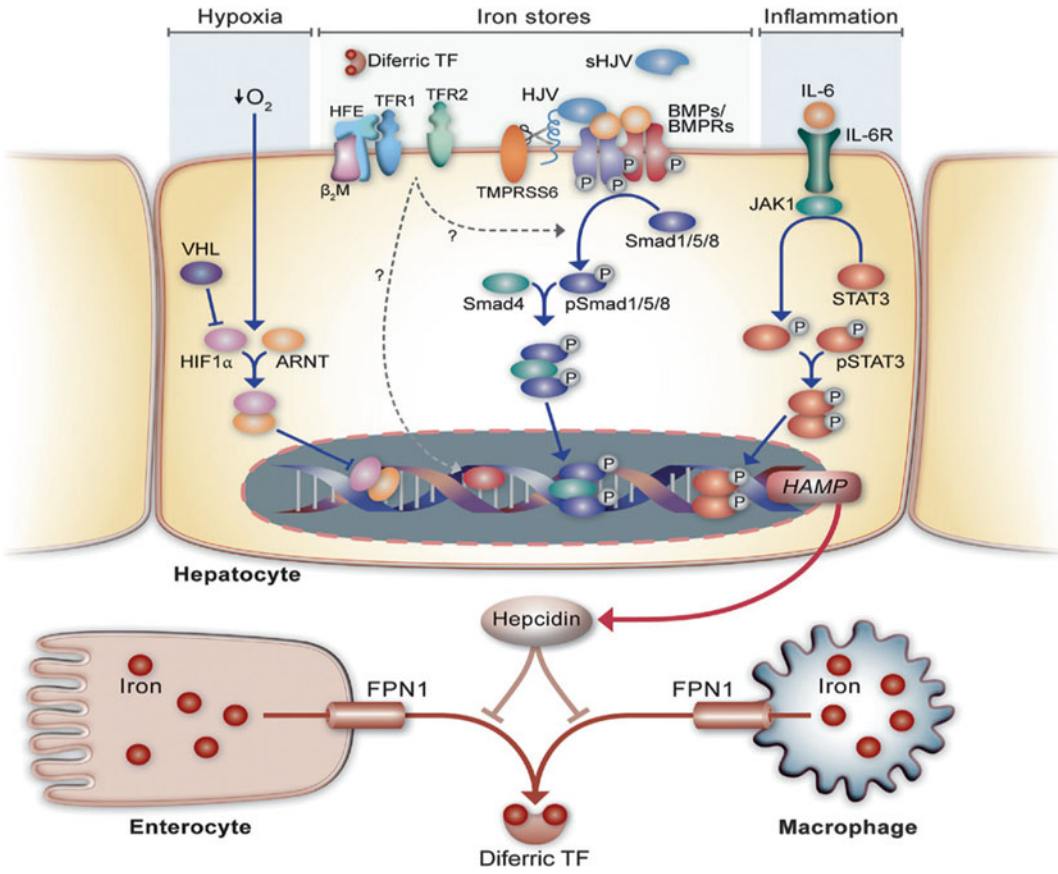


**Fig. 1** Pathways for the regulation of hepcidin in hepatocytes. A variety of systemic stimuli that reflect body iron requirements act on hepatocytes to alter the expression of the *HAMP* gene. The *BMP/SMAD* signaling pathway appears to play a central role in *HAMP* regulation. Proteins such as *HJV* and *TMPRSS6*, which are defective in human disorders of iron homeostasis, act through this pathway to increase or decrease hepcidin expression, respectively. *HFE* and *TFR2* are also mutated in human iron-loading disorders, but precisely how they alter *HAMP* expression is unclear. The effects of hypoxia and inflammatory cytokines are better defined. Hepcidin secreted by hepatocytes into the circulation travels to enterocytes, macrophages, and other cell types to determine how much iron they release into the plasma (From: Collins and Anderson 2012, with permission)

**Which of the following statements about gastrointestinal (GI) blood loss and evaluation of the etiology is correct?**

A. The patient should have endoscopic evaluation of the GI tract to identify a possible source of bleeding.
B. If all six FOBT results had been negative, further evaluation such as endoscopy would have been unnecessary.
C. Iron deficiency in patients on long-term anticoagulation with warfarin occurs only when there is an identifiable site of blood loss in the GI tract.
D. Blood loss from the GI tract is the only cause of iron deficiency anemia in men.

**Expert Perspective** The positive FOBT indicates the need for endoscopic evaluation of the GI tract to identify the source of blood loss. Even if there had been no evidence of blood loss by FOBT, endoscopic evaluation would still be indicated, because intermittent blood loss can be missed by FOBT. Although patients who have GI blood loss while taking anticoagulants usually are found to have an

identifiable source of bleeding, patients receiving long-term anticoagulation with warfarin can develop iron deficiency that is not associated with a specific lesion (Chen et al. 2014). The GI tract is the most common source of blood loss in men and postmenopausal women with iron deficiency. Bleeding from other sources can lead to iron deficiency anemia as well, including blood loss associated with the urothelial tract, hemobilia, or severe, recurrent epistaxis. Other causes include frequent blood donation, intravascular hemolysis such as in paroxysmal nocturnal hematuria (PNH) and elite athletes, and gastric resection (Skikne and Hershko 2012). Rarely, a picture of iron deficiency can develop in children with pulmonary hemosiderosis. (Causes of iron malabsorption are discussed in Case 2.)

The patient undergoes an esophagogastroduodenoscopy (EGD), which is normal. A colonoscopy shows a 2 cm polyp in the mid-transverse colon that is resected. Follow-up stool cards show six of six to be negative for occult blood.

**Question 4. Which of the following approaches to the treatment of the patient's iron deficiency anemia is appropriate?**

A. Transfusion of two units of packed red blood cells to raise the blood hemoglobin level above 8 g/dL
B. Intramuscular injection of iron dextran
C. Administration of an intravenous iron preparation
D. Oral iron replacement therapy with an iron salt such as ferrous sulfate for 6 months

**Expert Perspective** Transfusion of red blood cells for patients with iron deficiency anemia is rarely necessary. It is usually sufficient to administer iron replacement therapy. Patients who have developed anemia subacutely develop a compensatory mechanism by increasing production of 2,3-DPG, thereby shifting the oxyhemoglobin dissociation curve such that oxygen is released more readily (Tsai et al. 2010). In rare cases when the hemoglobin level is extremely low and the patient is suffering hemodynamic instability, red blood cell transfusions may be required, and it may be necessary under these conditions to administer the transfusions with close central venous pressure monitoring. In the case under discussion, the symptoms are relatively mild and the hemoglobin level is not so low that blood transfusion is necessary. Intramuscular administration of iron dextran is not necessary in patients whose GI tract is functioning normally and who are able to tolerate oral iron. Similarly, administration of intravenous iron usually is unnecessary in most cases of iron deficiency anemia, as oral iron replacement is typically effective. Oral iron preparations often are initially taken with meals, but if tolerated, it is preferable to take iron on an empty stomach, as absorption is better. Some clinicians recommend concomitant administration of vitamin C, which enhances absorption by binding iron in the acidic environment of the stomach for transport to the more alkaline duodenum where most iron absorption takes place (Collins and Anderson 2012; Gulec et al. 2014). Absorption of oral iron is enhanced in iron deficiency anemia (Cook et al. 1990), which facilitates iron replacement. The mechanism of this enhanced absorption is the result of upregulation of the duodenal iron transport molecules: divalent metal-ion transporter 1 (DMT1) and ferroportin (Fig. 2), as regulation of these transporters is iron dependent (Garrick and Garrick 2009; Theil 2011). This regulation favors a rapid increase in DMT1 expression in response to decreased iron availability, thereby quickly increasing the capacity of duodenal enterocytes to take up dietary iron. At the same time, decreased hepcidin production by the liver in response to iron deficiency permits increased ferroportin expression on the basolateral membrane of duodenal enterocytes, and this permits rapid transfer of iron to the systemic circulation to supply the need for iron by developing red blood cells in the bone marrow. The enhanced iron absorption under iron-deficient conditions thus facilitates correction of the hemoglobin deficit.

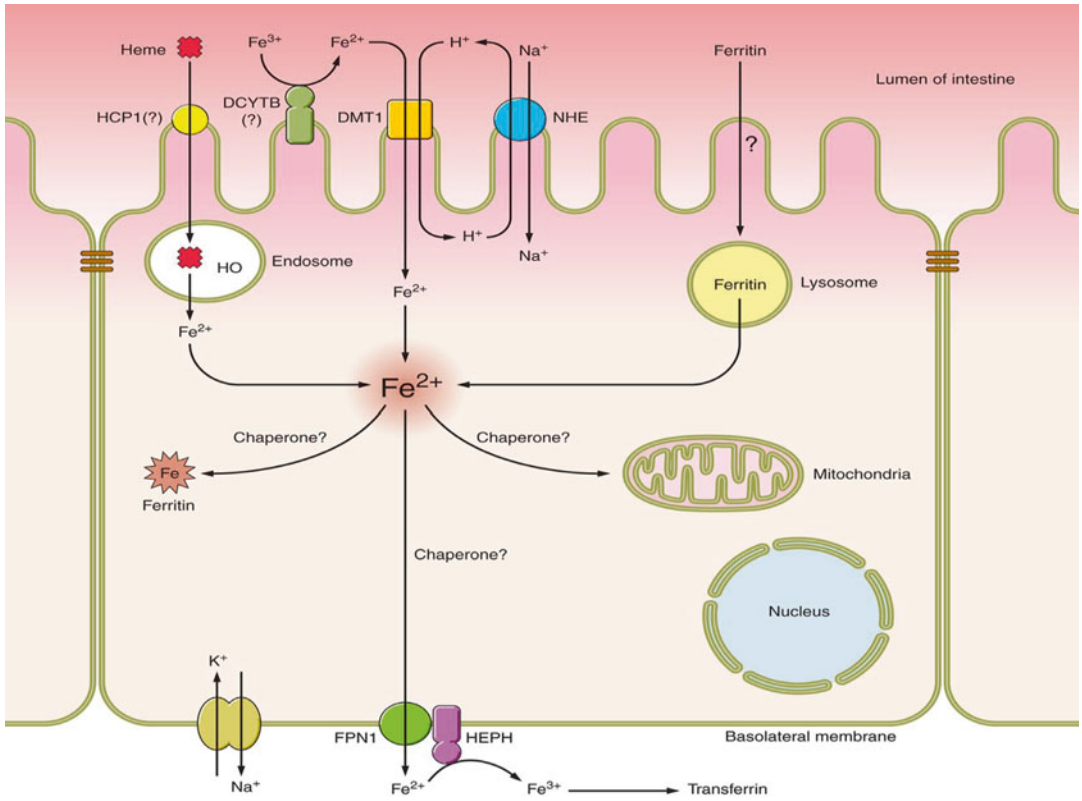


**Fig. 2** Mechanisms of iron absorption in the mammalian duodenum. A single enterocyte is depicted with the transport machinery responsible for assimilation of dietary iron. Iron may be derived from heme or ferritin or it may occur as free nonheme iron. Heme iron transport is probably mediated by endocytosis of heme followed by iron liberation from heme within endosomes by heme oxygenase (*HO*). How heme traverses the brush border or endosomal membrane has yet to be elucidated. Nonheme ferric iron must be reduced, possibly by duodenal cytochrome b (*DCYTB*) or other cell surface ferrireductases, and subsequently transported into cells via divalent metal-ion transporter 1 (*DMT1*). The proton gradient that fuels DMT1 activity is maintained by the combined actions of an apical sodium/hydrogen exchanger (*NHE*) and the basolateral Na_-K_-ATPase. Iron from ferritin is absorbed into enterocytes via an unknown mechanism and is likely then freed within lysosomes. Iron derived from all three dietary sources likely forms a single intracellular iron pool. Whether iron chaperones exist in enterocytes is unknown and thus how iron traffics within cells after absorbance is not clear. Iron destined for export traverses the basolateral membrane (BLM) via ferroportin 1 (*FPN1*). The exit of ferrous iron is functionally coupled with iron oxidation via hephaestin (*HEPH*) and possibly other ferroxidases. Ultimately, ferric iron then binds to transferrin in the interstitial fluids or in the vasculature and is distributed throughout the body (From: Gulec et al. 2014, with permission)

## Case 2: Approach to Diagnosis and Treatment of Refractory Iron Deficiency Anemia

A 40-year-old man complains of shortness of breath with exertion. On physical examination, vital signs are normal, but the patient has obvious pallor, with pale conjunctivae and nail beds.

| *Hematology laboratory report:* | |
|---|---|
| Hb = 8.5 g/dL | MCV = 72 fL |
| Hct = 0.25 | MCH = 23 pg |
| RBC = $3.1 \times 10^{12}$/L | MCHC = 31 g/dL |
| Reticulocytes = 3.0 % | RDW = 18.5 % |
| WBC = $8.0 \times 10^9$/L (normal: 4.5–$10.0 \times 10^9$/L) | |
| Platelets = $450 \times 10^9$/L (normal: 140–$440 \times 10^9$/L) | |

| *Peripheral blood smear:* | |
|---|---|
| RBC | Microcytosis and hypochromia, with increased anisocytosis and poikilocytosis, including "pencil-shape" cells. No polychromatophilia |
| WBC | Normal morphology |
| Platelets | Slightly increased |
| *Report of serum biochemical tests:* | |
| Serum ferritin = 8 μg/L | |
| Transferrin saturation = 3.9 %, with decreased serum iron (18 μg/dL) and increased TIBC (465 μg/dL) | |

The patient states that he consumes a normal western diet and denies hematochezia, melena, hematuria, or epistaxis. He is referred to a hematologist, who starts oral iron replacement therapy. After 6 weeks of oral iron therapy, while the patient is awaiting an appointment with a gastroenterologist, it is noted that the hemoglobin is 8.7 g/dL, essentially unchanged from the time of diagnosis.

**Question 5. Which of the following are possible causes of the lack of response to oral iron therapy?**

A. Celiac disease
B. Autoimmune atrophic gastritis
C. *Helicobacter pylori* infection
D. Inadequate adherence to oral iron therapy
E. All of the above

**Expert Perspective** Generally, a response to oral iron therapy would be expected within 4–6 weeks. An early indication can be seen within 7–10 days by examining the peripheral blood film for the appearance of polychromasia attributable to shift reticulocytes. Automated detection of a response is also available, by using CHr (Hershko and Camaschella 2014). A lack of response to oral iron therapy can be attributable to a variety of etiologies, including nonadherence to the medication regimen. Patients who have taken oral iron as prescribed and still fail to respond are considered to have refractory iron deficiency anemia. It is important to exclude concomitant conditions such as ACD, and measurement of the serum C-reactive protein (CRP) can be helpful in detecting ACD that does not have a clinically obvious cause. Other conditions that should be excluded are continued blood loss, factitious anemia, or use of proton pump inhibitors, which diminish gastric acid secretion and thereby impair iron absorption (Zhu et al. 2010). Iron malabsorption can occur in a number of other conditions, including *H. pylori* infection, autoimmune gastritis, celiac disease, and hereditary microcytic anemias.

**Question 6. Appropriate tests to identify the cause of refractory iron deficiency anemia in this case include all of the following except:**

A. *H. pylori* IgG antibodies
B. *H. pylori* fecal antigen
C. *TPRSS6* gene sequencing
D. Serum gastrin
E. Anti-endomysial antibodies or anti-TTG IgA antibody activity

**Expert Perspective** In a prospective study of patients with refractory IDA referred to a hematology outpatient clinic (Hershko et al. 2005), adult celiac disease was identified in 5 % and autoimmune atrophic gastritis was found in 26 %, about half of whom had coexistent *H. pylori* infection. *H. pylori* infection was detected in 55 % of the entire group. *H. pylori* infection alone was found in 19 %. About two-thirds of the patients with either autoimmune atrophic gastritis or *H. pylori* infection were refractory to oral iron treatment, and 100 % of patients with celiac disease were refractory.

It is recommended that all patients referred for unexplained refractory IDA should be tested for celiac disease, *H. pylori* infection, and autoimmune atrophic gastritis. This subject has recently been reviewed (Hershko and Camaschella 2014). In young patients or children with a microcytic hypochromic anemia refractory to oral iron treatment, but with a serum ferritin that is higher than would be expected in iron deficiency, a genetic evaluation is appropriate. The largest numbers of such cases reported (about 40) have mutations in the gene for transmembrane protease, serine 6

(*TMPRSS6*), which encodes matriptase-2, a transmembrane serine protease thought to cleave hemojuvelin, an activator of hepcidin expression (Fig. 2). In a genome-wide association study (GWAS), variants of *TMPRSS6* were associated with variations in hemoglobin levels (Chambers et al. 2009), and *TMPRSS6* variants have been associated with an increased risk of iron deficiency anemia (An et al. 2012). In a GWAS of persons with iron deficiency and control subjects, a *TMPRSS6* polymorphism was associated with serum biochemical iron measurements (McLaren et al. 2012). Iron-refractory IDA (IRIDA) is an autosomal recessive condition associated with *TMPRRS6* mutations, and diagnosis requires sequencing the exons and exon-intron boundaries of the *TMPRRS6* gene (Bertoncini et al. 2011). In this patient, the serum ferritin is low, as expected, which is not consistent with IRIDA. In addition, the patient is somewhat older than the usual age group in which IRIDA is seen.

The pathogenesis of *H. pylori*-associated IDA may be multifactorial, including occult GI blood loss and decreased iron absorption, possibly secondary to changes in the composition of gastric juice, including reduced gastric acidity. The diagnosis of *H. pylori* infection can be accomplished either by serology for *H. pylori* IgG antibodies or testing for fecal antigen. Patients having a positive result should have it confirmed by a urease breath test. Demonstration of *H. pylori* gastritis by endoscopic examination and biopsy is not mandatory. Patients with serologic evidence of celiac disease should have a duodenal biopsy and testing for HLA-DQ2 and –DQ8 genotypes. Studies have shown that there is an increased prevalence of serologic evidence for celiac disease in Caucasians but not Hispanics, suggesting that a personalized approach may be indicated in selecting tests for diagnostic evaluation of suspected refractory IDA (Murray et al. 2013). Patients with increased serum gastrin and anti-parietal cell or anti-intrinsic factor antibodies should be evaluated by EGD with mucosal biopsy.

**Case Continues** The patient is found to be positive for *H. pylori* IgG antibodies and has a positive urease breath test.

**Question 7. What is the most appropriate approach to treating the patient based on this diagnosis?**

A. Because the patient is unable to absorb oral iron, he should be treated with iron intravenously.
B. Transfusions of red blood cells should be administered, as the patient likely is bleeding from a peptic ulcer and may become hemodynamically unstable.
C. Treatment with a proton pump inhibitor should be started immediately to suppress gastric acid production.
D. So-called "triple therapy" should be administered to eradicate *H. pylori* infection.

**Expert Perspective** *H. pylori* infection can be effectively treated with triple therapy using a proton pump inhibitor plus the antibiotics clarithromycin and amoxicillin (Caselli et al. 2007; Malfertheiner et al. 2007; Fock et al. 2009). Although there may be a component of GI blood loss in patients having *H. pylori* infection, this is not an acute situation in most patients with refractory IDA. Administration of a proton pump inhibitor alone would not treat the underlying problem of the *H. pylori* infection.

Treatments for other causes of refractory IDA similarly target the underlying mechanism of the disease. Celiac disease is treated by adherence to a gluten-free diet, although iron replacement is best accomplished with intravenous iron (Mearin et al. 2010; Auerbach et al. 2013). There is no specific treatment for autoimmune atrophic gastritis, but *H. pylori* eradication in patients with *H. pylori* positivity results in improved gastric acid secretion, and remission of atrophic gastritis occurs in some (Annibale et al. 2002; Ito et al. 2002; Mera et al. 2005; Kodama et al. 2012). In patients with IRIDA, long-term treatment with oral iron may partially or completely correct the anemia (Cau et al. 2012; Khuong-Quang et al. 2013); IV iron has also been used.

**Question 8. Which of the following statements is correct regarding *H. pylori* eradication and iron replacement therapy?**

A. All patients will require oral iron therapy to achieve normal hemoglobin.
B. The patient should receive "total-dose" IV iron replacement to correct the anemia and replenish iron stores.
C. Successful eradication of *H. pylori* infection is associated with a restored ability to absorb iron, and the patient likely will respond to oral iron replacement therapy with correction of anemia.
D. Patients with *H. pylori* infection who also have autoimmune gastritis will still not be able to absorb oral iron after successful eradication of *H. pylori*.

**Expert Perspective** After eradication of *H. pylori* infection, patients achieve a normal hemoglobin concentration with oral iron replacement therapy, whether or not they have coexisting autoimmune gastritis (Hershko et al. 2007; Monzon et al. 2013). In some patients, the hemoglobin concentration returns to normal even without receiving oral iron. As a result of the restored ability to absorb oral iron, IV iron treatment is unnecessary. In contrast, no specific treatment is available for autoimmune gastritis alone, although some patients with concomitant *H. pylori* infection who undergo *H. pylori* eradication have an improved response to oral iron (Annibale et al. 2002; Mera et al. 2005; Kodama et al. 2012). Patients with autoimmune gastritis should also be monitored for development of a need for cobalamin treatment (Hershko and Camaschella 2014). Patients with celiac disease should be followed on a gluten-free diet (Rubio-Tapia et al. 2013) but are unlikely to benefit from oral iron; iron replacement is best accomplished with IV iron (Auerbach et al. 2013), and patients may require additional iron therapy if iron stores again become depleted. Patients with IRIDA usually experience only partial correction of the anemia after treatment with oral or IV iron. This likely reflects the suppressive effect of hepcidin on iron recycling which results from decreased ferroportin expression. Even in some adults who achieve a normal hemoglobin level after many years of treatment, microcytosis persists (Melis et al. 2008).

**Answers**

Question 1. B
Question 2. C
Question 3. A
Question 4. D
Question 5. E
Question 6. C
Question 7. D
Question 8. C

## References

An P, Wu Q, et al. TMPRSS6, but not TF, TFR2 or BMP2 variants are associated with increased risk of iron-deficiency anemia. Hum Mol Genet. 2012;21(9):2124–31.

Annibale B, Di Giulio E, et al. The long-term effects of cure of Helicobacter pylori infection on patients with atrophic body gastritis. Aliment Pharmacol Ther. 2002;16(10):1723–31.

Auerbach M, Goodnough LT, et al. Iron: the new advances in therapy. Best Pract Res Clin Anaesthesiol. 2013;27(1):131–40.

Bertoncini S, Blanco-Rojo R, et al. A novel SNaPshot assay to detect genetic mutations related to iron metabolism. Genet Test Mol Biomark. 2011;15(3):173–9.

Caselli M, Zullo A, et al. "Cervia II Working Group Report 2006": guidelines on diagnosis and treatment of Helicobacter pylori infection in Italy. Dig Liver Dis. 2007;39(8):782–9.

Cau M, Galanello R, et al. Responsiveness to oral iron and ascorbic acid in a patient with IRIDA. Blood Cells Mol Dis. 2012;48(2):121–3.

Chambers JC, Zhang W, et al. Genome-wide association study identifies variants in TMPRSS6 associated with hemoglobin levels. Nat Genet. 2009;41(11):1170–2.

Chen WC, Chen YH, et al. Gastrointestinal hemorrhage in warfarin anticoagulated patients: incidence, risk factor, management, and outcome. Biomed Res Int. 2014;2014:463767.

Collins JF, Anderson GJ. Chapter 71: Molecular mechanisms of intestinal iron transport. In: Johnson LR et al., editors. Physiology of the gastrointestinal tract. Oxford: Academic; 2012. p. 1921–48.

Cook JD. Clinical evaluation of iron deficiency. Semin Hematol. 1982;19(1):6–18.

Cook JD, Dassenko S, et al. Serum transferrin receptor as an index of iron absorption. Br J Haematol. 1990;75(4):603–9.

Fock KM, Katelaris P, et al. Second Asia-Pacific consensus guidelines for Helicobacter pylori infection. J Gastroenterol Hepatol. 2009;24(10):1587–600.

Gangat N, Wolanskyj AP. Anemia of chronic disease. Semin Hematol. 2013;50(3):232–8.

Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93(4):1721–41.

Garrick MD, Garrick LM. Cellular iron transport. Biochim Biophys Acta. 2009;1790(5):309–25.

Gulec S, Anderson GJ, et al. Mechanistic and regulatory aspects of intestinal iron absorption. Am J Physiol Gastrointest Liver Physiol. 2014;307(4): G397–409.

Hershko C, Camaschella C. How I treat unexplained refractory iron deficiency anemia. Blood. 2014; 123(3):326–33.

Hershko C, Hoffbrand AV, et al. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. Haematologica. 2005;90(5):585–95.

Hershko C, Ianculovich M, et al. A hematologist's view of unexplained iron deficiency anemia in males: impact of Helicobacter pylori eradication. Blood Cells Mol Dis. 2007;38(1):45–53.

Ito M, Haruma K, et al. Helicobacter pylori eradication therapy improves atrophic gastritis and intestinal metaplasia: a 5-year prospective study of patients with atrophic gastritis. Aliment Pharmacol Ther. 2002;16(8):1449–56.

Khuong-Quang DA, Schwartzentruber J, et al. Iron refractory iron deficiency anemia: presentation with hyperferritinemia and response to oral iron therapy. Pediatrics. 2013;131(2):e620–5.

Kodama M, Murakami K, et al. Helicobacter pylori eradication improves gastric atrophy and intestinal metaplasia in long-term observation. Digestion. 2012; 85(2):126–30.

Kroot JJ, Kemna EH, et al. Results of the first international round robin for the quantification of urinary and plasma hepcidin assays: need for standardization. Haematologica. 2009;94(12):1748–52.

Lok CN, Loh TT. Regulation of transferrin function and expression: review and update. Biol Signals Recept. 1998;7(3):157–78.

Malfertheiner P, Megraud F, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III consensus report. Gut. 2007;56(6): 772–81.

Markovic M, Majkic-Singh N, et al. Reticulocyte haemoglobin content vs. soluble transferrin receptor and ferritin index in iron deficiency anaemia accompanied with inflammation. Int J Lab Hematol. 2007;29(5): 341–6.

McLaren CE, McLachlan S, et al. Associations between single nucleotide polymorphisms in iron-related genes and iron status in multiethnic populations. PLoS ONE. 2012;7(6):e38339.

Mearin F, Balboa A, et al. Iron deficiency anemia and use of intravenous iron in digestive disease. Gastroenterol Hepatol. 2010;33(8):605–13.

Melis MA, Cau M, et al. A mutation in the TMPRSS6 gene, encoding a transmembrane serine protease that suppresses hepcidin production, in familial iron deficiency anemia refractory to oral iron. Haematologica. 2008;93(10):1473–9.

Mera R, Fontham ET, et al. Long term follow up of patients treated for Helicobacter pylori infection. Gut. 2005;54(11):1536–40.

Monzon H, Forne M, et al. Helicobacter pylori infection as a cause of iron deficiency anaemia of unknown origin. World J Gastroenterol. 2013;19(26):4166–71.

Murray JA, McLachlan S, et al. Association between celiac disease and iron deficiency in Caucasians, but not non-Caucasians. Clin Gastroenterol Hepatol. 2013;11(7):808–14.

Punnonen K, Irjala K, et al. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood. 1997;89(3):1052–7.

Rakel RE, Bope ET, editors. Conn's current therapy 2002. Philadelphia: Saunders; 2002. p. 357.

Rubio-Tapia A, Hill ID, et al. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol. 2013;108(5):656–76; quiz 677.

Skikne B, Hershko C. Iron deficiency. In: Anderson GJ, McLaren GD, editors. Iron physiology and pathophysiology in humans. New York: Humana Press; 2012.

Skikne BS, Punnonen K, et al. Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/log ferritin index. Am J Hematol. 2011;86(11):923–7.

Theil EC. Iron homeostasis and nutritional iron deficiency. J Nutr. 2011;141(4):724S–8.

Thomas C, Kobold U, et al. Serum hepcidin-25 in comparison to biochemical markers and hematological indices for the differentiation of iron-restricted erythropoiesis. Clin Chem Lab Med. 2011;49(2):207–13.

Tsai AG, Hofmann A, et al. Perfusion vs. oxygen delivery in transfusion with "fresh" and "old" red blood cells: the experimental evidence. Transfus Apher Sci. 2010;43(1):69–78.

Ullrich C, Wu A, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. JAMA. 2005;294(8):924–30.

Wilkins SJ, Frazer DM, et al. Iron metabolism in the hemoglobin-deficit mouse: correlation of diferric transferrin with hepcidin expression. Blood. 2006;107(4):1659–64.

WHO. Turning the tide of malnutrition: responding to the challenge of the 21st century. Geneva: World Health Organization, 2000;(WHO/NHD/00.7).

Zhu A, Kaneshiro M, et al. Evaluation and treatment of iron deficiency anemia: a gastroenterological perspective. Dig Dis Sci. 2010;55(3):548–59.