Management of Anemia

A Comprehensive Guide for Clinicians

Robert Provenzano
Edgar V. Lerma
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To all my mentors, and friends, at the University of Santo Tomas Faculty of Medicine and Surgery in Manila, Philippines, and Northwestern University Feinberg School of Medicine in Chicago, IL, who have, in one way or another, influenced and guided me to become the physician that I am …
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To my parents and my brothers, without whose unwavering love and support through the good and bad times, I would not have persevered and reached my goals in life …
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—Edgar V. Lerma

To my parents, my brothers, and my sons

—Robert Provenzano

Preface

The identification of anemia, in its many forms, has been well documented over the past century. Anemia caused by 'deficiencies' of iron, B12, folate, and others once identified had obvious treatment regimens that for the most part have not changed over the years. However, anemia of chronic disease (inflammation), genetically related anemia, and anemia related to chronic end-organ damage (lung, liver, or post-solid organ transplant) are only now being better understood and managed.

Breakthroughs in recombinant erythropoietin production in the late 1980s were thought to herald the answer to treatment of anemia of chronic disease but as recent literature has suggested was not the panacea once thought. Evidence of higher rates of cardiovascular and cerebral vascular events has tempered the clinicians' enthusiasm for their use and spurred further research into more novel, physiological pathways of management.

Our book provides the reader with an up-to-date, detailed analysis of the current science of anemia from anemia in children to anemia of the elderly and from anemia of inflammation to anemia of end-organ damage and anemia of obesity.

Understanding that knowledge in this space is ever changing; the editors' hopes are that these chapters will serve as a stable launching point for your education and future interest in this field.

Detroit, MI, USA — Robert Provenzano
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Disease State: Kidney Disease

1

Lynda Szczech

Overview

While kidney disease can be a result of many different etiologies, its functional marker is a rise in serum creatinine, a decline in estimated glomerular filtration rate (eGFR), or a change in urine to include proteinuria or proteinuria plus hematuria. Because serum creatinine is not linearly related to kidney function given that it is confounded by the amount of muscle mass of the person in which it is measured, it is necessary to estimate kidney function using one of three formulae that convert serum creatinine to estimated glomerular filtration rate (eGFR) or creatinine clearance (CrCl). The MDRD and CKD-EPI formulae are both used to calculate eGFR [1, 2], while the Cockcroft-Gault formula [3] can be used to calculate CrCl (Table 1.1). Both measures (eGFR and CrCl) are provided in units of milliliters per minute and measure how much blood is "cleaned" or "processed" by the kidney per minute. While there is no defined "normal" for eGFR and CrCl, key points to remember are that any values around 100 mL/min are likely not to represent a decline in kidney function. As eGFR and CrCl decline, an individual has less kidney function and more advanced levels of kidney disease and can be used to describe the stage of kidney disease for the individual (Table 1.2).

Epidemiology

In general, most kidney diseases are associated with an increased risk of anemia. The notable exception is polycystic kidney disease in which not only is anemia decidedly uncommon, but hemoglobin and hematocrit values are commonly in the upper range of normal. In individuals with one of the polycystic syndromes, the

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Table 1.1 eGFR and CrCl estimating equations

Method	Equation
Cockcroft-Gault	[(140—Age in years) × (Weight in kg) × (0.85 if female)]/(72 × Scr in mg/dL)
MDRD	$186 \times (\text{Scr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American})$
CKD-EPI	$A \times (\text{Scr}/B)^{C} \times (0.993)^{\text{Age}}$ A: African American female = 166; African American male = 163; White/other female = 144; White/other male = 141 B: Female = 0.7; Male = 0.9 C: If female and creatinine ≤0.7: −0.329; if female and creatinine >0.7: −1.209; if male and creatinine ≤0.7: −0.411; if male and creatinine >0.7: −1.209

Table 1.2 Prevalence of anemia based on stage of kidney disease

Stage	eGFR or CrCl	Prevalence of anemia
1	>90 with proteinuria	–
2	60–90	26.7%
3	30–60	41.6%
4	15–30	53.6%
5	<15 or on dialysis	75.5%

Based on data from [4]

presence of anemia should prompt an evaluation of causes of anemia unrelated to kidney disease.

For the most common causes of kidney disease, such as diabetes mellitus and hypertension, the likelihood of anemia is related to the stage of kidney disease. As kidney function declines (or stage of kidney disease increases), the likelihood of anemia due to kidney function increases (Table 1.2).

A key point here is that as detailed below, anemia "related" to CKD is really a diagnosis of exclusion. Therefore, in circumstances where CKD-related anemia is less likely (e.g., stage 2 kidney disease), other factors such as hemolysis in patients with autoimmune kidney disease or occult GI bleeding due to a malignancy in appropriate groups should be considered.

Pathophysiology

Because erythropoietin levels are not increased among persons with CKD and anemia as would be expected for their degree of anemia, it was assumed that this represented a failure in the ability of the kidney to respond with an appropriate elevation in erythropoietin due to inability to produce erythropoietin related to decreased functioning nephron mass. However, additional studies on the pathophysiology of anemia in kidney disease suggests a far more complex mechanism that involves a change in the relationship between the ability of the kidney to sense hypoxia and the production of erythropoietin.

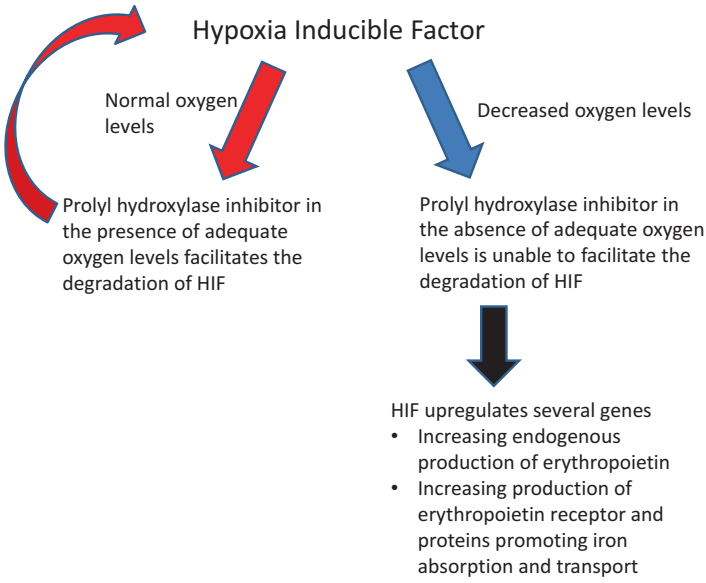

Fig. 1.1 Interactions between HIF, O_2, and prolyl hydroxylase

When the kidney senses hypoxia, through the production of hypoxia-inducing factors (HIF), it is able to affect a series of events that results in the stimulation of erythropoietin production (Fig. 1.1). The enzyme responsible for the degradation of HIF (prolyl hydroxylase) requires oxygen to function. In the presence of lower oxygen concentrations, it is less active in degrading HIF (Fig. 1.1) resulting in an increase in endogenously produced erythropoietin [5–7].

Screening for Anemia

As kidney function declines, the prevalence of anemia increases. Because anemia can be present along the entire spectrum of kidney function, it is recommended by current clinical practice guidelines that anemia be screened for using hemoglobin at least annually among all persons with CKD irrespective of etiology [8]. While there are multiple thresholds that have been used to define anemia and the threshold for the diagnosis may be higher than the threshold for treatment with an erythrocytosis-stimulating agent (ESA), nephrology clinical practice guidelines define anemia as <13.5 g/dL in adult males and <12.0 g/dL in adult females.

When screening for and treating anemia in CKD, it is important to utilize hemoglobin rather than hematocrit for a number of reasons. Mean corpuscular volume may be affected by hyperglycemia falsely elevating red cell volume and potentially affecting hematocrit. In addition, the variability within a sample for hematocrit is greater than hemoglobin. Issues such as this make the accuracy of hemoglobin measure more reliable.

Laboratory Evaluation of Anemia

It has been estimated that 16.8% of the US population has CKD [9]. Therefore, it cannot be underscored enough that it is important to consider in every patient the potential for other causes of anemia, consider what work up for anemia would be undertaken in the absence of kidney disease, and weigh on an individual basis how much of that work up needs to be performed in any given individual. For example, consider these two individuals.

- A 40-year-old black female with lupus has a hemoglobin of 9.5 and a creatinine of 1.2 mg/dL.
- A 60-year-old white male with autosomal dominant polycystic kidney disease presents with fatigue. His eGFR is 30 mL/min, and his hemoglobin is 10.6.

Each of these patients has CKD given their elevation in creatinine or decrease in eGFR. However, the degree to which you would expect anemia related to decreased kidney function in the woman with lupus is low given her mild CKD. Further, the man with PCKD might not experience anemia until late stage if at all. Therefore, the extent to which one should expect anemia should dictate whether a further diagnostic investigation should be performed. In the two examples, the likelihood of anemia related to their decreased kidney function alone is lower therefore additional work up relevant to their history is warranted. This might include a work up of anemia related to lupus such as hemolysis and a work up of anemia appropriate for a person who is 60 years such as occult gastrointestinal bleed.

Laboratory measures relevant to people with CKD include those measures in Table 1.3.

Imaging Studies or Biopsies That May Assist in Diagnosis and Treatment and Their Interpretation

Not all people with CKD will develop anemia. In those who do not develop anemia, imaging studies of the kidneys to evaluate for cystic changes, cystic diseases, or renal cell carcinoma may be considered. Among persons with CKD due to polycystic kidney disease, anemia is less likely to be present. Additionally, as CKD due to any etiology becomes more severe and among those patients with ESRD due to any etiology, acquired cystic changes occur and may be associated with increased endogenous erythropoietin levels and polycythemia. While this scenario is more of the exception than the rule, patients with ESRD without anemia should be expected to be seen at a prevalence of approximately 1.8% [10].

Among CKD patients with anemia, there are no imaging studies or biopsies that are indicated specifically for anemia attributed solely to kidney disease.

Table 1.3 Laboratory measures relevant to people with CKD

Categories	Specific tests	"Usual normal" ranges	Comments
Red cell parameters	MCV MCHC MCH	82–103 30–37 26–34	• In general, the anemia of CKD is a normochromic, normocytic anemia • Consider other causes of anemia if these parameters are outside of their normal ranges
Iron stores	Transferrin saturation Ferritin Content of hemoglobin in reticulocytes (CHr)	20%[a] 100 ng/mL[a] >29 pg/cell[a]	• Iron deficiency anemia is common among persons with kidney disease. This is directly relevant to the treatment of anemia • Serum ferritin is an acute phase reactant. Among persons who have CKD related to inflammatory processes such as lupus, serum ferritin may be elevated in the presence of iron deficiency due to the effect of inflammation on this parameter • Important to reassess periodically [e.g., every 3 months particularly if your patient is on erythropoiesis-stimulating agent (ESA therapy)] • Provides insight into internal distribution of iron (i.e., is it "available" for incorporation into hemoglobin and reticulocytes)
Reticulocyte response	Absolute reticulocyte count	3–7%	• Reticulocyte index = observed absolute reticulocyte count/normal absolute reticulocyte count
Vitamin deficiency	Vitamin B12, Folate	200 and 600 pg/mL 7–36 nmol/L	• Manifests as a macrocytosis

[a]For iron stores, rather than defining what is normal, current clinical practice guidelines provide thresholds to consider as goals for therapy or thresholds below which supplementation should be considered

Treatment of Anemia in CKD

The treatment of anemia as detailed in this chapter assumes that after consideration and investigation of the anemia, no additional non-CKD-related etiologies have been discovered (Table 1.4).

Goal hemoglobin is really only an appropriate consideration when erythropoiesis-stimulating agents (ESAs) are used as therapy. In the absence of ESAs, it is appropriate to replete the deficiencies identified (e.g., iron, B12, folate). However, when ESA therapy is used, the goal hemoglobin that is targeted is a matter of importance related to cardiovascular safety and should be guided by the product labeling

Table 1.4 Therapies for the treatment of CKD-related anemia

Treatment	Agents	Dose	Goal	Duration
Iron	Oral Intravenous • Iron dextran • Iron sucrose • Ferric gluconate • Ferumoxytol	ORAL: 325 mg three times a day is the dose traditionally recommended. Lower doses (e.g. 27 to 65 mg a day), however, may be just as effective and better tolerated. IV: usually 1 g total given in divided doses (check the package insert for the preparation administered)	Repletion of iron stores	Variable based on patient, route of administration, and dose received
Erythrocyte stimulating agents (ESA)	epoetin-alfa and -beta darbepoetin alfa	Starting dose 50–100 units/kg three times weekly IV or SC (epoetin-alfa) or 0.45 μg/kg IV or SC at 4-week intervals (darbepoetin)	Increase in hemoglobin sufficient to minimize the likelihood of transfusion	Monitor frequently for the ability to dose reduce or discontinue. Variable.

for the ESA therapy itself (e.g., epoetin-alfa, darbepoetin). At the time of this writing, the hemoglobin below which ESA therapy may be considered is <10 mg/dL and the goal is to not exceed 11 with a focus on correcting hemoglobin to a sufficient value such that the patient's risk of requiring a transfusion is minimized [11]. The importance of knowing and following the product labeling cannot be underscored enough given the recent safety data that has been published.

As previously discussed, in addition to the role that CKD could be playing in a patient's anemia, all other possible causes to include similarly common etiologies such as B12 and folate deficiency should be evaluated and treated as appropriate.

Iron

Iron repletion is an essential first step to the treatment of anemia due to CKD. In addition to the contribution of iron depletion to anemia directly, a patient must be iron replete to begin ESA therapy. Iron repletion (intravenous) will likely result in a rise in hemoglobin at 6 weeks that is variable but may be as much as 1.0 g/dL [12]. This response has been documented irrespective of the severity of initial anemia and

may decrease the subsequent dose of ESA required or perhaps obviate the need for the initiation of ESA therapy.

Oral iron repletion should be attempted before the administration of intravenous iron in patients with CKD. The ability to respond to oral iron supplementation is, however, blunted in clinical scenarios where patients have increased levels of inflammation. Inflammation results in increased hepatic production of hepcidin that blocks the egress of iron from intestinal cells into the circulation [13]. By blocking the exit of iron out of intestinal cells, these iron-rich enterocytes are sloughed off and iron is not effectively taken into the body. In patients in whom oral iron does not seem to be increasing iron stores, the administration of intravenous iron should be considered. The agents currently approved by the FDA for use in the US are listed in Table 1.4. Given the class boxed warning for all intravenous iron preparations for hypersensitivity and hypotensive reactions, careful monitoring should be used in a patient receiving IV iron.

Not only is it important prior to the initiation of ESA therapy to ensure a patient is iron replete, it is also essential to monitor a patient's iron stores during ESA therapy to assess their ability to remain iron replete. It is suggested that iron stores be monitored every month during the initial period of anemia correction in someone initiating ESA therapy and then approximately quarterly thereafter.

Erythrocyte-Stimulating Agents

Following iron repletion, consideration could be given toward the use of ESAs in a patient with CKD-related anemia. In general, if it is felt that ESA therapy is indicated to minimize the likelihood of red blood cell transfusion, therapy may begin if a patient's hemoglobin level is less than 10 g/dL. For persons with CKD, it is recommended that the dose of epoetin-alfa or darbepoetin be reduced or interrupted if the hemoglobin level exceeds 10 g/dL and that the patient be maintained on the lowest dose to reduce the need for transfusion. These directions have been guided by the FDA since 2006 due to safety issues that are expanded upon in the section "Controversies" below. The dose may be reduced effectively by either decreasing the units (mg) at each administration or maintaining the units (mg) and increasing the interval between each administration.

Relevant Clinical Practice Guidelines

National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines: http://www.kidney.org/professionals/KDOQI/guidelines_anemia/index.htm

KDIGO (Kidney Disease Improving Global Outcomes) guidelines: anticipated publication date 2012: http://www.kdigo.org/pdf/AnemiaConf_PublishedVersion_7-2-08.pdf

Controversies in the Use of ESA

Epoetin-alfa and darbepoetin received approval from the FDA for raising hemoglobin and improving the quality of life. Because higher hemoglobin was associated with better survival and lower risk of hospitalization in multiple observational studies, it was not until 1996 and 2006 that trials first suggested that the lower risk associated with higher hemoglobin between individuals did not equate to lowering the risk as a result of raising the hemoglobin within the individual. The effect of target hematocrit was first examined in the Normalization of Hematocrit study [14]. Dialysis patients were randomized in this trial to anemia correction using epoetin-alfa to a goal of either 30 or 42%. The trial was stopped early when the DMC felt it was unlikely that the higher target would have a better event-free survival (myocardial infarction or death) and the trend suggested that the group targeted to 42% were at greater risk.

The effect of hemoglobin target on a composite of death, heart failure, myocardial infarction, and stroke was examined in CKD patients in 2006 [15]. This trial was also stopped early when it was noted by the DMC that patients randomized to the higher target of 13.5 g/dL had a greater risk of the cardiovascular endpoint than those randomized to 11.3 g/dL. Secondary analyses suggested that this risk may be mediated through the higher dose of epoetin-alfa required by the group targeted to the higher hemoglobin [15].

Finally, the largest trial examined the effect of anemia correction to 13 g/dL as compared with placebo among patients with CKD, anemia, and type 2 diabetes mellitus [16]. While no difference was seen between groups with respect to overall cardiovascular risk, the group randomized to active treatment with darbepoetin to achieve the higher hemoglobin had a greater risk of stroke, and among those with a prior history of malignancy had a greater risk of death in subgroup analysis [17].

Taken together, these trials highlight the significant controversy that exists in the treatment of anemia using ESAs in CKD. When using ESAs, the healthcare provider should keep the following points in mind as therapy is tailored to the individual:

- Higher hemoglobin is associated with better outcomes in observational studies.
- Higher hemoglobin targets cause a greater risk of bad outcomes in randomized trials.
- Randomized trials are not consistent in demonstrating a quality of life benefit following the correction of anemia.

It should be noted that the increased risk of cardiovascular outcomes is seen in trials where ESAs have been the central therapy for the anemia. Therefore, concerns raised in these trials should be considered to be associated with ESA use rather than treatment of anemia through the repletion of iron or vitamin stores. In light of this, following repletion of iron or vitamins such as B12 and folate, and prior to the institution of ESA therapy, health care providers need to carefully consider if therapy with an ESA is warranted based on the presence of symptoms related to anemia.

Summary

- The anemia of CKD is usually normochromic and normocytic and is a diagnosis of exclusion. On diagnosis, careful consideration to all potential processes that could contribute to anemia should be given in a person with CKD.
- Iron deficiency should receive particular attention in a person with CKD as both functional and actual iron deficiency can contribute to anemia.
- Iron deficiency should be corrected prior to the administration of ESA for the treatment of anemia.
- Goals for treatment should include correction of all deficiencies. If ESA therapy is used, the hemoglobin goal should be set using the most up-to-date product labeling for the ESA (e.g., epoetin-alfa or darbepoetin).

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Iron-Deficiency Anemia

2

Anatole Besarab and Stefan Hemmerich

Introduction

Anemia is prevalent in most parts of the world. Iron-deficiency anemia (IDA) is one of the more common causes of anemia. It typically results from insufficient iron intake, poor gastrointestinal absorption, or overt or occult blood loss. Distinguishing iron deficiency from other causes of anemia is crucial for initiating appropriate treatment, as is identifying the underlying cause of iron deficiency. In this chapter, we will discuss normal iron hemostasis, the prevalence of iron deficiency, most common etiologies and therapies.

We will examine iron deficiency and its anemia from the perspective of advances in our understanding of systemic iron homeostasis, pathophysiological features, and treatment options. The focus will be on IDA in adults. Readers are referred elsewhere for information on the presentation, symptoms, and laboratory diagnosis of iron-deficiency anemia [1] and on issues that are specific to pregnancy [2].

Definitions and Diagnosis

Iron deficiency is a state in which reduction of iron stores precedes development of overt iron-deficiency anemia. It may persist without progression.

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Iron-deficiency anemia is a more severe condition in which low levels of iron are associated with anemia and the presence of microcytic hypochromic red cells in the circulation, the relative number of which reflects the severity of the iron deficiency.

Iron-restricted erythropoiesis refers to a state in which delivery of iron to erythroid precursors for Hb synthesis is impaired, no matter how replete the stores [3]. Iron stores may be normal or increased because iron is sequestered within macrophages as in the anemia of chronic disease (ACD). The latter, described in Chap. 4, is seen in patients with autoimmune disorders, cancer, infections, and chronic kidney diseases [4].

Separation of IDA from ACD is often difficult since both can co-exist, particularly in the elderly [5] and in patients with chronic kidney disease [6]. However, a substantial fraction of the anemia commonly found in the elderly patient occurs in the absence of iron deficiency or elevated hepcidin levels [7].

Functional iron deficiency is a state of iron-poor erythropoiesis in which there is insufficient mobilization of iron from stores in the presence of increased demands [8]. This is typically observed following treatment with erythropoiesis-stimulating agents (ESAs) [9].

Prevalence

Iron deficiency and iron-deficiency anemia (IDA) are common medical conditions seen worldwide [10]. The estimated prevalence of iron deficiency worldwide is twice as high as that of iron-deficiency anemia. IDA severely affects the lives of young children and premenopausal women (particularly those of low-income or in developed countries) [11]. In developing countries, iron deficiency and iron-deficiency anemia typically result from inadequate dietary intake and/or blood loss due to intestinal worm colonization, or both. In higher-income countries, certain eating habits such as vegetarian diet and chronic blood loss or malabsorption are the most common causes. Iron deficiency in developed countries is especially high in the elderly [10].

Clinical Features

Iron-deficiency anemia is chronic and frequently asymptomatic and thus may go completely undiagnosed. Weakness, fatigue, difficulty in concentrating, and poor work productivity are nonspecific symptoms ascribed to low delivery of oxygen to body tissues and decreased activity of iron-containing enzymes. The extent to which these non-hematologic effects of iron deficiency are manifested before anemia develops is variable. Signs of iron deficiency in tissue are subtle and may not respond to iron therapy. Iron deficiency has been reported to decrease cognitive performance and to delay mental and motor development in children but whether short-term treatment alters outcome is unclear [12].

Common signs and symptoms of IDA are:

- Difficulties with memory and concentration (cognitive)
- Fatigue, sluggishness, tiring easily, low energy level
- Feeling mildly light-headed, Feeling unusually cold (Cardiovascular)
- Mild shortness of breath with exertion that goes away with rest (cardiopulmonary)
- Pale conjunctiva, mucosa, or skin

Less common symptoms of iron deficiency include burning tongue (usually accompanied by zinc deficiency from malabsorption), restless leg syndrome, dyspnea in otherwise healthy adults, failure to thrive in babies and toddlers. Usually these are dominated by the symptoms coming from the primary cause of the IDA.

Severe iron-deficiency anemia in pregnancy is associated with an increased risk of preterm labor, low neonatal weight, and increased newborn and maternal mortality. Iron deficiency may predispose a person to infections, precipitate heart failure, and cause restless leg syndrome [13]. In patients with heart failure, iron deficiency has a negative effect on the quality of life, irrespective of the presence of anemia [14].

Iron deficiency can impact assessment of the diabetic state. Caution must be exercised in interpreting the results of HbA1c in patients of IDA and iron deficiency must be corrected before diagnosing diabetes and pre-diabetes solely on the basis of HbA1c criteria [15].

Iron Homeostasis and Physiology

Iron (Fe) is crucial to biologic functions, including respiration, energy production, DNA synthesis, and cell proliferation [16]. All cells need a small amount of iron; however, erythroid precursors require substantial amounts to synthesize hemoglobin. Accordingly, anemia is a prominent manifestation of iron deficiency. Three cell types are important in iron homeostasis: the duodenal enterocytes which absorbs iron, the hepatocyte that serves a depot function (removing excess iron from circulating plasma and safely storing it until it is needed), and the tissue macrophages that recognize and phagocytose old and/or damaged erythrocytes, recovering their iron for reuse and storage. Molecular signals coordinate the operations of each of these cell types. No efficient, regulated excretion mechanism for iron exists, emphasizing the importance of meticulous regulation of iron acquisition and distribution. Since excretory mechanisms for eliminating iron from the body are lacking and excess levels of iron in tissues may be toxic, iron absorption is limited to 1–2 mg daily. About 95% of the iron needed daily (about 25 mg per day in normal state) is provided through the recycling by macrophages that phagocytose senescent erythrocytes (Fig. 2.1). Fe absorption from water-soluble forms of iron is inversely proportional to Fe status in humans.

Enterocyte iron absorption is an extremely complicated process whose scope goes beyond the goal of this presentation. Understanding of some basic elements

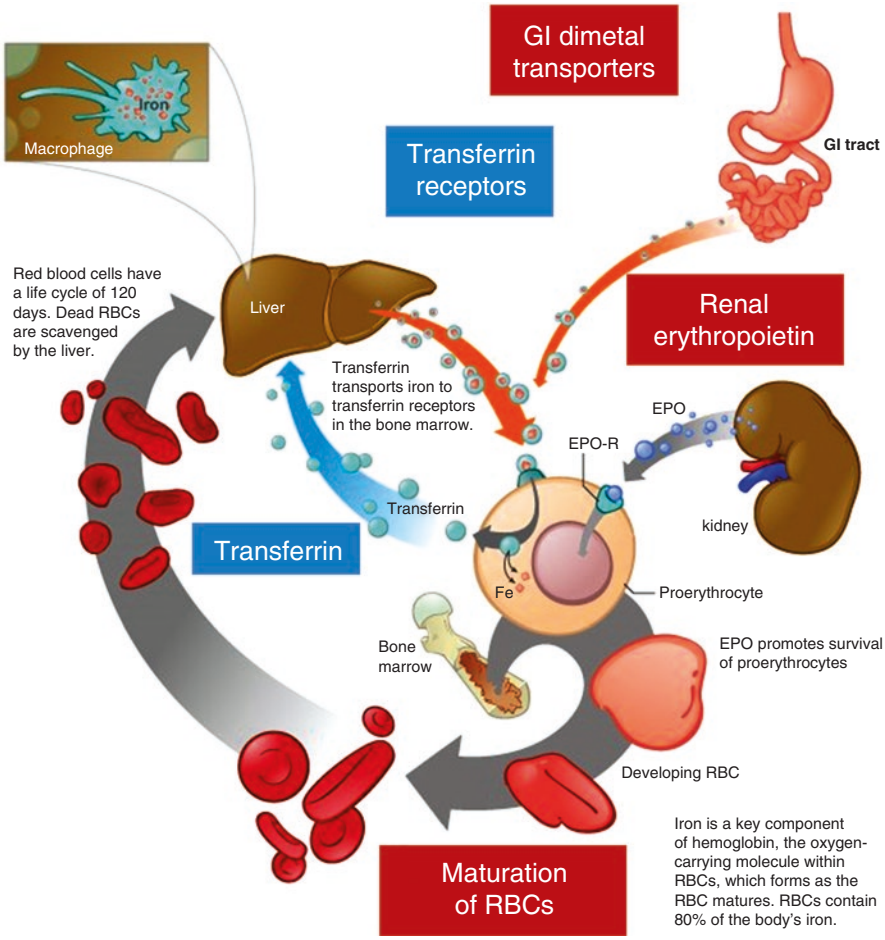

Fig. 2.1 Mechanisms of erythropoiesis and iron homeostasis

are important in managing any patient with iron deficiency [17]. Mammals absorb dietary iron through the duodenal epithelium which is organized in villous structures that maximize absorptive surface area. Enterocyte precursors are present at the bases of the villous crypts and migrate up the villous axis as they differentiate. Enterocytes have a brush border that additionally amplifies the surface area for absorption. Mature enterocytes live for only 1–2 days. Iron that accumulates within them is lost from the body when senescent enterocytes are shed into the gut lumen. The crucial export step is regulated by hepcidin, a hormone produced by the hepatocytes in response to iron stores.

Intestinal iron uptake from the gut lumen requires a number of transporters and the right valence of iron. Proteins for the uptake of heme and inorganic iron reside on the brush-border membrane. A key transporter is divalent metal transporter 1 (DMT1) which is activated by hypoxia-inducible factor 2α (HIF-2α) [18]; it requires

proton cotransport. Most non-heme iron in the diet is present as the Fe^{3+} form. DMT1 exclusively transports divalent metals, necessitating luminal conversion of Fe^{3+} to Fe^{2+}. This occurs through the action of duodenal cytochrome b (Dcytb), expressed in the intestinal mucosa by hypoxia, iron deficiency, or anemia. The intestine also absorbs heme iron from the diet; such heme absorption is a saturable, carrier-mediated process. The heme enterocyte importer, termed heme carrier protein 1 (HCP1), resembles bacterial proteins that transport metal-tetracycline complexes. Once dietary heme has entered the intestinal epithelial cell, it is cleaved by intracellular heme oxygenase 1 to release iron, the iron probably joining the same intracellular pool as non-heme iron.

Intracellular enterocyte iron is either stored in the multimeric protein ferritin or transported across the basolateral membrane of the enterocyte into the circulation. Ferroportin is the basolateral iron exporter as intestinal expression of ferroportin mRNA and protein increases in response to iron deficiency and hypoxia [19]. Selective inactivation of the murine ferroportin gene in intestinal cells has established that ferroportin is the major, if not only, intestinal iron exporter. Hepcidin binds to and internalizes this transporter.

To ensure that very little iron is free within cells, iron regulatory proteins (IRP1 and IRP2) control the posttranscriptional expression of genes modulating cellular iron uptake and storage. Cellular iron in excess of immediate needs is stored as an iron oxide solid within ferritin, a polymeric protein composed of varying ratios of heavy (H) and light (L) ferritin polypeptides [20]. The presence of IREs in DMT1 and ferroportin mRNAs during heme synthesis in erythrocytes suggests that their expression is likely controlled, at least in part, by cellular iron content. Data from animal models establish that the Tfn cycle is critical for iron uptake into erythroid cells [21].

Recycling of iron from senescent erythrocytes provides most of the iron utilized by developing red blood cells. Senescent red cells are removed from circulation by specialized macrophages in the spleen, bone marrow, and liver for the recovery of their iron. It is believed that accumulation of phosphatidylserine in the outer leaflet of the erythrocyte membrane seems to be a primary event [22] but Ca^{2+} flux, removal of sialic acids on the cell surface, and opsonization of red blood cells by autoantibodies are all possible signals for turnover [23]. The scavenger receptor CD36 [24] is a likely candidate for the macrophage-specific receptor. After the red cell has been internalized into an acidic phagosome, heme oxygenase liberates iron from heme; iron egress from macrophages is partly, if not entirely, mediated by ferroportin whose function is regulated by hepcidin.

Iron acquisition is thus tightly regulated by hepcidin [16]. The hepcidin homeostatic control mechanism regulates both duodenal enterocytes iron absorption (final step of transport) as well as macrophage recycling of iron (efflux of iron, mostly spleen, and bone marrow). Hepcidin functions as an acute-phase reactant that adjusts fluctuations in plasma iron levels caused by absorptive enterocytes and macrophages by binding to and inducing the degradation of ferroportin, which exports iron from cells [25]. Liver hepcidin expression increases in response to high circulating and tissue levels of iron (its physiologic signal) but it also increases during

systemic inflammation or infection. Increases in hepcidin levels induced by inflammatory cytokines, especially interleukin-6, explain the iron sequestration and reduced supply of iron to the bone marrow iron that occurs in the anemia of chronic disease. On the other hand, expression and thus production of hepcidin is decreased during states of expanded erythropoiesis, in iron deficiency, and with tissue hypoxia in response to signals originating in the bone marrow (erythroferrone), the liver, and probably muscle tissue and adipocytes [26]. Hepcidin is crucial in regulating total-body iron within normal ranges, avoiding both iron deficiency and excess.

In iron deficiency, the transcription of hepcidin is suppressed. This adaptive mechanism facilitates the absorption of iron (Fig. 2.1) and the release of iron from body stores. The rapidity of iron repletion varies with the degree and rapidity with which iron deficiency develops. Although spleen and bone marrow macrophages are the primary "reservoirs" for iron storage in the body, hepatocytes do appear to be a long-term reservoir for iron. However, since hepatocytes do not have cell membrane ferroportin, the release of iron from hepatocytes is much slower than from macrophages.

Iron circulates in plasma bound to the 80-kDa serum glycoprotein transferrin (Tfn) which contains two specific high-affinity Fe^{3+} binding sites with an overall association constant of 10^{20} M^{-1} at pH 7.4 [27]. In humans, transferrin consists of a polypeptide chain containing 679 amino acids and two carbohydrate chains and has both alpha helices and beta sheets that form two domains [28]. The amino acids which bind iron ion to the transferrin are identical for both of the binding sites; two tyrosines, one histidine, and one aspartic acid. For the iron ion to bind, an anion is required, preferably carbonate (CO_2^{-3}). In summary, iron-binding blood plasma glycoprotein controls the level of free iron in biological fluids. Diferric Tfn binds to a highly specific Tfn receptor (TfnR1) [29], permitting cellular uptake by receptor-mediated endocytosis and the formation of clathrin-coated pits that facilitate transferrin internalization into endocytic vesicles. As these endosomes become acidified, protein conformation changes at a pH of 5.5 cause iron to dissociate from Tfn [30]; Fe^{3+} is then reduced to Fe^{2+}, for transport from the endosome to the cytoplasm by the transporter DMT1. The Tfn cycle is completed when the endosome returns to and fuses with the plasma membrane, returning apotransferrin to the circulation and TfnR1 to the plasma membrane to start the cycle again.

The transferrin iron-bound receptor is a disulfide-linked homodimer. Transferrin plays a key role in areas where erythropoiesis and active cell division occur. It is also found in mucosal tissues; by binding iron and creating and a low free iron environment, it impedes bacterial survival in a process called iron withholding. Levels of transferrin decrease (acute phase reaction) during systemic inflammation, as well as with cancers and certain other diseases [31].

Etiology

The most common causes of iron deficiency are summarized in Table 2.1.

Table 2.1 The most common causes of iron deficiency

Cause	Examples
Physiologic	
Increased demand	Infancy, rapid growth in adolescence, menstrual blood loss, pregnancy, blood donation
Dietary or environmental	Insufficient intake, resulting from poverty, malnutrition, diet (e.g., vegetarian, vegan, iron-poor)
Pathologic	*Decreased absorption*: gastrectomy, duodenal bypass, bariatric surgery, *Helicobacter pylori* infection, celiac sprue, atrophic gastritis, inflammatory bowel diseases (ulcerative colitis, Crohn's disease)
	Chronic blood loss: gastrointestinal tract, including esophagitis, erosive gastritis, peptic ulcer, diverticulitis, benign tumors, intestinal cancer, inflammatory bowel diseases, angiodysplasia, hemorrhoids, hookworm infestation, obscure source
	Genitourinary system: heavy menses, menorrhagia, intravascular hemolysis (paroxysmal nocturnal hemoglobinuria, autoimmune hemolytic anemia, march hemoglobinuria, damaged heart valves, microangiopathic hemolysis (TTP/HUS)
	Systemic bleeding: hemorrhagic telangiectasia, chronic schistosomiasis, Munchausen's syndrome (self-induced hemorrhages)
Drug-associated	Glucocorticoids, salicylates, NSAIDs, proton-pump inhibitors
Genetic	Iron-refractory iron-deficiency anemia (IRIDA)
Iron-restricted erythropoietic	Treatment with erythropoiesis-stimulating agents (ESA), anemia of chronic disease, chronic kidney disease

Poverty, malnutrition, and famine are the main causes for anemia from iron deficiency in developing countries, especially in children and pregnant women [11]. Cereal-based diet decreases iron bioavailability as they contain phytates that sequester iron into a poorly absorbable complex. Other common causes in developing countries include hookworm infections and schistosomiasis, which cause chronic blood loss [10]. Strict vegan and vegetarian diets, malabsorption, and chronic blood loss resulting from heavy menstrual losses are also well-known causes of iron-deficiency anemia. Chronic blood loss from the gastrointestinal tract, including occult blood, especially in male patients and elderly patients, may reveal the presence of benign lesions, angiodysplasia, or cancer. Obscure gastrointestinal blood loss, especially from the small bowel, may be seen by means of video-capsule endoscopy [32]. This technique is increasingly used when conventional workups for iron-deficiency anemia is negative [33]. Persons who donate blood regularly are also at risk for iron deficiency, and their iron levels should be monitored.

In rare forms of intravascular hemolysis as in in paroxysmal nocturnal hemoglobinuria, iron is lost in the urine, the iron deficiency then aggravating the hemolytic anemia. Anemia develops in endurance athletes, perhaps from some hemolysis, blood loss, and/or mild inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDS), anticoagulants more so than antiplatelet agents can aggravate blood loss. Proton-pump inhibitors (PPIs) by reducing acid secretion impair iron absorption (Table 2.1) [34].

Multiple causes may be operative in any given person. Low iron intake in the presence of intestinal infections with nematodes may result in severe anemia [35]. Blood losses may combine with the anemia of inflammation [5]. In end-stage kidney disease (ESRD), iron-deficiency anemia results from dialyzer blood loss (average of 1–2 g elemental Fe per year), increased hepcidin from reduced hepcidin clearance and from inflammation, and PPIs and anticoagulant use. In elderly persons, the anemia correlates with advanced age and multiple related conditions, including iron deficiency [36] inflammatory disorders, decreased levels of erythropoietin, and cancer [7, 30]. Obesity, now reaching epidemic proportions in Western societies, is associated with mild iron deficiency [7]. The processes include subclinical inflammation, increased hepcidin levels, and decreased iron absorption [37].

In most cases, resistance to oral iron therapy is due to disorders of the gastrointestinal tract (Table 2.1). Partial or total gastrectomy or surgical procedures that bypasses the duodenum can produce such resistance. Bariatric surgery, such as laparoscopic Roux-en-Y gastric bypass, which is performed in obese patients is an emerging cause of iron deficiency (up to 45% of subjects) [38] and anemia because the procedure effectively removes an active iron absorption site from the digestive process and increases gastric pH [39]. Lifelong nutritional monitoring and iron supplementation are advisable [40].

Infection with *Helicobacter pylori* infection leads to decreased iron absorption (Table 2.1) as the microorganism competes with its human host for iron, reduces the bioavailability of vitamin C, and may lead to micro-erosions that cause bleeding [41]. Since it is estimated that half the world's population is infected with *H. pylori*, clinicians should be aware of the possibility of infection and provide treatment in order to eradicate this acquired source of iron-resistant iron-deficiency anemia.

The prevalence of celiac disease and its atypical manifestations, which include iron-deficiency anemia, appear to be increasing worldwide [42]. Whether gluten allergy contributes to iron deficiency is unclear. The incidence among iron-replete participants for a positive anti-transglutaminase antibodies is negligible; however, gluten sensitivity by this antibody test was found in 2.5% of participants with iron deficiency and seemed to occur in Caucasians [43]. In another study of a series of patients with iron-refractory iron-deficiency anemia, 5% of participants had gluten sensitivity [44]. These findings suggest that gluten sensitivity may be associated with secondary iron-refractory iron-deficiency anemia. Similarly, autoimmune atrophic gastritis is another rare cause of iron-refractory iron-deficiency anemia, resulting from an immune reaction against gastric parietal cells and intrinsic factor, should be considered as a possible albeit unlikely cause of iron-refractory microcytic anemia [44]. In patients with inflammatory bowel disease (IBD), anemia may be iron-resistant, but it is multifactorial from a combination of deficiencies in iron, folate, and vitamin B_{12}, inflammation, and side effects from drug therapy.

An uncommon but important entity with respect to our understanding of iron sensing by the liver is an entity known as iron-refractory iron-deficient anemia (IRIDA). It is a rare autosomal disorder characterized by the absence of a hematologic response (an increase of <1 g of hemoglobin) after 4–6 weeks of treatment

with oral iron [45]. IRIDA is caused by a mutation in TMPRSS6 [45], the gene encoding transmembrane protease serine 6, also known as matriptase-2, which inhibits the signaling pathway [46] that activates hepcidin [47]. Loss-of-function mutations in TMPRSS6 have been reported in many families [48]. In these families, constitutively high production of hepcidin is noted. Hepcidin blocks the intestinal absorption of iron despite the presence of anemia. Anemia is variable, more severe in children, and unresponsive to treatment with oral iron. Microcytosis is striking, transferrin saturation is very low in the presence of normal or borderline-low ferritin levels and high hepcidin levels [49]. The diagnosis ultimately requires sequencing of TMPRSS6. Although IRIDA represents <1% of the cases of iron-deficiency anemia seen in medical practice, knowledge of this condition is valuable to clinicians, since it clarifies how essential the suppression of hepcidin is to the body's response to pharmacologic iron. IRIDA points to the existence of genetic susceptibility to iron deficiency. Variants of TMPRSS6 have been associated with modulation of serum hepcidin levels in individual persons [50] as well as variation in iron levels in population studies [51].

Diagnostic Procedures

The traditional laboratory measures used to determine iron status and iron deficiency and related conditions (e.g., functional iron deficiency, iron-deficiency anemia, IRIDA, and anemia of chronic diseases) are now well established. Older studies of iron status in blood donors used various biochemical markers including serum iron, serum transferrin, transferrin saturation (TSAT), and serum ferritin levels [52]. In these studies, iron depletion was considered to be present if the ferritin concentration was below 12 μg/dL. However, this cut off failed to identify iron deficiency in over one-third of blood donor cases. Other investigators have found that a higher ferritin concentration between 22 and 40 μg/dL [53] better reflects functional iron depletion. These findings were based on more sensitive measures of iron status such as serum (soluble) transferrin receptor (sTfR) levels, which reflect the functional iron compartment and have been shown to correlate with depleted iron stores in marrow preparations [54].

Cook et al. described the use of the ratio of sTfR to ferritin (R/F) [55] for assessing body iron stores and determining iron deficiency from a small capillary blood specimen requiring no venipuncture. Analysis showed a single normal distribution of body iron stores in US men aged 20–65 years (mean ± 1 SD, 9.82 ± 2.82 mg/kg). Distribution analysis in US women aged 20–45 years indicated two populations; 93% of women had body iron stores averaging 5.5 ± 3.35 mg/kg whereas the remaining 7% of women had a mean tissue iron deficit of 3.87 ± 3.23 mg/kg. Quantitative estimates of body iron greatly enhance the evaluation of iron status and the sensitivity of iron intervention trials in populations without inflammation. Moreover, it eliminates the need for a TSAT determination.

In another study of almost 1700 male and female blood donors, studies were performed to see if better criteria could be developed [56]. Absent iron stores (AIS)

was defined as plasma ferritin level of less than 12 μg/L. The logarithm of the ratio of soluble transferrin receptor to ferritin of at least 2.07 (≥97.5% in FT/RA males) was used to define iron-deficient erythropoiesis (IDE). Receiver operating characteristics analysis was performed to assess selected RBC indices (e.g., percentage of hypochromic mature RBCs, proportion of hypochromic mature RBCs [%HYPO], and hemoglobin [Hb] content of reticulocytes [CHr]) in identifying AIS and IDE; %HYPO and CHr detected IDE with comparable sensitivity, 72% vs. 69%, but differed in specificity: %HYPO 68% and CHr 53%. For detecting absolute iron deficiency, sensitivity was improved to 85% for %HYPO and 81% for CHr but specificity was reduced for both. Venous Hb at (<12.5 g/dL) had high specificity but poor sensitivity for iron deficiency, whether relative or absolute. The sensitivity of hemoglobin measurements is poor because anemia associated with nutritional iron deficiency is relatively mild, resulting in extensive overlap in Hb values between healthy and iron-deficient persons [57]. A plasma ferritin level of less than 26.7 μg/L was a good surrogate for assessing IDE. The most recent guidelines for the differential diagnosis of microcytic anemias have been published elsewhere [58]. The current clinical consensus is to use a ferritin level of <30 μg/L as the most sensitive and specific test for identification of iron deficiency. Levels are even lower in patients with iron-deficiency anemia.

However TSAT is still used by many to determine iron status [27]. A value of less than 16% indicates an iron supply that is insufficient to support normal erythropoiesis. However, in determining iron status, it is important to consider the entire picture rather than using a single result. In nephrotic syndrome, urinary loss of transferrin, along with other serum proteins can manifest as iron-resistant microcytic anemia.

Diagnosis of iron-deficiency anemia in the context of inflammation is challenging and cannot be determined on the basis of the results of a single test since many of the indicators are either positive (ferritin) or negative (transferrin) acute phase reactants. As a result, higher cutoff levels for ferritin are used to define iron-deficiency anemia accompanied by inflammation [59, 60], with the best predictor being a ferritin level of less than 100 μg/L. Higher ferritin cutoff levels are used in the diagnosis of iron deficiency in other conditions (e.g., <300 μg/L for heart failure [61] and for chronic kidney disease in the presence of a transferrin saturation level of less than 30%) [62]. The assessment of iron stores through iron staining of bone marrow specimens obtained by means of biopsy is an option that is used infrequently. ELISA kits for measuring plasma hepcidin are available [63] but there is significant variation from kit to kit. At present, C-reactive protein (CRP) level may be the most reliable screening tool to identify patients with inflammation.

The R/F ratio also has limited value in individuals with inflammation or liver disease although sensitivity/specificity of a value of 0.6 was superior in predicting response to IV iron than either TSAT <20% or ferritin <100 ng/mL, singly or used together [64]. More recently, a meta-analysis performed by the US Agency for Health Care Research (AHCR) [65] indicated that in CKD stage 3 or higher, both CHr and % hypochromic red cells have a similar or better overall test accuracy compared with classical markers (TSAT or ferritin) to predict a response to IV iron

treatment (as the reference standard for iron deficiency). In addition, CHr (with cutoff values of <28 pg) and % hypochromic red cells (with cutoff values of >10%) have better sensitivities and specificities to predict iron deficiency than classical markers (TSAT <20 or ferritin <100 ng/mL). There is also evidence that sTfR has a similar test performance compared with classical markers (TSAT or ferritin) to predict a response to IV iron treatment. Across studies, a high degree of heterogeneity exists in the test comparisons, definitions for the reference standard (a response to IV iron treatment), iron status of the study populations (assessed by TSAT or ferritin), and background treatment. This heterogeneity limits final conclusions about the optimal test for iron deficiency.

The AHCR concluded that based on results from two randomized clinical trials, there is a reduction in the number of iron status tests and IV iron treatments administered to patients when CHr was used to guide iron management compared to when guided by TSAT or ferritin [65]. These results suggest that CHr may be a suitable alternative marker of iron status for guiding iron treatment, and could potentially reduce the frequency of iron testing and potential harms from IV iron treatment, particularly in those with some degree of inflammation.

Therapy

Oral Iron

The benefit of treating iron deficiency without the presence of anemia remains uncertain. Small studies show that the administration of intravenous iron (oral iron not studied) improves fatigue in women without anemia whose ferritin levels are in the iron-deficient range [66]. Some studies with limited number of participants and quite heterogeneous also suggest that oral iron supplementation benefits physical performance in women of reproductive age [67]. Patients with IDA should receive iron supplementation. However, emerging data suggest that nonabsorbed iron could be harmful by modifying the gut microbiota, increasing the concentration of intestinal pathogens [68]. Patients with severe iron-deficiency anemia producing cardiovascular symptoms, such as heart failure or angina, should receive red-cell transfusions to treat the tissue hypoxia of anemia. One unit of packed red cells also provides approximately 200 mg of iron helping to correct partially the iron deficiency.

Prevention of iron deficiency in at-risk populations is practiced in some parts of the world by fortifying foods with iron compounds [69], for instance iron sulfate (FeS) which is soluble or iron pyrophosphate (FePP) which is less soluble. In human studies, plasma ferritin is a strong negative predictor of Fe bioavailability from FeS but not from FePP. Thus, more soluble Fe compounds demonstrate better overall absorption and can be used at lower fortification levels and because their absorption is upregulated in Fe deficiency, they innately "target" Fe-deficient individuals in a population [70]. Both addition of ascorbate and Na_2EDTA enhance iron absorption at molar ratios of 0.6:1 to 0.7:1 relative to fortification iron [71].

The administration of oral iron is a convenient, inexpensive, and effective means of treating stable patients. The low hepcidin levels in patients with uncomplicated iron-deficiency anemia ensure effective iron absorption and the recovery of hemoglobin levels; however, 3–6 months of treatment are often required for the repletion of iron stores and the normalization of serum ferritin levels. Mean values of absorption rates from 100 mg Fe in healthy males and females are ~5.0%, whereas in latent iron deficiency and in iron-deficiency anemia mean values of 10% and 13% are obtained, respectively. The maximum absorption rate is 20–25% [72]. With the conventional dose of 180–200 mg per day, less than 20 mg is absorbed explaining the long duration of therapy needed in most patients. Of note, studies of dosage amounts and frequency have shown that with increasing dose, fractional absorption decreases, whereas absolute absorption increases. A sixfold increase in iron dose from 40 to 240 mg only increases the amount of iron absorbed from 6.7 to 18.1 mg in non-anemic young women with plasma ferritin ≤20 μg/L. Providing lower dosages (40–80 mg Fe) and avoiding twice-daily dosing maximize fractional absorption. In fact, the hepcidin response to oral iron and its duration of the hepcidin response supports alternate day supplementation [73].

Among the myriad preparations on the market, iron sulfate is most frequently used; iron gluconate and iron fumarate are also effective. The recommended daily dose for adults with iron deficiency is 100–200 mg of elementary iron; for children, the dose is 3–6 mg per kilogram of body weight of a liquid preparation; for both groups, the supplement should be administered in divided doses without food. Addition of vitamin C is believed to increase absorption. Long-term use of oral iron is limited by side effects, including nausea, vomiting, constipation, and metallic taste; these side effects are frequent and, although not severe, are bothersome to some patients. Although oral iron may cause dark stools, it does not produce false-positive results on tests for occult blood. Failure of oral iron treatment typically results from premature termination of treatment, lack of compliance with the regimen, or discontinuation by the patient. Truly refractory response to treatment is uncommon and requires other, specific treatments, such as the eradication of infection with *H. pylori* or the introduction of a gluten-free diet in patients with celiac disease, may restore the capacity for iron absorption [43].

None of the markers of iron status are predictive of which patients will or will not have a response to oral iron therapy. Iron absorption studies in iron-deficient patients and healthy persons have been performed using iron radioisotopes [72], but they are no longer used in clinical practice due to radiation exposure and logistical difficulties. The oral iron challenge test in which either a low dose of 10–20 or a higher dose of 60 mg of oral iron is administered and serum iron levels are measured 1–3 h afterward is rarely used. Criteria include either the maximal increase in serum iron or the area under the curve [74, 75]. More recently, hepcidin has been used to predict the likelihood of response to oral iron in clinical studies. Those with low hepcidin levels were likely and those with high levels were unlikely to respond [76]. However, hepcidin tests are a specialty test and not routinely available for clinical use. The earliest response may be in the indices of RBC hemoglobin content in reticulocytes (CHr). A study in rheumatologic diseases and

iron-deficiency anemia showed that a change in CHr and in serum levels of iron and transferrin saturation may predict the response to the administration of oral iron after 1 week of therapy [77].

One of the most frequent obstacles to oral iron repletion in daily practice is patients' lack of adherence to what is prescribed. In this sense, individualized rather than "recommended" oral daily doses of 100–200 mg iron in divided doses might increase compliance. A dose of >120 mg per day is usually too much in that it greatly surpasses the maximum ability of the gut to absorb iron [77, 78] and it induces dose-dependent gastrointestinal, side effects that can be prevented by simply decreasing the dose. Administration of elemental iron at doses as low as 15 mg a day has been shown to correct anemia as effectively as administration of higher doses, with fewer adverse effects [79].

Parenteral Iron

Published evidence does not support the use of intravenous (IV) iron over oral iron to treat deficiencies in non-hemodialysis-dependent patients, even those with CKD [80]. Intravenous iron (IV Fe) therapy has traditionally been limited by the possibility of hypersensitivity reactions (including anaphylaxis), especially those formulated with varying molecular weight dextrans. IV iron has a variety of risks [81]. Acute anaphylaxis can be life-threatening and severe anaphylactoid reactions manifested by skin eruptions, hypotension, and gastrointestinal symptoms may precipitate hospitalization. In addition to acute effects, there is also concern over the cumulative effects over months and years on organ systems related to labile plasma iron effects in generating reactive iron species [82]. These are less likely in those without kidney disease who get iron more frequently.

The development of newly approved, safer iron formulations is modifying this clinical practice even in those with normal renal function. The transient side effects of IV Fe supplementation include nausea, vomiting, pruritus, headache, and flushing; myalgia, arthralgia, and back and chest pain. These usually resolve within 48 h, even after total dose administration (vida infra) [83]. Hypersensitivity reactions are rare [81, 83], as are severe or life-threatening reactions [84]. The pathophysiological features of these reactions are uncertain and might be exacerbated by released free iron [85, 86], a phenomenon is much less likely to occur with currently used formulations. Predisposing conditions are rapid infusions, a history of atopy, and drug allergy. To minimize risk infusion rate should be slow, the patient carefully observed, and trained health care personnel must be present in an environment with access to resuscitation facilities [84]. The use of a test dose is no longer recommended as it may provide false reassurance when larger amounts of iron are given as total dose infusions; premedication with antihistamine is also not advised because it may cause hypotension, tachycardia, and somnolence [87], effects often attributed to the IV Fe itself [88].

Although clinical trials are reassuring with regard to the efficacy and side-effect profile of intravenous iron, concerns persist with regard to the long-term biologic

effects of iron and its effects on the generation of oxygen radicals, patient susceptibility to infections [89], and the potential such treatment would have to worsen conditions such as type 2 diabetes and other chronic metabolic disorders [90]. Longer term (years) well-designed, randomized, controlled trials are needed to verify the long-term effects of intravenous iron supplementation [89]. In the interim, intravenous iron should be used only when the benefits outweigh the risks.

Why is IV iron even necessary? IV Fe circumvents the problem of iron absorption allowing Hb to increase more quickly than oral iron particularly when given in amounts of ~1000 mg elemental Fe over short periods of time [61, 91, 92]. Administration of amounts of 500 mg or more is often referred to a total dose infusion. Another advantage is that in some patients the total dose required (up to 1000 mg) can be provided in a single infusion. The dose needed is calculated with this formula:

Dose (in mg) = body weight in kilograms × 2.3 × hemoglobin deficiency (target hemoglobin level − patient hemoglobin level) + 500 to 1000 mg iron for tissue iron repletion.

The cost of IV Fe therapy is high, but the number of hospital or clinic visits that are required is decreased [92]. Patients with malabsorption and genetic IRIDA [vide supra] may require intravenous iron. IV Fe administration is also preferred when a rapid increase in hemoglobin level is required or when oral iron cannot keep up with chronic blood loss, as is the case in patients with hereditary hemorrhagic telangiectasia. Active inflammatory bowel disease is an emerging indication for IV Fe as some forms of oral iron are not only ineffective but may also increase local inflammation [93].

Intravenous iron has become the standard in the management of anemia in patients with chronic kidney disease who are receiving dialysis and treated with ESAs. IV Fe supplementation may eliminate or delay the need for ESAs in some patients with chronic kidney disease who are not receiving dialysis [6, 94]. Erythropoiesis-stimulating agents are also used in selected patients with low-risk myelodysplastic syndrome and in patients with cancer who are receiving chemotherapy: in these circumstances, iron supplementation is usually limited to patients with concomitant iron deficiency or to those in whom there is no response to erythropoiesis-stimulating agents. The mechanism by which IV iron enhances the effect of ESAs is unclear. One hypothesis suggests that increased iron in macrophages leads to the overexpression of ferroportin by means of the iron-responsive element−/iron-regulatory protein system, which enhances the mobilization of iron for use in erythropoiesis [3].

Intravenous iron should be avoided in the first trimester of pregnancy because of the lack of data on safety [95]; it has an acceptable side-effect profile when used later in pregnancy.

There has been interest in the role of IV Fe in CHF. A multicenter European trial of patients with iron deficiency and chronic heart failure showed that the use of intravenous iron supplementation led to improvements in physical performance, New York Heart Association functional class [61], and quality of life independently from the correction of anemia [96]. However, larger and longer studies are needed

to determine if there is added benefit in terms of mortality, hospitalization, and on renal function.

Conclusions

Anemia is a worldwide prevalent disorder. Distinguishing iron deficiency from other causes of anemia is important so the appropriate treatment is initiated. Signs of iron deficiency in tissue are subtle and symptoms are not specific. The molecular regulatory processes for iron homeostasis have pointed to the importance of iron sensing by hepatocytes which regulates hepcidin levels and marrow erythroblasts which through erythroferrone send a signal to suppress hepcidin when iron is needed for Hb synthesis. In the absence of inflammation, a TSAT <15% and a ferritin <30 ng/mL indicate iron deficiency. Inflammation through its effects on transferrin and ferritin alters these parameters. Availability of iron at the level of the bone marrow is best assessed by the reticulocyte hemoglobin content or the percent of hypochromic cells.

Treatment of iron deficiency must first focus on treating the underlying cause, if possible. Oral therapy is the standard of care but we are learning that lower dose of <100 mg/day less frequently may be as effective as doses of 200 mg/day and produce less side effects, fostering compliance. Iron-refractory iron-deficient anemia and that accompanying chronic inflammation may require parenteral iron intravenously. With the latter, hypersensitivity reaction are uncommon and therefore should be performed in an environment with access to resuscitation facilities and trained health care personnel are present. The role of iron in congestive heart failure and in some myopathic states is still being evaluated.

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Nutritional Anemia in Adults and in Elderly Patients

3

Emmanuel Andrès

Introduction

Anemia is a common condition, especially in middle age and in elderly patients, and its prevalence increases with age [1]. It affects quality of life in all patients, cognitive and physical function in frailty patients, and is a comorbid condition that affects other diseases (e.g., heart disease, cerebrovascular disorders) [2, 3]. Anemia is even associated with a risk of death. Thus, anemia should not be accepted as an unavoidable condition or a consequence of aging.

In adults and in the elderly, many underlying conditions can lead to anemia, but the most often ones are nutritional deficiencies, in practice iron deficiency, vitamin B9 (folic acid) deficiency, and vitamin B12 (cobalamin) deficiency [4]. Recognition of these disorders and deficiencies is essential for optimal treatment.

In this chapter, we report and discuss the current literature of nutrition-deficiency anemia, also call nutritional anemia, in middle age and in elderly patients in developed countries.

Definition of Nutritional Anemia

The *World Health Organization* (*WHO*) defines anemia in the adult as hemoglobin (Hb) concentration <12 g/dL for non-pregnant women and <13 g/dL for men [2]. In elderly, an Hb concentration <12 g/dL is commonly considered as an "established" anemia (under the strict sense of the academic definition), regardless of the sex of the patient [5]. Although low hemoglobin levels are often seen with advancing age,

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anemia should not be assumed to be a normal consequence of aging. Age may be associated with compromised hematopoietic reserve and consequently with an increased susceptibility to anemia in the presence of hematopoietic stress induced by an underlying disorder [6]. In clinical practice, an Hb level <10 g/dL is often considered to be a cut off level where investigation, particularly in the elderly, and treatment should be performed [7]. Indeed, at this Hb level, several recent studies have shown a benefit from investigating the anemia [7].

Nutrition-deficiency or nutritional anemia refers to types of anemia that can be directly attributed to nutritional disorders [4, 8]. Thus, the term "nutritional anemia" covers any anemia resulting from a dietary deficiency of materials essential to red blood cell formation, for example: iron; vitamins, especially vitamin B9, but also vitamin B12 and vitamin C (in scurvy), and more rarely, vitamin A, vitamin E and vitamin B2 (riboflavin), vitamin B3 (in pellagra), vitamin B6 (pyridoxine); selenium, zinc and copper; and protein [8].

Prevalence of Nutritional Anemia

The prevalence of anemia increases with advancing age, especially after age 60–65 years, and rises sharply after the age of 80 years [2, 6]. In this population, anemia represents, in developed countries, a public health problem. Results from the third *National Health and Nutrition Examination Survey* (*NHANES III*) carried out in the United States indicate that the prevalence of anemia among men and women aged 65 or more was 11% for men and 10.2% for women [1]. Survey findings indicate further that most anemia among the middle-aged and elderly is mild; only 2.8% of women and 1.6% of men had an Hb <11 g/dL [9]. Results from the *NHANES III* also indicate that nutritional anemia represents at least one-third of all causes of anemia [1].

Results from the Framingham cohort indicates a slightly lower prevalence of anemia among older men living in the United States. In this group of 1016 subjects 67–96 years of age, the prevalences of anemia in men and women were 6.1% and 10.5%, respectively [10]. In this study, nutritional anemia was also a major cause of anemia as well as anemia of inflammation and anemia in the setting of renal failure.

In a French nationwide study of 1351 patients hospitalized in departments of internal medicine, anemia was present in 874 (65%) patients according to the *WHO* definition, and 573 (42%) patients had Hb levels <11 g/dL [11].

Etiology of Anemia, with a Focus on Nutritional Anemia

Because middle age and elderly patients often have several associated co-morbid conditions and are commonly taking a variety of medications, some of which may contribute to anemia, the precise etiology of anemia is frequently difficult to

determine, even after extensive investigations, including bone marrow biopsy [12, 13]. Thus in the literature and in our experience, the etiology of anemia can be identified in only approximately 80% of the cases, in spite of the use of new tools such as video capsule [7, 14].

A significant proportion of middle age and elderly anemic patients, around 30–50%, are presumed to have multiple causes for their anemia [14, 15]. In the cases for which the cause of anemia has not been established, patients may have received an inadequate diagnostic work-up.

In adult and in the elderly, causes of anemia are divided into three main broad groups: (1) nutrient deficiency or nutritional anemia, most often iron-deficiency anemia; and/or (2) anemia of chronic disease as renal failure, chronic inflammation, chronic heart failure; and (3) unexplained anemia [2, 3, 9].

In the aforementioned *NHANES III* study, 34% of all anemia in adults and elderly patients are caused by iron, vitamin B9, and vitamin B12 deficiency, alone or in combination (nutritional anemia) [1]. In this study, 12% of all anemia are related, perhaps at least associated, with renal insufficiency, 20% with chronic diseases, and in 34% of the cases, the cause remained unexplained. About 60% of nutritional anemia is associated with iron deficiency and most of those cases are the result of chronic blood loss from gastrointestinal lesions in developed countries [1]. The remaining cases of nutrition-deficiency anemia are usually associated with vitamin B12 and/or vitamin B9 deficiency and are easily treated.

In Table 3.1, we report our personal experience (retrospective study) of the etiology of anemia in 300 hospitalized patients ≥65 years old [4, 7].

As see below, rare unknown causes of nutrition-deficiency anemia also include several other vitamin deficiencies (vitamins A, B2, B3, C, and E), selenium, zinc, or copper [8]. These later etiologies are nevertheless not well-studied in the literature, and to date, few not-well-documented date are available, except for the theoretical and pathological aspects of anemia.

Table 3.1 Etiology of anemia in patients older than 65 years ($n = 300$), hospitalized in an internal medicine department (tertiary reference center)

Etiology	Prevalence (%)
Chronic inflammation (chronic disease)	23.0
Iron deficiency	18
Renal failure	9
Liver disease and endocrine disease (chronic disease)	7
Posthemorrhagic	7
Folate deficiency	6
Myelodysplasia	5
Vitamin B12 deficiency	4
Unexplained causes	21

Based on data from [7]

Iron-Deficiency Anemia in Middle Age Patients and in the Elderly

Iron-deficiency anemia, the most common cause of anemia in middle age patient and in the elderly [1], results usually from chronic gastrointestinal (GI) blood loss mainly caused by: esophagitis, gastritis, ulcer, related or not related to nonsteroidal anti-inflammatory drug intake and/or chronic *Helicobacter pylori* infections, varices (portal hypertension), colorectal cancer or premalignant polyps, or angiodysplasia [15, 16]. GI blood loss is often occult and is not ruled out by negative fecal blood tests. GI tract abnormalities can be identified in the majority of patients with iron-deficiency anemia [14].

In 40–60% of patients, the source is in the upper GI tract [18, 19]. The blood loss is in the colon in 15–30% of cases. The source is not found in the remaining 10–40% of patients with GI blood loss. Fortunately, these patients do well with iron replacement and repeat investigation is not often needed, especially in frailty elderly and too sick elderly [15]. In middle age patient, repeat GI investigation with upper and/or lower endoscopy and video capsule may be of interest, with the detection of the etiology of bleeding in an additional 20% of cases [19, 20]. It is important because one-third of the iron-deficiency anemia is related to GI malignancies. In young women, iron-deficiency anemia is often in relation with heavy menstrual bleeding, or meno-metrorrhagia [21].

Table 3.2 presents our experience of the evaluation of the GI tract in 90 patients with chronic blood loss, follow-up in an internal medicine department (in a referral center) [7]. Bleeding disorders and particularly anticoagulants (vitamin K antagonists) may cause iron deficiency in the elderly (around 20% in our experience) [7, 14].

H. pylori infection and chronic gastritis, especially atrophic gastritis, are significantly associated with unexplained iron-deficiency anemia, as celiac disease and

Table 3.2 Results of the evaluation of the gastrointestinal tract in elderly patients (≥65 years) with chronic blood loss ($n = 90$), hospitalized in an internal medicine department

Etiology	Prevalence (%)
Esophagitis and Mallory Weiss syndrome	4.4
Gastritis, atrophic gastritis and ulcer related or not related to NSAID use and/or *Helicobacter pylori* infection	30
Varices related to portal hypertension	9
Angiodysplasia	2.25
Colon diverticula	4.5
Colorectal benign and pre-malignant polyps	5.5
Colorectal cancer	5.5
Inflammatory bowel disease	2.25
Unexplained causes	36.6

NSAID nonsteroidal anti-inflammatory drug
Based on data from [14]

rarely, large amount of tea (at least 2 L par day) and vegetarian diet [22]. Older persons may also become iron deficient because of inadequate dietary intake or inadequate absorption or bioavailability of iron (with anti-acids) [15]. Heme iron from animal sources is better absorbed than non-heme iron from plant sources. Vitamin C in food enhances iron absorption from non-heme sources. The tannins and polyphenols in tea and coffee can inhibit iron absorption. Any elderly subject whose dietary intake is poor and has recent unexplained weight loss is a candidate for increased medical surveillance. Nevertheless, iron-deficiency anemia is rarely the result of dietary deficiency in industrialized countries [23]. Without blood loss, anemia takes several years to develop.

Chronic blood loss from the genitourinary tract and chronic hemoptysis may result in iron deficiency but are much less common causes [15].

Vitamin B9 Deficiency Anemia in Middle Age Patients and in the Elderly

A regular diet contains 500–700 μg of vitamin B9. On average, 50–60% of dietary folic acid is absorbed in the duodenum and jejunum. Vitamin B9 deficiency usually develops as a result of inadequate dietary intake, of malabsorption, as in celiac disease, or of malnutrition [1, 15]. The body contains very little vitamin B9, with stocks expected to last 4–6 months. Patients usually have a history of weight loss, poor weight gain, and weakness.

In addition, several drugs (methotrexate, cotrimoxazole, sulfasalazine, anticonvulsant) and alcohol may cause deficiency of folic acid [7]. Pregnancy is also a common cause of vitamin B9 deficiency, but is generally prevented in industrialized countries by vitamin B9 supplementation.

Vitamin B12 Deficiency Anemia in Middle Age Patients and in the Elderly

Both vitamin B12 and folic acid deficiency are common among middle age patient and in the elderly, each occurring in at least 5% of the patients [15]. The Framingham study demonstrated an incidence of 12% among elderly people living in the community [24].

In adult and elderly patients, the etiologies of cobalamin deficiency are represented primarily by food-cobalamin malabsorption (FCM) and pernicious anemia and, more rarely, by intake deficiency and malabsorption [25]. In our study, in which we followed more than 200 elderly patients with a proven deficiency, FCM accounted for about 60–70% of the etiologies of cobalamin deficiency, and pernicious anemia 15–25%. Figure 3.1 presents the principal causes of cobalamin deficiency in 172 elderly patients (median age: 70 years) hospitalized in the university hospital of Strasbourg, France [26, 27].

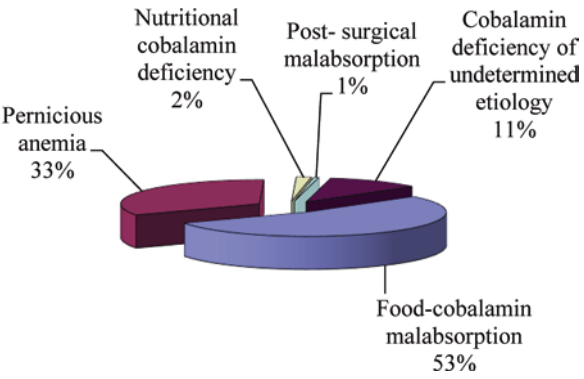

Fig. 3.1 Etiologies of cobalamin deficiency in 172 elderly patients hospitalized in University Hospital of Strasbourg, France. Based on data from [25, 29]

Initially described by Carmel in the 1990s, the FCM is characterized by the inability to release cobalamin from food and/or intestinal transport proteins, particularly in the case of hypochlorhydria, where the absorption of "unbound" cobalamin is normal ("mal digestion" of the food-cobalamin) [28]. As we have recently indicated, this syndrome is defined by cobalamin deficiency despite sufficient cobalamin intake from food and a normal Schilling test, where the latter rules out malabsorption or pernicious anemia [29].

FCM is caused primarily by atrophic gastritis. Over 40% of patients older than 80 years of age have gastric atrophy that might or might not be related to *H. pylori* infection [28]. Other factors that commonly contribute to FCM in middle age patient and elderly people include: chronic carriage of *H. pylori* and intestinal microbial proliferation (in which case vitamin B12 deficiency can be corrected by antibiotic treatment); long-term ingestion of antacids, including h_2-receptor antagonists and proton-pump inhibitors, and biguanides (metformin); chronic alcoholism; surgery or gastric reconstruction (e.g., bypass surgery for obesity); partial and pancreatic exocrine failure [29].

In our experience, the clinical manifestations of FCM are not very different from those of cobalamin deficiencies associated with other causes, e.g., Biermer's disease [29]. However, it should be emphasized that Carmel first believed that FCM was associated with moderate cobalamin deficiency, leading to only "subtle" clinical symptoms ("subtle cobalamin deficiency") [30]. Our published data contradict this assertion [26, 27].

Clinical Presentation of Nutritional Anemia

The onset of symptoms is usually insidious, anemia settling in general over several weeks or months, and many patients adjust their activities. Fatigue, dispend, and other typical symptoms often occur gradually. In elderly, these symptoms of anemia are likely to be overlooked. In this situation, pallor can be helpful but hard to detect [15]. The symptoms of other diseases may be exacerbating if anemia is also present.

Anemia in older individuals is associated with a very wide range of complications, including increased risk for mortality, cardiovascular disease—anemia has been reported to worsen angina and congestive heart failure, cognitive dysfunction related to cerebrovascular insufficient, longer hospitalization for elective procedures and comorbid conditions, reduced bone density, and falls and fractures [6, 31]. Not surprisingly, anemia also has a significant effect on quality of life in the elderly.

Moreover, symptoms related to nutrition-deficiency as iron, vitamin B12, and folic acid might be present outside the hematological sphere. Table 3.3 presents features related to vitamin B12 deficiency in elderly patients [25]. It should be noted that vitamin B12 deficiency may be present even in the absence of anemia. The symptoms of vitamin B9 deficiency are nearly indistinguishable from those of cobalamin deficiency.

Iron deficiency is responsible for changes in hair, nails, mucosa and tongue as pruritus, chronically sustained inflammation, dermatitis herpetiformis, photodermatitis, and Plummer's syndrome [32].

Nutritional anemia is generally hyporegenerative and represents the consequence of the hematopoietic system inability to replace the peripheral blood loss [12, 15]. Patients with nutritional anemia often have mild to moderate anemia, with Hb levels

Table 3.3 Manifestations related to vitamin B12 deficiency (with the exception of hematological manifestations)

Neuro-psychiatric manifestations	Digestive manifestations	Other manifestations
Frequent: polyneurites (especially sensitive ones), ataxia, Babinski's phenomenon	Classic: Hunter's glossitis, jaundice, LDH, and bilirubin elevation ("intramedullary destruction")	Under study: atrophy of the vaginal mucosa and chronic vaginal and urinary infections (especially mycosis), venous thromboembolic disease, angina (hyperhomocysteinemia)
Classic: combined sclerosis of the spinal cord	Debatable: abdominal pain, dyspepsia, nausea, vomiting, diarrhea, disturbances in intestinal functioning	
Rare: cerebellar syndromes affecting the cranial nerves including optic neuritis, optic atrophy, urinary and/or fecal incontinence	Rare: resistant and recurring mucocutaneous ulcers cobalamin deficiency	
Under study: changes in the higher functions, even dementia, stroke and atherosclerosis (hyperhomocysteinemia), parkinsonian syndromes, depression, multiple sclerosis		

Based on data from [25]

Table 3.4 Hematological manifestations of vitamin B12 deficiency in patients with documented cobalamin deficiency ($n = 201$), hospitalized in an internal medicine department (tertiary reference center)

Parameters	Values
Hemoglobin level (g/dL)	10.3 ± 0.4 (4.9–15.1)
Mean erythrocyte cell volume (fL)	98.9 ± 25.6 (76–142)
Reticulocyte count (%)	15.2 (1–32)
White cell count (/mm^3)	6200 ± 4100 (500–20,000)
Platelet count (10^3/mm^3)	146 ± 42 (27–580)
Anemia with Hb level <12 g/dL	37%
Anemia with Hb level <6 g/dL	2.5%
Anemia and macrocytosis (MECV >100 fL)	33.8%
Isolated macrocytosis (VGM >100 fL)	17%
Microcytosis (VGM <80 fl)	5%
White cell count <4000/mm^3	14%
Neutrophile count <1000/mm^3	3%
Thrombopenia (<150 × 10^3/mm^3)	10%
Neutrophile hypersegmentation	32%
Megaloblastosis	60%
Life threatening manifestations	9%

Based on data from [33]

between 8 and 10 g/dL. In practice, because of the multifactorial etiologies of anemia, particularly in elderly, the erythrocytes are frequently normocytic: mean erythrocyte cell volume (MECV) between 80 and 100 fL in anemia of exclusive iron deficiency, the erythrocytes are usually microcytic (MECV <80 fL) in anemia of exclusive vitamin B9 and/or vitamin B12 deficiency, the erythrocytes are usually macrocytic (MECV >120 fL). Other hematological manifestations may be associated as presented in Table 3.4, with the example of cobalamin deficiency [33].

A low serum iron level, an increased total iron-binding capacity, and a low serum ferritin level (<15 ng/mL) accompany iron-deficiency anemia [17]. In cases associated with inflammation, the transferrin receptor—ferritin index is the most appropriate parameter to measure [17].

In cobalamin deficiency, serum vitamin B12 level is low (<200 pg/mL), serum methylmalonic acid and homocysteine levels are increased [30]. In vitamin B9 deficiency, the red cell acid folic concentration is more reliable than the serum level and therefore should be measured [30].

Treatment of Nutritional Anemia

Treatment of nutritional anemia requires particular attention to establish the correct cause [15]. In iron-deficiency anemia, iron supplementation should be initiated, in association with the treatment of the underlying cause of bleeding. Standard therapy for iron deficiency is oral administration of a 300 mg tablet of ferrous sulfate (60 mg of elemental iron) [34, 35]. Intravenous iron replacement can be helpful in patients

with iron deficiency that fails to respond to oral replacement. Vitamin C enhances iron absorption. Intravenous iron replacement can be helpful in patients with iron deficiency that fails to respond to oral replacement. Parenteral iron may also be used when there is intolerance or non-compliance with oral preparations. Intravenous iron sucrose is reasonably well tolerated, even when administered in boluses [35].

In adult and elderly patients, vitamin B12 deficiency anemia may be treated by vitamin B12 supplementation, booth parenterally or orally [36, 37]. Our team has developed an effective oral treatment for both of food-cobalamin malabsorption and pernicious anemia using a crystalline cobalamin (cyanocobalamin). The main results of our oral cobalamin treatments studies are summarized in Table 3.5 [36]. A systematic review conducted under the auspices of the *Cochrane Metabolic and*

Table 3.5 Personal experience on oral cobalamin therapy: results on hematological manifestations

Study characteristics (number of patients)	Therapeutic modalities	Results
Open prospective study of well-documented vitamin B12 deficiency related to food-cobalamin malabsorption ($n = 10$)	Oral crystalline cyanocobalamin: 650 µg per day, during at least 3 months	Normalization of serum vitamin B12 levels in 80% of the patients
		Significant increase of Hb levels (mean of 1.9 g/dL) and decrease of mcv (mean of 7.8 fL)
Open prospective study of low vitamin B12 levels not related to pernicious anemia ($n = 20$)	Oral crystalline cyanocobalamin: 1000 µg per day during at least 1 week	Normalization of serum vitamin B12 levels in 85% of the patients
Open prospective study of well-documented vitamin B12 deficiency related to food-cobalamin malabsorption ($n = 30$)	Oral crystalline cyanocobalamin: between 1000 and 250 µg per day, during 1 month	Normalization of serum vitamin B12 levels in 87% of the patients
		Significant increase of Hb levels (mean of 0.6 g/dL) and decrease of MCV (mean of 3 fL); normalization of Hb levels and ECV in 54% and 100% of the patients, respectively
		Dose effect—effectiveness dose of vitamin B12 ≥ 500 µg per day
Open prospective study of low vitamin B12 levels not related to pernicious anemia ($n = 30$)	Oral crystalline cyanocobalamin: BETWEEN 1000 and 125 µg per day during at least 1 week	Normalization of serum vitamin B12 levels in all patients with at least a dose of vitamin ≥250 µg per day
		Dose effect—effectiveness dose of vitamin B12 ≥ 500 µg per day
Open prospective study of low vitamin B12 levels related to pernicious anemia ($n = 10$)	Oral crystalline cyanocobalamin: 1000 µg per day, during at least 3 months	Significant increase of serum vitamin B12 levels in 90% of the patients (mean of 117.4 pg/mL
		Significant increase of Hb levels (mean of 2.45 g/dL) and decrease of MCV (mean of 10.4 fL)

Hb hemoglobin, *MCV* mean cell volume
Based on data from [36]

Endocrine Disorders Review Group supports the efficacy of oral cobalamin therapy, with a daily dose of 2000 and 1000 μg initially and thereafter a weekly dose of 2000 and 1000 μg of vitamin B12 [37].

In vitamin B9 deficiency, therapeutic doses vary between 1 and 5 mg per day [38]. Usually, treatment is continued for at least 3–6 months, provided that the underlying causes of the deficiencies have been corrected.

Food sources of nutrients are best for prevention of nutritional anemia, but often supplementation is necessary, especially for the elderly [39]. The *USA National Academy of Sciences* recommends acid folic and vitamin B12 supplements (fortified cereal) in elderly.

Conclusions

In conclusion, nutritional anemia represents around one-third of all anemias in middle age and elderly patients. About two-thirds of nutritional anemias are associated with iron deficiency and most of those cases are the result of chronic blood loss from gastrointestinal lesions. The remaining cases of nutritional anemia are usually associated with vitamin B12, most frequently related to food-cobalamin malabsorption, and/or acid folic deficiency. Treatment of nutritional anemia is easy with nutrient-deficiency replacement but requires particular attention to discerning the cause.

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Anemia of Chronic Disease

4

Anatole Besarab and Stefan Hemmerich

Abbreviations

ACD	Anemia of chronic disease
AMPK	Adenosine monophosphate-activated kinase
BFU-E	Burst-forming unit erythroid
BMP	Bone morphogenetic protein
CFU-E	Colony-forming unit erythroid
CFU-GEMM	Colony-forming unit-granulocyte, erythrocyte, monocyte, megakaryocyte
CKD	Chronic kidney disease
COX 2	Cyclo-oxygenase 2
CRP	C-reactive protein
DMT-1	Divalent metal transporter 1
EPO	Erythropoietin
EPOR	Erythropoietin receptor
ERFE	Erythroferrone
ESA	Erythropoiesis-stimulating agents
ESRD	End stage renal disease
FP	Ferroportin
GDF	Growth differentiation factor
GM-CSF	Granulocyte-monocyte colony stimulating factor
Hb	Hemoglobin

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HLA	Human leucocyte antigen
HMBGB1	High mobility group box 1
HMGB	High mobility group box
hsCRP	High-sensitivity CRP
ID	Iron deficiency
IDA	Iron-deficiency anemia
IFN	IFN
IgG	Immunoglobulin G
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRE	Iron-responsive element
IRE/IRP	Iron-responsive element/iron regulatory protein
JAK/STAT	JANUS-associated kinase/signal transducer and activator of transcription
LPI	Labile plasma iron
LPS	Lipopolysaccharides
MAPK	Mitogen-activated protein kinases
Mb	Myoglobin
mRNA	Messenger RNA
NFκB	Nuclear factor kappa B
NO	Nitrous oxide
NTBI	Non-transferrin bound iron
PAF	Platelet activating factor
PDGF	Platelet-derived growth factor
RA	Rheumatoid arthritis
RES	Reticuloendothelial system
ROS	Reactive oxygen species
s-EPO	Serum EPO levels
SLC4A1	Soluble carrier family 4 (anion exchanger) member 1 (Diego blood group)
Smad	Small body size/mothers against decapentaplegic
SOCS	Suppressor of cytokine signaling
STAT	Signal transducer and activator of transcription
TfR	Transferrin receptor
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Introduction

Anemia of chronic disease (ACD) also known as “anemia of chronic inflammation” is the second most prevalent anemia [1]. ACD is often present in hospitalized patients [2] with its prevalence as cause of anemia exceeded only by iron-deficiency

anemia (IDA) [1, 3–5]. It may be difficult to delineate the true prevalence rate of ACD which may be even higher as ACD is often confused with IDA and is virtually always a diagnosis of exclusion. The incidence of ACD increases with age, affecting 77% of the elderly in whom no clear cause of anemia is found, thus indicating a multifactorial etiology [6, 7]. ACD develops in patients in whom their current illness elicits an active immune/inflammatory response leading to reduced iron uptake at varying sites and should be clearly differentiated from those patients in whom their illness (e.g., cancer) or its treatment (e.g., cytotoxic drugs) is the major cause for the anemia [7]. ACD is seen in many disease states such as malignancies, inflammatory, chronic infections, and autoimmune diseases [1, 4, 5], reflecting the multiplicity in pathogenic pathways that lead to the anemia state. Severity of ACD is usually mild to moderate anemia in patients diagnosed with other chronic disease conditions, which may not always be purely inflammatory. A list of the possible causes of ACD is shown in Table 4.1.

Pathogenic mechanisms vary within each class of disorders listed in Table 4.1. However, each of the individual factors to be discussed subsequently plays some role in the eventual cause of anemia. One part of the pathophysiological process reflects one overarching goal: deprive the invading cells of iron, whether these cells are cancer cells or external pathogens. Iron is an important nutrient for proliferation of mammalian cells as well as infectious agents. However, it is the red blood cell which becomes the innocent bystander. To truly understand the processes that lead to anemia, the reader is referred to Chap. 2 on Iron-Deficiency Anemia in which the crucial role of normal iron metabolism in erythropoiesis is described. However, in order to fully understand ACD, the effects of inflammation on response to EPO must be understood as well. Disorders in EPO secretion and action and shortening of red cell lifespan also play a role in the pathogenesis of ACD. The main therapy is treatment of the underlying disorder and red cell transfusions in severe anemia. In more severe (protracted) anemia that leads to impaired quality of life and

Table 4.1 Disorders producing anemia of chronic disease and prevalence of anemia within each category

Associated disorder	Estimated prevalence (%) of the disorder
Infections (acute and chronic): viral infections, including HIV; bacterial; parasitic; fungal	18–95
Cancer: hematological and solid tumors	30–77
Autoimmune:	8–71
Rheumatoid arthritis	
Systemic lupus erythematosus	
Connective-tissue diseases	
Vasculitis	
Sarcoidosis	
Inflammatory bowel disease (IBD)	
Chronic rejection after solid-organ transplantation	8–70
Chronic kidney disease and inflammation	25–30

has an impact on the mortality and survival rate, recombinant erythropoiesis-stimulating agents (ESAs) are used.

Importance of Iron

Adequate hemoglobization of red blood cells, an iron-requiring process, is essential for normal erythropoiesis. Total body iron is 50 mg/kg body weight, or approximately 3500 mg for a 70-kg man [8]. Sixty-five percent this iron is distributed within red cells as hemoglobin (Hb); 10% as myoglobin (Mb), cytochromes, and enzymes; and the remainder in the RES, liver, and bone marrow. To meet the daily requirement of producing 300 billion new erythrocytes, differentiating erythroblasts require approximately 20–30 mg/d iron, most of which is obtained from the recycling of senescent red blood cells (RBCs) by phagocytic macrophages of the reticuloendothelial system (RES) [9]. Heme from these cells is metabolized by heme oxygenase, and the Fe^{2+} released is sequestered by ferritin [8].

Only 10% of the dietary iron intake of 15–20 mg/dL is present as relatively bioavailable heme compounds, which are readily absorbed into enterocytes and degraded by heme oxygenase to release Fe^{2+}. The remaining non-heme iron exists in the relatively unavailable Fe^{3+} state and must be reduced to Fe^{2+} by ferrireductase in conjunction with ascorbic acid; iron is then transported into the enterocyte by divalent metal transporter (DMT)-1 [8]. Cytosolic iron, whether present in duodenal enterocytes, macrophages, or hepatocytes, moves to circulating transferrin via an exporter, ferroportin (FP)-1, during which Fe^{2+} is oxidized to Fe^{3+}, and bound to plasma transferrin [10]. In order to provide sufficient iron for reticulocyte production, transferrin-bound iron must be recycled 6–7 times daily [8]. Iron enters the erythroblast when two transferrin molecules bind TfR-1, which then undergoes endocytosis into a clathrin-coated siderosome [8]. The siderosome is acidified, releasing Fe^{3+} that is again reduced to Fe^{2+} by ferrireductase and exported to the cytoplasm via DMT-1. The apotransferrin:TfR-1 complex is recycled to the cell membrane and released into the circulation [8, 11]. Meanwhile, cytosolic iron enters the mitochondrion, where ferrochelatase catalyzes its insertion into protoporphyrin IX to form heme, the critical component of Hb [8, 12].

Two models of iron homeostasis have been described. One model postulates that plasma iron is sensed by duodenal crypt enterocytes via the TfR [8]. When iron is scarce, low cytosolic iron induces the transcription of TfR-1, DMT-1, and FP-1 mRNA, all of which stimulate iron absorption. A second, compatible regulatory model proposes that iron absorption is downregulated by hepcidin, a 25-AA polypeptide produced by hepatocytes when iron is abundant. Hepcidin binds to FP-1 in enterocytes, macrophages, and hepatocytes themselves, promoting the JAK-2-mediated tyrosine phosphorylation, internalization, and degradation of FP-1; thus, hepcidin inhibits both the efflux of iron from the duodenum into the plasma as well as the mobilization of iron from the RES [13]. Hepcidin expression is itself regulated at critical points in the homeostatic loop. Specifically, hepcidin transcription is inhibited *by HIF* during tissue hypoxia, by soluble hemojuvelin during iron

deficiency, and by EPO, GDF-15, and twisted gastrulation (TWSG-1) during erythroblast maturation. Conversely, hepcidin transcription is stimulated by iron-mediated production of bone morphogenetic proteins (BMPs), lipopolysaccharide (LPS), and IL-6 in states of systemic inflammation [8, 9, 14, 15].

In summary, iron metabolism is balanced by two regulatory systems. One functions systemically and relies on the hormone hepcidin and the iron exporter ferroportin. The other predominantly controls cellular iron metabolism through iron-regulatory proteins that bind iron-responsive elements in regulated messenger RNAs. To optimize iron delivery to cells, the two systems must "tango" together in a coordinated manner [15], while at the same time avoiding iron deficits or excess [16, 17]. Tight regulation of iron is necessary because iron is highly toxic and human beings can only excrete small amounts through sweat, skin and enterocyte sloughing, and fecal and menstrual blood loss [18].

Importance of Erythropoietin

The other important component for red cell production is the effect of the hormone EPO. In adults, 90% of the EPO produced in response to anemia originates from the kidney, specifically from a population of interstitial peritubular fibroblasts with neuron-like morphology (pericytes) in the inner cortex and outer medulla [19, 20]. In adults, the population of interstitial peritubular fibroblasts in the inner cortex and outer medulla [19, 21] regions tends to be especially susceptible to hypoxia [22, 23]. EPO expression in other cell types is normally suppressed by transcription factors that bind the G-A-T-A tetranucleotide sequence in the core promoter region of the gene (GATA transcription factors) [20]. Mild hypoxia, rather than increasing the transcription of EPO mRNA within each cell, actually stimulates the brisk recruitment of previously quiescent clusters of cells within the kidney, each of which then generates EPO at a fixed rate [24]. Moderate hypoxia, however, fuels additional EPO production by the liver, mainly from hepatocytes near the central veins and, to a lesser extent, from stellate or Ito cells [19, 21].

RBC production (erythropoiesis) takes place in the bone marrow. It begins with the EPO-independent differentiation of the multipotent hematopoietic stem cell into multipotential colonies containing erythroid cells, megakaryocytes, neutrophilic and eosinophilic granulocytes, and monocyte-macrophages (CFU-GEMM). Some of these colonies in turn develop into the burst-forming unit-erythroid (BFU-E) [25]. The BFU-E is the first cell type in the erythroid lineage to express the EPO-receptor (EPO-R), and EPO is required for its survival and subsequent proliferation into several colony-forming units-erythroid (CFU-E), a process which requires 10–13 days. Of all the erythroid precursors, the CFU-E has the highest membrane density of EPO-R and additionally expresses TfR and GATA-1 [26]. In the presence of EPO, GATA-1 promotes transcription of an anti-apoptotic protein allowing the CFU-E to multiply. Conversely, in the absence of EPO, pro-apoptotic caspases are activated, and the CFU-E dies. If the CFU-E survives, it first differentiates into the proerythroblast, which again requires exposure to EPO to escape apoptosis. The

proerythroblast has a large nucleus and expresses numerous membrane adhesion molecules [27]. During maturation, the nucleus condenses and many of these adhesion molecules are lost. Thus in this long almost 2-week period in which stem cells differentiate and proliferate into erythroblast, EPO is the main biological driver for making enough cells and different EPO-dependent signals are used as the cells divide, differentiate, and avoid apoptosis. Very little Hb is actually present within any of these cells.

Terminal erythroid differentiation occurs as the erythroblast progresses through the basophilic, polychromatic, and orthochromatic normoblast stages. Thirty-two daughter cells arise from each erythroblast and ultimately become reticulocytes. During this sequence, the cell rapidly acquires Hb and various proteins that promote membrane elasticity and stability [27]. The orthochromatic normoblast extrudes its pyknotic nucleus, which is ingested by a macrophage, and after 2–3 days becomes a reticulocyte [24]. This enucleated, multi-lobed reticulocyte is transformed into a biconcave erythrocyte during the next 2–3 days, initially in the bone marrow and then in the circulation. At the erythroblast stage, the cells send a signal, erythroferrone, to the liver to suppress hepcidin, thus permitting iron to be absorbed from the gut and released from macrophage stores for incorporation into heme in the developing RBCs [28].

Although the pathophysiological processes operative in ACD are still incompletely understood, they are thought to be mediated through the actions of various cytokines especially tumor necrosis factor (TNF), interleukins (IL)-1 and −6, and interferon (IFN) [1]. These cytokines, as well as the acute phase protein, hepcidin released by the liver, inhibit iron release from the marrow macrophages for incorporation into heme by the post erythroblast stage of erythropoiesis. The cytokines also directly induce the modulation of translation/transcriptions of genes involved in iron homeostasis, either directly or via production of labile radicals [7].

The diagnosis of ACD requires a good knowledge of the processes that affect production of the key molecules and of course interpretation of the results of the circulating iron status parameters as well as ferritin and hepcidin assays. An important step in ACD diagnosis is distinguishing ACD from iron-deficiency anemia [29]. A diagnosis of ACD does not suffice for anemia observed in all chronically ill patients [30], because of the temptation to label all ill patients in whom a definite cause of anemia cannot be discerned as ACD. A diligent effort should be made to unravel the underlying cause of anemia in all patients as well as to rule out iron deficiency. The success of management of either condition is therefore reliant on making correct diagnosis, as each would require different treatment protocols [31, 32]. In addition to treating the underlying condition, targeting other inflammatory pathways may be beneficial to achieve rapid resolution of anemia [33, 34].

Pathogenesis of Anemia of Chronic Disease

The pathogenic mechanisms vary [2] as each of the pathways depends largely on the etiological process ongoing in the patient (see Table 4.1). However, each of the individual factors plays some role in the eventual cause of anemia. These processes

include bone marrow invasion by tumors or infective agents, alteration of iron metabolism and diversion of body iron, hemophagocytosis, reduction in erythropoiesis secretion, and diminished response to EPO stimulation [1, 6, 7]. In addition, red blood cell survival in the circulation decreases, magnifying the effect of underproduction of red blood cells.

A list of the major cytokines involved in ACD and their mechanisms of effecting these actions is given in Table 4.2.

Iron Dysregulation (Reticuloendothelial Iron Blockade

This is a major causal pathway in ACD, eventuating in the presence of a hypochromic microcytic red cell picture similar to what is found in iron deficiency. Microbial invasion, malignancies and autoimmune disorders cause activation of CD3 T lymphocytes and macrophages which release cytokines including IFN-γ (from T cells), tumor necrosis factor- (TNF-)α, interleukin- (IL-)1, and IL-6 (from monocytes) [35, 36]. TNF-α is secreted also by neutrophils, macrophages, T-cells and natural killer cells in response to stimulation by IL-2, granulocyte-monocyte colony stimulating factor (GM-CSF), and platelet associated factor (PAF), and is inhibited by IL-6, transforming growth factor and prostaglandin E2 [37]. Bacterial LPS and IL-6 induce the hepatic cells to release hepcidin [38], which enhances breakdown of ferroportin, leading to blockade of the duodenal iron transfer [13, 39, 40]. Both molecules also upregulate the expression of divalent metal transporter (DMT-1), a trans-membrane protein involved in the uptake of iron by enterocytes [40, 41] and by macrophages [34]. They also inhibit the expression of ferroportin 1, the only iron exporting system in mammalian cells [13], which reduces export of iron from the

Table 4.2 Effects of cytokines on iron metabolism and erythropoiesis

Cytokines	Effects on iron or erythropoiesis
TNF-α	Inhibits EPO production
	Stimulates ferritin synthesis
	Enhances degradation and phagocytosis of effete red cells
	Direct inhibitor of erythropoiesis
IFN-γ	Inhibits production of EPO
	Increases intracellular iron (via DMT1) and inhibits ferroportin
	Increased NO production and iNOS mRNA expression
Interleukin-6	Increases iron uptake via DMT-1 activation
	Reduces transferrin receptor by decreasing TfR RNA expression
	Downregulates expression of SLC-4a1 in erythroid precursors
Interleukin 4 and 10	Increases ferritin via action on IRE/IRP
Interleukin 22	Influences hepcidin production

macrophages to the growing erythroid precursors; this action is influenced by hepcidin (vide infra) [14, 42, 43].

As part of the "acute phase reaction" IFN-γ stimulates ferritin transcription while simultaneously inhibiting transferrin receptor (TfR) mRNA expression via an IRE/IRP-independent process [44, 45]. It also increases expression of DMT-1, which is involved in the active transport of ferrous molecules from the lumen to the duodenal endothelial cell cytoplasm [46]. As a result of these effects, absorbed iron is retained in the intestinal endothelial cells which are eventually shed while serum ferritin levels remain elevated despite an apparent scarcity of iron. Within the RES and especially within marrow macrophages cytoplasmic iron is retained whereas the post-normoblast proliferating erythroid precursors remain iron-deprived.

Hepcidin, as described earlier, is a molecule produced by the liver that inhibits iron absorption from the duodenum as well as iron release by the bone marrow macrophages [38, 42, 44, 45]. This action to regulate intestinal iron absorption as well as plasma and tissue iron concentration results from its ability to bind to and lead to internal degradation of its receptor—ferroportin [42, 45, 47–51]. Hepcidin, by simultaneously affecting both the influx of iron into plasma from duodenal enterocytes and efflux of iron into macrophages, lowers serum transferrin-bound iron more than the effect of either action alone. This response can occur quite quickly. A mechanism of regulation termed the "mucosal block" phenomenon is the ability of an initial dose of ingested iron to block absorption of a second dose given 2–4 h later. Studies indicate that mucosal block is a fast-response endocytic mechanism destined to decrease intestinal iron absorption during a high ingest of iron and is mediated by changes in serum iron signals via hepcidin [52].

IL-22 is yet another cytokine that also influences hepcidin liver production. Injection of mice with exogenous mouse IgG1 Fc fused to the N-terminus of mouse IL-22 (Fc-IL-22), an IL-22R agonist with prolonged and enhanced functional potency, induced hepcidin production [53]. This response was independent of IL-6 and was attenuated in the absence of the IL-22R-associated signaling kinase, Tyk2. Antibody-mediated blockade of hepcidin partially reversed the effects on iron biology caused by IL-22R stimulation. Taken together, these data suggest that exogenous IL-22 also regulates hepcidin production to physiologically influence iron usage. Further support for the physiological role of IL-22 comes from studies of IL-22 knockout mice subjected to an acute inflammatory stimulus via administration of LPS. In the absence of IL-22, there was a response of hepcidin (probably via IL-6) but the hypoferremic response to LPS was blunted [54].

In summary, hepcidin downregulates intestinal trans-epithelial iron transport by causing an ubiquitin-dependent proteosome degradation of intestinal DMT-1 [42]. The effect on DMT-1 and on ferroportin occurs by internalizing and degrading these membrane receptors and thus inhibiting release of iron by the macrophages while reducing absorption of iron by the intestinal mucosal cells [1, 55, 56]. This is actually a defense mechanism that the body adopts to produce a hypoferremic state that denies bacterial and cancer cells their much-needed iron. This, as well as the pathway involving IL-6, and to a lesser extent IL-22, are considered to be the major contributors for the development of ACD. In patients on hemodialysis (HD), a

significant reduction of hepcidin levels follow administration of ESAs but not intravenous iron [57]. This effect of ESA indicates that despite inflammation, the feedback loop between increased bone marrow erythropoiesis and decreased liver hepcidin production remains intact.

Abnormal hepcidin metabolism is found in virtually all inflammatory conditions. However, some conditions such as uremia or obesity are not usually considered to be inflammatory in nature. In obesity, chronic low-grade inflammation exists and enhances hepcidin production. Adipose tissue is known to secret interleukin-6 and leptin that triggers hepcidin production [58]. It was found that adipose tissue also expresses hepcidin and hemojuvelin, a regulator of hepcidin production. These recent findings suggest that adipose tissue may have an important role in erythropoiesis particularly in obesity that is still poorly clarified.

Reduced Erythropoiesis

As important as iron restriction is in the genesis of ACD, other mechanisms contribute as well since anemia of inflammation is usually normocytic and normochromic, while diseases associated with overexpression of hepcidin, alone, are often microcytic and hypochromic. These differences in erythrocyte parameters suggest that anemia in many inflammatory states is not fully explained by hepcidin-mediated iron sequestration [59]. Studies suggest that chronic anemia associated with inflammation may benefit from interventions protecting the number of erythrocytes produced in addition to anti-hepcidin interventions aimed at enhancing iron availability.

Red blood cell formation is inhibited by several cytokines including IFN-γ, transforming growth factor (TGF-β), and TNF-α. The action of TGF-β and TNF-α is mediated via the p38 mitogen-activated protein kinase (MAPK) pathway, whereas IFN-γ acts via the Janus-associated kinase (JAK/STAT) pathway [44]. Activation of this pathway leads to production of intracellular factors which enhance apoptosis eventually resulting in myelosuppression. Other cytokines affecting red cell development include IL-1 and IL-6.

IFN-γ suppresses red cell development, IFN-γ induces apoptosis in erythroid precursors by increasing nitric oxide production and inducible nitric oxide synthase mRNA production [50]. IFN-γ, and to a lesser extent IFN-α and β, have been observed to induce apoptosis of the erythroid burst-forming (BFU-E) and colony-forming units (CFU-E). This action is mediated in part via the action of ceramide and due to reduction in EPO receptors in precursor erythroid cells [7]. Other modalities of IFN action include reduction in quantity and activity of EPO as well as reduced expression of other growth factors such as stem cell factor.

In an experimental model of ACD, anemia was associated with a 50% reduction in EPO-stimulated differentiation of EPO-R^+ cells (pre erythroblast) to erythroblasts [60]. This suppression required accessory cells, including antigen-presenting cells, which activated other cells to produce pro-inflammatory cytokines. In vitro neutralization of IFN-γ, but not IL-12, TNF-α, IFN-α, IL-1α, or IL-1β, abrogated

the erythropoietic suppression induced by inflammation. The anemia observed was also associated with reduced RBC survival in vivo, as demonstrated by a seven- to eightfold higher turnover of biotinylated RBCs as compared to that in control animals. In vivo IFN-γ neutralization confirmed that IFN-γ contributed to erythropoietic suppression but not to reduced RBC survival. In a totally different model of anemia, that of acute blood-stage malaria, a role for IFN-γ and IL-4 were found in STAT6-induced erythropoietic suppression [60].

A role for IFN-γ had been proposed in the macrophage activation syndrome (MAS), a devastating cytokine storm syndrome complicating many inflammatory diseases, characterized by fever, pancytopenia, and systemic inflammation. Murine models of MAS indicated that IFN-γ was the driving stimulus for hemophagocytosis and immunopathology. A study investigated the inflammatory contributors to a murine model of Toll-like receptor 9 (TLR-9)-induced fulminant MAS produced by IL-10 receptor blocking antibody and a TLR-9 agonist. IFN-γ-knockout mice developed immunopathology and hemophagocytosis comparable to that seen in wild-type mice [61]. These results showed that both fulminant MAS and hemophagocytosis can arise independently of IFN-γ, IL-12, or type I IFNs and that IFN-γ-mediated dyserythropoiesis, not hemophagocytosis, is the dominant cause of anemia in this model of MAS.

TNF-α levels are often increased in ACD. In rheumatoid arthritis (RA), inflammatory cytokines, particularly TNF-α, IL-1, and IL-6, are thought to contribute to the pathogenesis of ACD. The role of TNF-γ was examined in vivo using a chimeric monoclonal antibody to block its action. In RA patients with ACD, administration of the blocking antibody led to a dose-dependent increase in Hb levels compared to placebo and these changes were accompanied by a reduction in both EPO and IL-6 levels [62], supporting the role of TNF-α in the causation of ACD. TNF-α appears to act directly on bone marrow red cell precursors rather than on suppression of EPO production. In addition to systemic levels, in patients with ACD, increased local production of cytokines including TNF-α by marrow T-lymphocytes may also occur [63].

IL-6 is one of the more important cytokines mediating the pathogenesis of ACD [36, 56]. It is a potent inhibitor of TNF-α and induces the transcription of ferritin that leads to increased retention and iron storage within the reticuloendothelial system. As stated previously, IL-6 inhibits erythropoiesis through the inhibition of absorption and uptake of iron [39]. Serum IL-6 is elevated in ACD and it correlated well with parameters of disease activity such as erythrocyte sedimentation rate and C-reactive protein (CRP). Previous studies in chronically ill animal species, however, had suggested the existence of other pathways of anemia induction in which suppression of TNF elevation did not prevent occurrence of anemia [33, 64, 65], and that the IL-6 pathways for anemia were not hepcidin-dependent [30], thus suggesting either direct inhibition of erythropoiesis or the existence of other yet to be established pathways. In patients with RA, growth of the erythroid colony growth burst-forming units of erythroblasts (BFU-E) is impaired in those with ACD but not in non-anemic RA controls [65]. Furthermore, studies indicate that local production of pro-inflammatory cytokines in the bone marrow, not merely circulating IL-6 and

TNF-α may be associated with the development of ACD in RA [66]. IL-6 downregulates the expression of the SLC4a1 gene in late erythroid precursors, and thereby reduces Hb synthesis [35]. It also reduces the mitochondrial mass and function in the developing erythroid progenitors [59].

Patients surviving sepsis develop persistent anemia but the molecular mechanisms have been unknown until recently. The role of a ubiquitous nuclear protein, high mobility group box 1 (HMGB1), that is released by activated macrophages/monocytes, and functions as a late mediator of sepsis has gotten recent attention [67]. Circulating HMGB1 levels are elevated in a delayed fashion (after 16–32 h) in septic animals. Administration of recombinant HMGB1 to mice recapitulates many clinical signs of sepsis, including fever, derangement of intestinal barrier function, lung injury, and lethal multiple organ failure. Administration of anti-HMGB1 antibodies or inhibitors (e.g., ethyl pyruvate, nicotine, stearoyl lysophosphatidylcholine, and Chinese herbs such as Angelica sinensis) protects mice against lethal endotoxemia, and rescues mice from lethal experimental sepsis even when the first doses are given 24 h after onset of sepsis [68]. In mice that survive polymicrobial gram-negative sepsis, a hypochromic, microcytic anemia with reticulocytosis develops. The bone marrow of sepsis survivors accumulates polychromatophilic and orthochromatic erythroblasts. Circulating TNF-α and IL-6 are elevated for 5 days after the onset of sepsis, and serum HMGB1 levels are increased from day 7 until at least day 28. Administration of recombinant HMGB1 to healthy mice produces anemia which is ameliorated by administration of anti-HMGB1 monoclonal antibodies after onset of sepsis (hematocrit 48.5 ± 9.0% vs. 37.4 ± 6.1%, $p < 0.01$, Hb 14.0 ± 1.7 g/dL vs. 11.7 ± 1.2 g/dL, $p < 0.01$). Together, these results indicate that HMGB1 mediates anemia by interfering with erythropoiesis, suggesting a potential therapeutic strategy for anemia in sepsis.

Activin B production by hepatic cells is markedly increased during inflammation. Activin B binds to the bone morphogenetic protein (BMP) receptor type 1; receptor activation acting via the Smad and JAK-STAT transmembrane proteins to cause upregulation of hepcidin expression [69].

Both IL-6 and hepatic hepcidin expression have been found to be significantly increased in various malignancies [51]. Thus the iron sequestration from hepcidin, which inhibits the export of iron from the enterocytes, hepatocytes, and marrow macrophages [30, 55], is an important component in the anemia associated with cancer.

Diminished Response to Erythropoietin

As described under erythropoiesis, in some cases of ACD, the erythropoietic response (Hb achieved to exogenous administered EPO or ESA levels) is not commensurate to the degree of anemia; this phenomenon is termed "blunted EPO response, ESA hyporesponse or resistance," and was first observed in sickle cell patients with chronic kidney disease (CKD), where ESAs even at very high dose did not correct the anemia. Similar but less severe hyporesponse occurs in those with

thalassemia minor. In both, the hyporesponse may be contributed to by hemolysis. An association between EPO-resistant anemia and inflammation [70] as manifested by increased levels of inflammation markers, such as CRP, IL-6, IFN-γ, and TNF-α in patients with CKD has also been noted.

Hypoferremia, Reduced Erythrocyte Survival, and Hypoxia

IFN-γ and bacterial LPS upregulate the expression of DMT-1 in a dose-dependent manner [71], thereby enhancing the uptake of unbound iron into the enterocytes and the monocyte/macrophages. Within the monocytes, iron retention occurs as expression of ferroportin mRNA is downregulated by hepcidin. In chronically ill patients, higher levels of both TNF-α and IL-6 correlate with lower levels of serum iron [70], thus creating a prevailing atmosphere of hypoferremia. At the same time that iron is limited for Hb synthesis within erythrocyte precursors, the rate of erythrophagocytosis increases. The latter is a process designed to remove senescent and damaged red cells in normal situations; in ACD cellular damage is caused by cytokines, endotoxins and reactive oxygen species. Some animal experiments have revealed that sublethal doses of TNF-α may cause phagocytosis of erythrocytes by macrophages. TNF-α rapidly primes human monocytes for enhanced release of O-(2) and erythrophagocytosis and suggest that TNF-α activates monocytes through autocrine or paracrine mechanisms at the inflammatory sites inasmuch as TNF-α is primarily produced by activated monocytes/macrophages [72–74].

Red blood cells (RBCs) often have a short circulating survival in inflammatory conditions, especially in those on HD since ongoing blood losses in the dialysis circuit account to the yearly loss of approximately 5–6 L of blood at a hematocrit of 33%. Fluctuations in EPO levels from exogenous ESAs contribute to shortened RBC lifespan because a decline in the level of EPO triggers the preferential destruction of newly formed RBC, a process termed neocytolysis [75]. Hypochromic RBCs are vulnerable to more rapid turnover in all forms of IDA because iron deficiency increases exposure of the phagocytic signaling molecule phosphatidylserine, loss of deformability, and increased oxidative stress [76]. Additionally, IFN-γ further drives the development of anemia by inhibiting not only erythroid colony growth, but also shortening the lifespan of erythrocytes via increased turnover in the spleen [77].

Another mechanism for reduced survival of RBCs involves effects of hypoxia per se. Hypoxia leads to increased transcription of hepcidin mRNA [78]. This process is thought to be mediated through platelet-derived growth factor (PDGF) [79]. Furthermore, the production of free oxygen radicals in inflammation causes release of pro-inflammatory cytokines as discussed previously which leads to increased hepcidin. Other pro-inflammatory cytokines like IFN-gamma cause increased expression of inducible nitric oxide mRNA and subsequent production of NO [50]. This in conjunction with the production of superoxide in inflammation may cause reversal of the effects of hypoxia on hepcidin production. This induces nitric oxide-mediated apoptosis of red cell precursors and thus worsens anemia.

Specific Inflammatory Entities

Chronic inflammation is a common feature of end-stage renal disease (ESRD) that is gaining increasing attention as a major cause of morbidity and mortality [80]. Hyporesponse of some degree is most commonly seen in those with CKD, particularly with advanced disease. Even in patients not on dialysis, cytokine levels are often increased. In one study of 50 anemic patients, 23 received ESA treatments. Levels of TNF-α were found to be significantly higher and serum albumin significantly lower with higher IL-6 and IL-8 in anemic compared to non-anemic patients. Further analysis by multiple logistic regression found that anemic patients treated with ESAs had significantly higher odds for being in the upper two quartiles for IL-6, IL-8, and TNF-α compared to non-anemic patients [81].

Blunted erythropoiesis to exogenous ESA can be partly explained by the fact that the cytokines, bacterial LPS and IFN-γ [82], induce formation of nitric oxide (NO) and oxygen-free radicals, which directly inhibit expression of EPO in vitro. These reactive oxygen species (ROS) are thought to inhibit the EPO-inducing transcription factors as well as possibly damage EPO-producing cells. Silymarin, which modulates immune cells by inhibiting prostaglandin and prostacyclins production as well as neutrophil and monocyte activation and mobilization, has the capacity to reverse this trend [83].

Recently our understanding of iron metabolism has indicated that "iron" itself can be a source of oxidative stress. As discussed earlier, living organisms have evolved sophisticated mechanisms to maintain appropriate iron levels within cells and within their body. Labile plasma iron (LPI) represents a component of non-transferrin-bound iron (NTBI) that is both redox active and chelatable, capable of permeating into organs and inducing tissue iron overload with ROS. HD patients are particularly susceptible to the effects of parenteral iron since they get intravenous iron as part of repletion or maintenance iron therapy to prevent hyporesponse to ESA [84]. The LPI measures the iron-specific capacity of a given sample to produce reactive oxygen species; a test result of >0.6 units of LPI in a sample of blood indicates a potential for iron-mediated production of ROS. HD patients with LPI units ≥0.6 have higher serum iron, ESA dose, ferritin, high-sensitivity CRP (hsCRP), hepcidin, and lower hemojuvelin levels. In these HD patients, NTBI correlated with direct markers of inflammation, hsCRP ($r = 0.37$, $p < 0.01$), IL-6 ($r = 0.43$, $p < 0.001$), and with ferritin ($r = 0.41$, $p < 0.001$) [85].

CKD

In normal adults, weekly production of endogenous EPO is ~700 IU which can increase acutely with high altitude exposure, 1.8-fold (at 3000 m) and 3.0-fold (at 4000 m) [86]. Uremia is characterized by an inflammatory state [87]. Not surprisingly then, in adults with advanced CKD/ESRD, exogenous EPO dose varies from 1000 to more than 40,000 IU/week when administered thrice weekly, yet normal hematocrits are not attained [88]. Attaining the latter requires a further two- to

threefold increase in EPO dosage [89]. The frequency of moderate anemia (Hb < 12 g/dL in women and <13 g/dL, WHO definition) [90] increases with severity of kidney disease [90, 91]. The frequency and dose of exogenous ESA also increase with CKD severity [92] although the starting doses and maintenance doses of ESA are 40–50% lower than those used a decade ago in response to the alert that higher doses producing harm [93, 94].

The source of inflammation is multiple. Currently, a basal level of inflammation is believed to originate primarily from abnormal bacterial function in the gut [95]. Intestinal dysbiosis, alteration in barrier function, and bacterial translocation seem to account for CKD-related systemic inflammation [96]. CKD in animals is manifested by systemic inflammation, including increased plasma levels of pentraxin-2 and activated antigen-presenting cells, CD4 and CD8 T cells, and Th17- or IFN-γ-producing T cells in the spleen as well as regulatory T-cell suppression. CKD-related systemic inflammation in these animals is associated with intestinal dysbiosis of proteobacterial blooms, translocation of living bacteria across the intestinal barrier into the liver, and increased serum levels of bacterial endotoxin [97]. By fermenting undigested products that reach the colon, the intestinal microflora produce indoles, phenols and amines, among others, that are absorbed by the host, accumulate in CKD and have harmful effects. These gut-derived uremic toxins and the increased permeability of the intestinal barrier in CKD are associated with increased inflammation and oxidative stress and may be involved in various CKD-related complications, including cardiovascular disease, anemia, mineral metabolism disorders, or the progression of CKD [97]. Eradication of facultative anaerobic microbiota with antibiotics prevented bacterial translocation, significantly reduced serum endotoxin levels, and fully reversed all markers of systemic inflammation to the level of nonuremic controls [96].

Much of the enteric effect in uremia is mediated by the gasotransmitter hydrogen sulfide (H_2S), which via adenosine monophosphate-activated kinase (AMPK) normally suppresses the inflammatory activation of signal transducer and activator of transcription 3 (STAT3) and thereby hepcidin via AMPK. Pharmacological and genetic activation of AMPK ameliorated hepcidin production, corrected iron dysregulation, and relieved hypoferremia and anemia in both acute and chronic models of inflammation [98]. AMPK suppresses STAT3/hepcidin activation by promoting proteasome-mediated JAK2 degradation, which is dependent on the intact function of suppressor of cytokine signaling 1 (SOCS1) and increased interactions between SOCS1 and JAK2. Importantly, metformin, an AMPK activator, is associated with decreased serum hepcidin content and anemia morbidity in Chinese type 2 diabetes mellitus patients [98] and perhaps should be carefully investigated in CKD in those with inflammatory component to their anemia.

Although "general state of uremic inflammation in CKD" with effects on muscle (via toll-like receptors) [99] affects symptomatology and quality of life, the inflammation associated with microbial infection in dialysis patients [100] is of special importance since CKD patients are more susceptible to such than those without kidney disease [101]. As part of the host-defense system, macrophages generate DMT-1 and neutrophils release apolactoferrin and neutrophil gelatinase-associated

lipocalin (NGAL) [10], all of which remove iron from the circulation and decrease its availability to iron-dependent microorganisms [102]. NGAL is a 25 kDa protein which, similarly to hepcidin, sequesters iron during the acute phase response and binds siderophores, high-affinity iron chelators produced by bacteria [10]. In addition, cytokines such as IL-1β and TNF-α also stimulate the translation of presynthesized ferritin subunit transcripts during the acute phase response [103]. Presumably, this ferritin-mediated iron trapping is protective in states of acute inflammation, such as bacterial infection, but maladaptive in states of chronic inflammation, such as CKD, congestive heart failure (CHF), and anemia related to inflammation.

A contributing effect to diminished response in HD patients is the bacteriological quality of the water used to create the dialysate. The switch from conventional to online-produced ultrapure dialysate resulted in a lower bacterial contamination with a significant decrease of CRP and IL-6 blood levels [104]. These reductions are accompanied by a significant and sustained reduction of the exogenous EPO dosage required to correct anemia. Using multiple regression analysis, IL-6 levels have a strong predictive value for exogenous EPO dosage [104]. HD patients who are exposed to endotoxin, impure dialysate, and bioincompatible membranes may develop refractory anemia.

In advanced CKD, Hb levels correlate inversely with mortality particularly in the presence of hyporesponsiveness or refractiveness to ESA therapy. Achieved Hb is a surrogate for outcomes with those less than 11 g/dL faring less well once they reach dialysis dependency [105]. Hyporesponse to ESA is also costly. The average per patient cost of anemia in CKD management increases fivefold when comparing patients with Hb >12 g/dL to those patients with Hb <10 g/dL [106]. Additional costs in the latter are driven by comorbidities and the heavier resource utilization by such patients.

Periodontal disease is rampant among dialysis patients and this state produces higher erythrocyte sedimentation rate (ESR) and higher CRP levels which associate with lower Hb levels refractory to dose escalation of ESA. Again this relative hyporesponse and the inflammatory parameters improve with dental treatment of disease [107]. HD patients who are underdialyzed ($Kt/V < 1.3$) show an association between "dialysis adequacy" and Hb, suggesting that the elimination of uremic toxins is required to sustain adequate erythropoiesis [108, 109].

Inflammation has recently been associated with atherosclerosis and malnutrition in ESRD, and this link has led to the development of the malnutrition, inflammation, atherosclerosis (MIA) hypothesis [110]. This describes a syndrome whereby raised levels of pro-inflammatory cytokines (such as IL-1, IL-6, and TNF-α) are a common link between malnutrition, inflammation, and atherosclerosis [110]. Several inflammatory proteins as well as the inflammatory cytokine, especially IL-6 have been linked with this diminishing response to exogenous EPO. Anemia appears to be an important element linking elevated cytokine levels with poor patient outcomes [111].

Several mechanisms for cytokine-induced anemia have been proposed, including intestinal bleeding, impaired iron metabolism, and suppression of bone marrow erythropoiesis and EPO production. These latter effects suggest that

pro-inflammatory cytokines may be an important cause of lack of response to recombinant human EPO therapy. Statistically significant differences were found between responders and nonresponders to recombinant EPO therapy for total lymphocyte and CD4+ T-lymphocyte counts, albumin (lower in nonresponders) and CRP (higher in nonresponders) levels [46]. Such EPO-resistant anemia is associated with the increased risk of ESRD, cardiovascular events, and death [112, 113].

The issue has been what is a safe dose of exogenous ESA to administer and to what Hb level. Randomized clinical trials clearly showed there was a danger in taking all comers to higher Hb levels and all post hoc analyses of these trials identified hyporesponsiveness as the culprit. Now some additional analyses point to an additional effect of high ESA dose independent of "just" hyporesponsiveness.

Cancer: Bone Marrow Infiltration

Anemia secondary to cancers occur via three basic mechanisms: (1) reduced production of red cells (either by tumor invasions, effect of cytotoxic drugs, suboptimal nutrition or cytokine-based inhibition); (2) increased red cell loss (hemolysis or hemorrhage); and (3) miscellaneous etiologies including nutritional deficiencies of Vitamin B_{12} and folate [30]. In most cases these mechanisms overlap; however, the major mechanism is cancer-driven inflammation [114]. Marrow invasion by malignant cells leading to physical obstruction and destruction of the bone marrow microenvironment seems to ultimately occur in most cancers. However, in some cases of malignancies, significant anemia is observed in the absence of marrow invasion or scarcity of vital nutrients. This signifies that other pathways may be important in the pathogenic processes leading to anemia in cancers. Secretion by the tumor of cyclooxygenase (COX- 2), as well as of vascular endothelial growth factor (VEGF), GM-CSF, IL-6, and TNF-α, which lead to cancer cachexia and anemia [115], may all play a role. Celecoxib, a COX-2 inhibitor, has also been noted to counteract the anemia and cachexia associated with ACD [115]. The point of action of several cytokines involved in ACD is shown in Table 4.2.

Growth differentiation factor 15 (GDF-15), a member of the transforming growth factor beta super family [116], is an inhibitor of leukocyte integrin. GDF-15 is strongly upregulated by stimuli that deplete cells of iron and this response is specifically antagonized by the reprovision of iron. GDF-15 exhibits greater sensitivity to iron depletion than hypoxia, and responses to hypoxia and iron depletion are independent of HIF and IRP activation, suggesting a novel mechanism of regulation [117]. GDF-15 has been observed to have a direct relationship with serum hepcidin levels in multiple myeloma cancer patients with anemia [116]. GDF-15, interleukin-6, and EPO in multiple myeloma patients all increase significantly when these patients are anemic with levels decreasing markedly following successful chemotherapy [117].

Hyporesponsiveness to ESAs is a major limitation to the treatment of cancer-related anemia. Enhanced GDF-15 levels contribute to cancer progression and metastasis. Paradoxically, GDF-15 has been found to suppress hepcidin expression

[118] and is further associated with angiogenesis, disease progression, and hematopoiesis [118, 119]. Tumor progression in turn results in even more GDF-15 secretion, which, by downregulating hepcidin expression, results in iron overload in some cancer patients, a phenomenon also found in some patients with sideropenic anemia due to chronic blood loss. This has been proposed to be a major mechanism of anemia in cancer-driven inflammation, as the serum level of GDF-15 correlates to a large extent with the degree of anemia in cancer patients [118, 119]. In the case of infective agents like Malaria and HIV, toxic products of these parasites directly suppress erythropoiesis. These organisms and malignant cells also competitively deprive the erythroid precursors of available iron. The invading microbial cells require iron as an important component of several iron-containing enzymes needed for protein synthesis and proliferation.

Cardiopulmonary

Decompensated CHF in CKD patients is associated with increased levels of IL-1, IL-6, IL-18, TNF-α, endotoxins, aldosterone, angiotensin II, soluble adhesion molecules, and the soluble receptors TNFR-1, TNFR-2, IL-6R, and gp130, as well as volume overload, all of which contribute to an "inflammatory" state and worsening anemia [111, 113]. Even without CKD, cytokines contribute to the anemia present in CHF [110]. In models of ischemia–reperfusion injury, pathological changes in the myocardial tissue were associated with increased expression levels of TNF-α, IL-6, and IL-1β in the myocardium, and with increased serum levels of these mediators [120].

Mechanical circulatory assist devices (MCADs) are increasingly utilized independently of cardiac transplantation in the management of heart failure. MCAD support has been correlated with elevated plasma levels of inflammatory cytokines TNF-α, IL-1β, and IL-6, which have separately been found to inhibit EPO-induced erythrocyte (RBC) maturation. Previous analysis of hematological parameters for MCAD-supported patients concluded that an amplified inflammatory response impedes RBC proliferation and recovery from hemolytic anemia. In a study of 78 MCAD-supported patients implanted for greater than 30 days [121], Hb, RBC distribution width (RDW), mean corpuscular volume (MCV), and cardiac index were retrospectively analyzed. Hb, a conventional marker for anemia, declined with MCAD placement and remains below the clinically defined, minimum normal value whereas in an inverse fashion, the RDW rose above maximum normal measure, signifying an increased fraction of juvenile RBCs [121]. Thus, a response of erythropoiesis does occurs in reaction to the onset of anemia manifested by an increased production of immature RBCs, but the patient's inflammatory cytokine response to the implanted device does not enable full compensation of the MCAD-induced anemia, perhaps by inhibition of full EPO effects.

Idiopathic pulmonary fibrosis (IPF) is a chronic inflammatory process characterized by severe derangement of gas exchange in the advanced stages of disease. However, erythrocytosis is infrequent in IPF, unlike in other pulmonary diseases

producing hypoxemia. In a small study, nine patients (six men and three women) with IPF and profound hypoxemia ($pO_2 < 65$ mmHg) were sex- and age-matched to 34 healthy volunteers [122]. Hb was comparable between the two studied groups. By contrast, serum TNF-α, IL-6, and IL-8 values were significantly higher in patients with IPF. Sera from IPF patients induced a significant growth inhibition of BFU-E arising from mononuclear cells of either patients or control subjects [122]. Overall, the findings suggested that there are an increased number of primitive erythroid progenitors in IPF. These, however, fail to proliferate and differentiate in vivo, suggesting ineffective erythropoiesis, and consequently Hb levels do not rise in proportion to the severity of hypoxemia. Perhaps, cytokines released from alveolar macrophages have not only local but also systemic effects, since the serum of these patients directly suppressed erythropoiesis [122]. Whether the suboptimal erythropoietic response to hypoxia is entirely attributed to this suppression is unknown since several other factors could synergistically or additively interfere with erythropoiesis.

Rheumatoid Arthritis

Erythroid colony growth, using BFU-E as a parameter, is impaired in anemic RA patients but not in nonanemic RA controls [65]. Levels of IL-6 and TNF-α are also significantly higher in the supernatant of bone marrow cultures of RA patients with ACD compared to controls indicating that local pro-inflammatory cytokine production in the bone marrow may be as important as systemic levels in the development of ACD in RA [65]. Both systemic and local cytokines may contribute to hyporesponse to EPO since systemic levels of EPO may be "appropriately" elevated for the degree of anemia [123].

Inflammatory Bowel Disease (IBD

Anemia is a frequent complication in inflammatory bowel disease (IBD) and severely impairs the quality of life of affected patients. The etiology of anemia in IBD patients often involves a combination of iron deficiency (ID) and ACD with elements of other nutritional deficiencies [8]. Despite guidelines recommending screening for and treatment of anemia in IBD patients, current data suggest that anemia remains underdiagnosed and undertreated. Surprisingly, anemia was not diagnosed even though the median Hb value was <11 g/dL in both ulcerative colitis (UC) and Crohn's disease (CD) in one study [124]. This study showed that lab results allowed further differentiation of the type of anemia in 70% of anemic patients. At the time of first diagnosis, an iron-deficiency anemia was diagnosed in 26 of 68 patients with anemia [20 CD, 4 UC, and 2 indeterminate colitis (IC)], but only nine patients received subsequent iron therapy [124]. After 1 year, 27 patients were identified to have an iron-deficiency anemia (19 CD, 8 UC), 20 of them were treated with iron (71.4%). Of nine patients with proven iron-deficiency anemia at

time of first diagnosis and subsequent administration of iron, five (55.5%) continued to have iron-deficiency anemia despite treatment for 1 year. In total, 38 patients (54.3%) did not receive any iron substitution at all despite proven iron-deficiency anemia, and only 13 patients of 74 (17.6%) patients were treated with intravenous iron [124]. Even patients with diagnosed iron-deficiency anemia were infrequently and inconsequently treated with iron preparations, despite the high impact on the quality of life [124].

Besides basic laboratory parameters (see section "Laboratory Diagnosis"), the concentration of soluble TfR (sTfR) and novel parameters such as the sTfR/log ferritin index can help to differentiate ID from ACD [125]. Once identified, causes of anemia should be treated accordingly.

Aging

Anemia is one of the characteristics of the frailty phenotype and is often observed in elderly patients. In the elderly, anemia is usually of multifactorial origin, including chronic inflammation, CKD, nutrient deficiencies, and iron deficiency (approximately two-thirds of all cases), but in some cases no identifiable cause is found. In the elderly, the classic diagnosis of anemia based on the mean corpuscular volume associated with a low Hb level might be inaccurate. In frail elderly patients, all investigations should be carefully considered, invasive examinations undertaken only where justified to uncover the underlying cause, and treat it whenever possible [36]. The aging process itself might be an intrinsic factor in the development of anemia, possibly through the age-related dysregulation of certain pro-inflammatory cytokines such as IL-6 which then directly inhibits EPO production or possibly decreases response by an interaction with the EPO receptor [126]. In such patients, the anemia is often mild, with a Hb level > 10 g/dL, and well tolerated. However, a substantial fraction of the anemia found in the elderly appears to occur in the absence of iron deficiency or elevated hepcidin levels [6].

Laboratory Diagnosis

Initial assessment of ACD involves a good history of the illness [30, 31] as well as general investigations to rule out other causes of anemia. Traditionally, the distinction between different causes of anemia is based on a hematological algorithm starting with the interpretation of the mean corpuscular volume (MCV). Accordingly, micro-, macro-, or normocytic erythrocyte conditions may hint at different causes for anemia. Review of the morphology of the blood film as well as red blood cell indices (MCV, mean cell Hb, mean cell Hb concentration) and bone marrow, reticulocyte count, stool analysis, serum bilirubin and lactate dehydrogenase assay, and assessment of renal function, are required. Important indices of iron status such as serum iron, total iron binding capacity (TIBC),

transferrin saturation (TSAT), ferritin, and more recently the ratio of TfR to log of ferritin are important in differentiating ACD from IDA and other causes of hypochromic, microcytic anemia such as the thalassemias. This requires an in-depth understanding of the stimuli and regulatory pathways of production of the various molecules that are usually assayed (see pathogenesis of anemia and Table 4.2). Some writers have also emphasized a key potential role for early markers of impaired erythropoiesis markers, such as the Hb content in reticulocytes (CHr) or reticulocyte Hb equivalent, measures that are not available as part of conventional laboratory tests [127].

Blood Smear Morphology

Even though the initial red cell morphology shows a normochromic and normocytic picture, with time this evolves into one that is hypochromic and microcytic. Severity of anemia in ACD is usually moderate with Hb usually 8–11 g/dL, rarely decreasing to Hb less than 7 g/dL. In these patients with suspected ACD, other causes of external blood loss or destruction should be looked for. The reticulocyte count (or better still the reticulocyte index) is usually reduced in ACD as well as in IDA. The blood smear may provide information on the underlying cause of ACD; thrombocytosis in cases of chronic hemorrhage, toxic granules in neutrophils in severe sepsis, hypersegmented neutrophils in mixed nutritional deficiency or folate/Vitamin B_{12} deficiency found in malignant conditions.

Iron Indices

Separation of IDA from ACD is often difficult since both can co-exist, particularly in the elderly [5, 6]. The major difference between ACD and IDA is that there is an absolute lack (serum ferritin below 30 ng/mL) [2] of iron in IDA, whereas the pathogenesis of ACD is multifactorial with the inflammatory component producing iron-limited erythropoiesis. Iron stores may be normal or increased because iron is sequestered within macrophages as in the anemia of chronic disease (ACD). The latter is also seen in patients with autoimmune disorders, cancer, infections, and CKD [5].

In ACD, transferrin may remain unchanged or increase slightly, serum iron decrease thereby reducing TSAT; in IDA, transferrin increases markedly while serum iron falls, again reducing TSAT. In both IDA and ACD the erythrocyte-free protoporphyrin is increased, but in ACD, serum ferritin and marrow-stainable iron are increased [128], whereas in IDA these two parameters are decreased. The hypoferremia in ACD is due to trapping of iron in the cells of the reticulo-endothelial system and the low serum iron consequently results in lower transferrin saturation. The level of the transport protein transferrin is therefore increased in IDA, but reduced or normal in ACD [2].

Serum Ferritin/Ferritin Receptor

Ferritin is an acute phase protein, and its levels are increased in chronic inflammatory states [129]. In the absence of inflammation, ferritin is a good measure of body iron status. The normal level of serum ferritin is usually between 15 and 300 μg/L. In patients with ACD, serum ferritin is usually increased secondary to inflammation and the redistribution into and retention of iron by the reticulo-endothelial cells.

The sTfR is a fragment of the membrane TfR, whose levels seem to correlate with the level of the latter [130]. The production of both molecules is regulated by the availability of intracellular iron via the iron regulatory element protein (IRE/IRP) interaction. Therefore, low iron availability leads to increased TfR and sTfR.

Though the level of sTfR is also affected by inflammatory cytokines, it is still usually useful in differentiating ACD from IDA [3]. This is because the pro-inflammatory cytokines and the erythroid iron deficiency both affect the sTfR in opposite directions, therefore balancing out their impact. As a result, concentration of sTfR and novel parameters such as the sTfR/log ferritin index can guide the challenging task of differentiating between ID and ACD [130]. Because the sTfR in ACD is usually lower and the ferritin is higher than in IDA, the ratio is similar to the values in non-anemic patients (1–2). By contrast, the ratio is very high in pure IDA. Skikne et al. [130] demonstrated that subjects with ID anemia or ACD + ID anemia had significantly higher sTfR and sTfR/log ferritin index values than subjects with ACD only. ROC curves produced cutoffs of 21 nmol/L for sTfR and 14 nmol/L for the sTfR/log ferritin index. The sTfR/log ferritin index was superior to sTfR.

Hepcidin

As previously described, hepcidin is a 25-amino acid chain protein, produced predominantly by hepatocytes and to a lesser extent by activated neutrophils and macrophages. Its hair-pin structure is maintained by disulfide bonds between four cysteine amino acid residues in the chain. Hepcidin synthesis is induced by iron overload and inflammation and inhibited by anemia and hypoxia [78]. It acts by inhibiting export of iron from the iron-containing duodenal enterocytes and from macrophages by binding to and inducing endocytosis of ferroportin (the sole iron exporter in mammalian cells), resulting in its breakdown/degradation. This is thought to be a major mechanism for the anemia of chronic disease. Some authors believe that serum hepcidin is the most accurate serological marker for the differentiation of ACD and IDA.

The important laboratory differences between ACD and IDA as well as the expected results in situations where both co-exist are listed in Table 4.3.

Functional iron deficiency (FID) is a state in which there is insufficient iron incorporation during erythropoiesis, despite normal total body iron stores, and it is a major component of ACD. Therefore, besides “true” iron-deficiency anemia, FID

Table 4.3 Serum levels that differentiate ACD from IDA

Variable	ACD	IDA	Both
Iron	Reduced	Reduced	Reduced
Serum transferrin	Reduced/normal	Increased	Reduced
TSAT	Reduced	Reduced	Reduced
Ferritin	Increased	Low	Reduced/normal
Soluble TfR	Normal	Increased	Normal/increased
sTfR/logFerritin	Low (<1)	High (>2)	Intermediate (1–2)
Cytokine levels	Increased	Normal	Increased
Hepcidin	High	Low	Intermediate

should be considered if serum ferritin is >100 μg/L and TSAT is below 20% [131, 132]. Again, sTfR/log ferritin may help discriminate absolute from functional ID anemia [5, 127, 132]. In patients with evidence of inflammation and intermediate serum ferritin levels (<100 μg/L but >30 μg/L), a value of sTfR/log ferritin >2 may indicate a combined anemic status, whereas a ratio < 2 is consistent with normal total body iron stores [5].

Erythropoietin Assay

EPO is produced by the renal EPO-producing cells in response to anemia as well as reduced oxygen tension. The assay results for serum EPO levels (s-EPO) in ACD vary with etiology for the ACD. Patients with inflammation have a higher mean s-EPO (69 mIU/mL) than patients with neoplasia (43 mIU/mL) and those with infection (27 mIU/mL), the latter not differing significantly from values found in normal individuals. In those with inflammation as well as iron deficiency, s-EPO are the highest (72 mIU/mL) [131]. These values have to be interpreted in light of the inverse relationship between Hb and s-EPO levels in a variety of anemias without inflammation or infection. With chronic bacterial infection, s-EPO levels decrease over time, an effect mediated by TNF-α [133]. Serum-EPO levels also appear to be suppressed by fever. Overall, inadequate EPO production may contribute to the pathogenesis of ACD secondary to infection. A similar finding of inadequate EPO response has also been noted in rheumatoid arthritis. With moderately severe anemia (Hb < 10 g/dL), s-EPO in RA patients with ferritin <20 ng/mL averaged about 65 compared to 104 mU/mL to iron-deficient controls ($P < 0.05$) [134]. In systemic lupus erythematosus (SLE), anemia can be caused by many factors including autoimmune hemolysis. s-EPO response is blunted in almost half of anemic SLE patients, particularly those with ACD and autoimmune hemolytic anemia [135].

The main use of s-EPO is its predictive value to determine whether a response to EPO therapy, if used, is likely to occur following administration of recombinant human EPO for 2 weeks or more. A basal EPO level of >100–150 mIU/mL or a ferritin level of >400 ng/mL is predictive of a poor response to EPO in 88% of patients not receiving concomitant anticancer drugs [136]. High serum s-EPO at treatment initiation (>100–150 mIU/mL) predict resistance, especially in hematological

malignancies [137], since subcutaneously administered exogenous recombinant EPO, even at doses up to 40,000 IU, only increases time-averaged circulating EPO levels by less than a factor of 2–3 when a level of five- to tenfold higher is needed (unpublished observations). Unfortunately, the thromboembolic risk from exogenous ESA may reside in the peak levels achieved [138] which precludes dose escalation. Although patients with cancer-related anemia show higher s-EPO than patients without anemia, there is extreme variability among individuals [137].

Vitamin Deficiencies and Other Causes

In addition to the ID and inflammation that may be present in ACD, anemia in IBD patients can result from impaired Vitamin B_{12} and folic acid absorption. Especially in cases of macrocytic anemia, Vitamin B_{12} and folic acid level measurements should be performed during screening visits. Vitamin B_{12} and folic acid deficiency is not uncommon in IBS: it is found in up to 33% and 29% of Crohn's [139, 140] and 16% and 8.6% of IC patients [141], respectively. Possible causes include ileal dysfunction following proctocolectomy and ileal pouch-anal anastomosis, bacterial overgrowth and reduced intestinal transit time.

Additionally, myelosuppressive medication in those with cancer and IBD patients on thiopurines and sulfasalazine should always be considered in the diagnostic workup of anemia [142].

Treatment

The overriding goal of anemia therapy in ACD is to improve oxygen-carrying capacity of blood (Hb concentration) thereby reversing cardiac compensatory mechanisms that might result in deleterious consequences, and to improve quality of life. The presence of moderate to severe anemia is associated with poorer prognosis in patients with CKD, cancers, CHF, and kidney disease. Hb levels of ≤8 g/dL in CKD patients on HD doubles the risk of death compared with patients who had Hb of 10–11 g/dL [142]. However, the ultimate therapeutic goals of "normalization of Hb levels and repletion of iron stores" may not be appropriate in all forms of ACD, specifically in CKD where normalization has been shown to produce "harm" compared to partial correction of anemia when ESA are used [89, 143]. More reasonable goals may be an Hb increase of 2–3 g/dL and attainment of a transferrin saturation of >30%. These can be achieved more quickly in those with IBD (4–6 weeks) compared to ESRD (several months).

Epidemiologically, anemia is associated with increased all-cause and cardiovascular mortality and higher rates of left ventricular hypertrophy (LVH), CHF, major adverse cardiovascular events (MACEs), and progression to ESRD in patients with CKD [144]. Among European HD patients (DOPPS), the relative risk (RR) for hospitalization and death increased by 4 and 5%, respectively, with each 1 g/dL decrease in Hb within the 10–13 g/dL range [145]. Patients who have their Hb concentration

optimized to between 10 and 12 g/dL have improved survival and better treatment outcome [146].

In those with existing heart disease, anemia is an independent risk factor for cardiovascular events and death [147]. Treatment of anemia improves quality-of-life symptoms, exercise tolerance [148], and left ventricular hypertrophy [149], the latter known to be associated with mortality [149]. Muscular function may be impaired in iron deficiency. Muscular dysfunction seen in diastolic CHF may not be due to anemia alone. In animals, some degree of iron deficiency contributes to muscular dysfunction [150]. Muscle fatigue is a prominent feature in advanced CKD. Studies on muscle and nerves date back 30 years. Early explanation for the high CPK levels in uremia invoked abnormalities in metabolism, the weakness being secondary to skeletal muscle abnormalities, hypothyroidism, and vitamin D deficiency [151]. There is reason to believe that ID may be the more important component. In addition, adequate iron supplementation seems to have nonhematological benefits, such as improved cognition, thermoregulation, immune function, and exercise adaptation, as well as decreased restless legs syndrome [152].

Iron Therapy

It is important to remember that iron deficiency can co-exist with ACD. For instance, in patients with non-dialysis-dependent CKD, one study of 114 CKD patients showed anemia in 72, of which one-third were classified as having iron-deficiency anemia [132]. Thus although inflammatory factors contribute to anemia in renal patients in all stages of renal failure through impaired EPO production, iron deficiency is often overlooked. In another study of 54 epoetin- and iron-naive ACD patients in whom iliac crest biopsy of bone marrow were performed looking for iron distribution (Perls' stain), final assessment found that 26 had iron-deficiency anemia, 21 anemia of chronic disorders, and only seven had normal iron stores [153]. Low hepcidin and high ferroportin expression by erythroblast and macrophage were seen in iron-deficiency anemia, while the opposites were true in anemia of chronic disorders. However, even though the hepcidin-ferroportin system seems regulated by the ferritin-driven hepcidin system, serum hepcidin and peripheral iron indices were of little help in describing bone marrow iron status [153]. Similarly although blood studies showing low serum ferritin levels (<30 μg/L), high transferrin levels, and a decreased MCV index are routinely used to evaluate for ID in IBS and RA, it is important to note that serum ferritin levels up to 100 μg/L do not adequately exclude ID. The TfR/log ferritin ratio is a better indicator of co-existing ID [65, 130, 132]. Some studies suggest that measurement of hepcidin or its precursor may be a good indicator of the inflammatory component present in ACD [154].

Links between iron accumulation and the formation of toxic free radicals and progressive tissue damage have been suggested, and an excess of iron (as well as deficiency) may lead to a higher risk for cardiovascular events. These issues merit careful consideration, as do studies of the relative efficacy and safety of IV and oral

iron supplementation, and any predictive relationship between baseline values of Hb and iron handling and response to supplementation.

Rational use of iron should minimize the development as well as correct iron deficiency (functional or absolute) if present. Iron therapy may not be very effective in many disease processes leading to ACD since the pathogenesis involved is a relative (functional) deficiency in the availability of iron to the red cell precursors and not an absolute deficiency. In addition, several microorganisms and tumor cells utilize the excess iron for their cellular proliferation. Iron is known to have an inhibitory effect on the immune system by downregulating IFN-γ-mediated pathways [155, 156]. Iron, particularly intravenous, also enhances the production of hydroxyl radicals which cause tissue and endothelial damage. The conundrum frequently is how to provide iron without producing harm from too much iron.

Normal diets usually provide sufficient iron supplies in the form of elemental and heme iron. As previously stated, in patients with active inflammation, particularly those with IBD, inflammation lowers iron absorption via changes in hepcidin. In addition, changes in mucosa in IBD leads to maldigestion and malabsorption which aggravates ID. Despite these considerations, in most forms and stages except very severe/advanced ACD, oral iron substitution can be effective when disease activity is moderate and the anemia is mild [157, 158]. In fact, because of widespread availability, low cost, established safety profile, and good bioavailability in its reduced form, many physicians tend to favor oral iron substitution as a first-line therapy. Frequently used oral iron supplements are ferrous fumarate (325 mg tablets containing 106 mg elemental iron per tablet), ferrous sulfate (325 mg tablets containing 65 mg elemental iron per tablet), and ferrous gluconate (325 mg tablets containing 36 mg elemental iron per tablet). However in situations of poor oral intake or deficient or proven malabsorption, parenteral iron may be used [158, 159].

Parenteral iron can be administered intravenously or intramuscularly [159–162]. Multiple IV iron preparations exist. Agents available globally include sodium ferric gluconate complex in sucrose, iron sucrose, ferumoxytol, ferric carboxymaltose, and two iron dextrans. All are effective in replenishing or increasing iron stores. Intravenous iron is usually administered as a loading (repletion) dose of 0.5–1.3 g elemental iron. Rate of infusion varies. Low molecular iron dextran is given as a slow infusion over several hours. The other parenteral irons can be given quicker and sometimes within 15 min allowing a slow "push" depending on the preparation used. Iron dextran when compared to other preparations is associated with a higher incidence of anaphylactoid reactions, particularly the high molecular form. Overall, however, the rate of anaphylactoid reactions is about 0.2% whereas less severe other adverse reactions (e.g., pruritus, rash, urticaria, or wheezing) occur in 3.7% of infusions [163], particularly if iron is infused rapidly. Because the major side effect is the severe and sometimes life-threatening anaphylactoid reactions that may occur during any infusion, an emergency tray must be available on site [164]. Jectofer, a brand of intramuscular iron is sometimes used but this needs to be given as a deep intramuscular injection using a Z (zig-zag) technique. This is to avert the undesired aftermath of leaving a dark patch around the injection site, especially in fair-skinned individuals, or the development of sterile abscesses.

Patients with inflammatory bowel disease [165] and CKD (if GFR is >30 mL/min/1.73m^2) [166] are known to benefit from oral iron with or without ESA. This response is based on the observation that some degree of absolute iron deficiency may exist in patients with ACD, and also from the inhibitory effect of iron on TNF-α as it confers better outcome in rheumatoid arthritis and CKD [34, 37]. Iron therapy therefore should be considered in patients with ACD who also have an absolute deficiency of iron and may be tried on individuals who are not responding to EPO, as functional iron deficiency may be involved.

Red Cell Transfusion

Blood transfusion remains an important short-term management option especially in individuals with severe anemia (Hb < 6.5 g/dL) associated with cardiac decompensation. However, long-term use of red cell transfusion has been associated with increased mortality, mainly due to iron overload and immune activation by HLA antigens, particularly in kidney patients who may be eventually transplanted [167]. One must also recognize the small risk of immune hemolytic anemia which can occur in patients who have no antibodies detectable by routine procedures [direct (DAT) and indirect (IAT) antiglobulin tests] [168].

Erythropoiesis-Stimulating Agents

EPO stimulates proliferation of erythroid precursors—BFU-E in the marrow via its action on bone morphogenetic protein (BMP-SMAD) [31, 169] and the JAK-STAT5 pathway. BMP is a member of the TGF-β family which binds to serine threonine kinase receptors and transduce signals via the SMAD. In hepatocytes BMP binds to hemojuvelin which acts as its signaling component and thus induces expression of hepcidin, with consequent decrease in iron absorption [169]. Induction of the JAK-STAT pathway indirectly inhibits the pro-inflammatory effects of hepcidin by inhibiting IL-6 production by monocytes [170]. Erythropoietic agents have been approved for use in ACD due to cancers or anticancer chemotherapy as well as in patients with CKD and HIV [130]. The mode of action involves counteracting the antiproliferative effects of the pro-inflammatory cytokines, as well as stimulation of iron uptake and heme biosynthesis in erythroid precursors. These agents should be used in conjunction with iron and the target Hb should be between 11 and 12 g/dL, as previous studies have shown that levels above this are associated with poorer treatment outcome. However, the common side effects of EPO should be assessed in each patient and include thromboembolic complications, iron deficiency, and influenza-like syndrome. In those with CKD, raised blood pressure requiring antihypertensive therapy and cerebral convulsion/hypertensive encephalopathy have been observed. Background knowledge of ferrokinetics and the dynamics of the molecules involved in iron metabolism are necessary for the investigation, interpretation of results, and optimal management of patients with ACD.

Assurance of Normal Vitamin Status

Any deficiency of folate and/or Vitamin B_{12} should be corrected. Less often assessed is the role of Vitamin D. Vitamin D deficiency is common in CKD/ESRD. The simplest approach may be to assure adequate Vitamin D status. Hepcidin levels have been found to inversely correlate with Vitamin D status in CKD [171]. In some patients with CKD and suboptimal response to EPO, vitamin D therapy leads to improved anemia with reduced requirement for EPO. This response is reportedly present in patients independent of parathyroid hormone levels.

With respect to Vitamin D, the active moiety (after liver conversion to 25-OH-Vitamin D) is the renal and local tissue formation of 1,25 dihydroxy Vitamin D_3 or calcitriol [172]. Calcitriol had been observed to directly increase proliferation of erythroid precursors via the activation of 1α hydroxylase [171, 173]. Activation of vitamin D receptors on immune cells leads to release of interleukin-10 from bone marrow stromal and accessory cells. Recent studies have shown that calcitriol downregulates hepcidin and increases ferroportin expression. These would facilitate transport of iron from tissue to bone marrow. High doses of vitamin D have been found to decrease the pro-hepcidin cytokines, IL-6 and IL-1β, in a dose-dependent manner [172]. This has the effect of inducing proliferation of erythroid progenitor cells as well as inhibiting inflammation [171].

Erythroferrone (ERFE), a regulator of hepcidin synthesis and iron homeostasis, has been found to downregulate the expression of hepcidin mRNA in thalassemic mice. Ablation of ERFE ameliorates the iron overload and restores hepcidin levels in this situation [174].

Improvement in anemia as well as response to ESAs had also been observed with dialysis membranes coated with Vitamin E [175]. Vitamin E is an antioxidant. A common cause of relative ESA refractiveness in HD patients is the use of catheters as vascular access for dialysis. Patients using a tunneled, cuffed catheter for HD, compared to those who use an arteriovenous fistula, are more likely to show relative ESA hyporesponsiveness, presumably from inflammation associated with a subclinical infection or the presence of foreign material in the vasculature [176]. Inflammation-associated ESA-hyporesponsiveness has been treated with statins, vitamins C and E, oxpentifylline (also referred to as pentoxifylline), ultrapure water and biocompatible membranes in HD patients, and transplant nephrectomy in patients with failed allografts [177]. In HD patients, the pre-dialysis Hb and hematocrit do not reflect the average interdialytic values due to extracellular volume removal. Postdialysis Hb typically increases on average by 1 g/dL; increases of 2–3 g can be seen if the predialysis Hb > 12 g/dL and more than 3 kg of excess fluid is removed [178].

Targeting the Cytokines

Efforts directed at inhibition of hepcidin and other pro-inflammatory cytokines are currently being made in an attempt to overcome their inhibitory effects on the

reticuloendothelial cells and on erythropoiesis. In addition, molecules which are able to bind IREs and IRPs and therefore tilt the balance toward more physiological iron homeostatic processes than those usually observed in IDA are being developed. In light of the putative role of pro-inflammatory cytokines, anti-cytokine agents may prove useful to optimize the efficacy of recombinant ESA in anemic chronic renal failure patients. Other potential therapeutic strategies include minimizing exposure to causes of inflammation from various co-morbid conditions, such as persistent infections and chronic heart failure as well as the rational use of iron [179].

Omega-3 polyunsaturated fatty acids can downregulate the production of TNF-α and IL-6 in rheumatoid arthritis [180] and diabetes mellitus [181], an effect largely mediated via its action on the transcription factor nuclear factor kappa (NF-κB) and can improve the ACD associated with these disease states. The poly-unsaturated fatty acids have the advantage of being safe with little or no known adverse effects.

Hepcidin is a major target in the management of anemia of chronic disease due to its central role in pathogenesis [32, 172, 182]. High levels of hepcidin correlate with failure of ESAs. Inhibition of hepcidin might improve response to EPO. A novel synthetic anti-hepcidin agent (NOX-H94) was shown to lead to resolution of anemia in animal studies [183]. Combined treatment with hepcidin inhibitors and ESAs was found to be superior to treatment with ESAs alone in animal models [32]. Various other approaches aimed at pharmacological control of hepcidin expression are being investigated, targeting different regulatory steps. They include hepcidin sequestering agents (antibodies, anticalins, and aptamers), inhibitors of BMP/SMAD or of IL6/STAT3 pathway or of hepcidin transduction (siRNA/shRNA) or ferroportin stabilizers [184]. In addition, since heparins and heparin sulfate proteoglycans are important in hepcidin expression and modulation of the BMP6/SMAD pathway, they too may have a role clinically.

The feasibility or proof of concept of using an antihepcidin l-oligoribo-nucleotide, lexaptepid, to block the inflammation-induced increase in hepcidin was evaluated in a randomized, double-blind, placebo-controlled trial of 24 normal individuals injected with 2 ng/kg *Escherichia coli* LPS [185]. Injection 0.5 h later of 1.2 mg/kg lexaptepid as compared to placebo prevented the decrease in serum iron during experimental human endotoxemia despite similarity of the LPS-induced inflammatory response. At 9 h, following injection, serum iron had increased by 15.9 ± 9.8 μmol/L from baseline in lexaptepid-treated subjects compared with a decrease of 8.3 ± 9.0 μmol/L in controls ($P < 0.0001$). Clinical trials of various other novel therapeutic entities are ongoing in anemia of CKD and other inflammatory conditions.

GDF-15 has also been proposed as a suitable target due its central role in mutagenesis, angiogenesis and metastasis, as well as ACD in various malignancies [122]. Combination therapy has become more appealing since the revelation of a hepcidin-independent pathogenic pathway for the development of ACD [67].

Finally, guanosine 5′-diphosphate (GDP) has also been found to be a promising hepcidin-binding agent. In vitro studies revealed that GDP caused ferroportin

stabilization and increased the ferroportin-mediated cellular iron efflux of HepG2 and Caco-2 cells. Furthermore, the co-administration of GDP and $FeSO_4$ ameliorated the turpentine-induced anemia of inflammation in mice as indicated by increased Hb level, serum iron, ferroportin expression and decreased ferritin level [186]. These results suggest that GDP is a promising natural small-molecule inhibitor that targets hepcidin–ferroportin complex, and that could therefore be incorporated with oral iron supplement regimens to ameliorate ACD.

Other therapeutic approaches include inhibition of the expression of hepcidin-encoding genes. The genes coding for fatty acid desaturases which are the rate-limiting enzymes in the synthesis of omega-3 fatty acids also present another interesting therapeutic target.

Emerging Therapies

Since the discovery of the hypoxia inducible factor (HIF) pathway and its regulation via HIF prolyl hydroxylase enzymes in the context of erythropoiesis and iron metabolism, seven different prolyl hydroxylase inhibitors (HIF-PHIs) have been developed, four of which are in active clinical trials [187]. The rationale for targeting this pathway in treating anemia is that endogenous EPO is stimulated while simultaneously hepcidin production is suppressed and transferrin synthesis increased. The latter permits enhanced transport of iron. In a number of Phase 2 clinical trials, these agents have been found to be effectively correct and maintain Hb in CKD-associated anemia [187–195]. CKD-associated anemia is in part related to the inflammation present in "uremia." In both nondialysis CKD [194] and incident HD [189] patients, the effective dose of the HIF-PHI roxadustat was independent of the baseline CRP level. With ESAs, dose needed to achieve target Hb of 10–12 g/dL increases exponentially with CRP. With time, no doubt such agents will find a niche in treatment of other disorders producing ACD.

Summary

The understanding and consequent diagnosis of anemia of chronic disease has continually fazed many physicians. Several pro- and anti-inflammatory cytokines and hormones suppress erythropoiesis. The majority of these control iron release from the intestinal mucosa and marrow macrophages via ferroportin. Future treatment options should be targeted at the genes encoding these cytokines as well as using anti-inflammatory food supplements: fatty acids and Vitamin D. The use of iron supplementation in conjunction with ESAs may also be beneficial.

Conflict of Interest Nothing to disclose.

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Current and Novel Drugs for Management of Anemia

5

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Introduction

Anemia is defined as an abnormally low concentration of blood hemoglobin, developing either because of increased destruction or decreased production of red blood cells, or a combination of the two. The presence of anemia is associated with various poor clinical outcomes in various patient populations, irrespective of its etiology. Correcting anemia can result in improved clinical outcomes, and the treatment of low blood hemoglobin levels is an important therapeutic goal in patients with various underlying diseases. Such treatment can occur in the form of blood transfusions, resulting in immediate correction of anemia; this form of therapy is usually reserved for conditions where the anemia is severe and its correction is needed immediately. Since blood used for transfusions is a relatively scarce resource and transfusions are associated with a number of complications (e.g., volume overload, iron overload, transmission of infectious agents, acute transfusion reactions, etc.), when possible it is generally preferable to treat anemia by correcting its underlying cause(s). Anemia can develop both a result of increased destruction or decreased production of red blood cells (RBC), and the cause-specific therapy of these two mechanisms are distinctly different. This chapter reviews the pharmacologic agents used to treat anemia developing as a result of decreased red blood cell production.

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Physiologic Underpinnings of Red Blood Cell Production

The dynamic development of various therapeutic options for anemia in the past decades was fueled by a growing understanding of the complex physiology underlying RBC production, along with the recognition of the various disease states' effects on these physiologic processes. RBC production is a complex process which involves the coordinated acquisition and incorporation of various building blocks into cells. Figure 5.1 illustrates the complex processes underlying RBC production, highlighting its key elements, while Fig. 5.2 summarizes the pathologic processes affecting RBC production. The latter are also the key intervention points for therapeutic strategies, such as pharmacologic replacement of erythropoiesis-stimulating agents (ESA) and iron, modulation of hypoxia-inducible factor (HIF) levels, alleviation of inflammation, testosterone replacement, and hepcidin inhibition.

Erythropoietin

Erythropoietin (EPO) was identified as a vital cog in erythropoiesis in the early 1940s and named "erythropoietin" in 1948 [1]. EPO is produced by cortical peritubular fibroblasts located near the proximal tubular cells in the outer medulla and

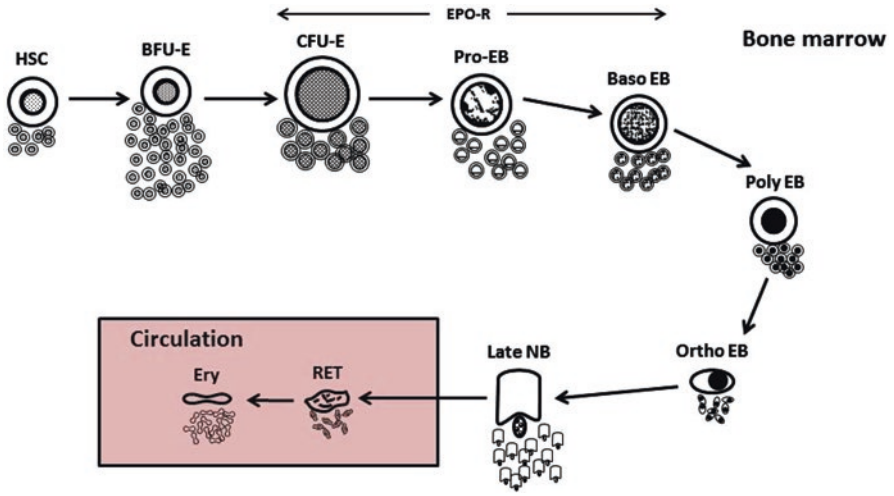

Fig. 5.1 Normal erythropoiesis. The hematopoietic stem cell (HSC), once committed, differentiates into the blast-forming units—erythroid (BFU-E) that in turn differentiates into the colony-forming unit—erythroid (CFU-E). The proerythroblast (Pro-EB), the next stage, is the last nonspecific progenitor cell. This then goes through multiple stages of maturation through the basophilic (Baso EB), polychromatophilic (Poly EB), and orthochromatic (Ortho EB) erythroblasts before losing the nucleus during the late normoblast (Late NB) stage. Then the cell is extruded into the circulation as a reticulocyte (Ret) which then becomes the erythrocyte (Ery). EPO-dependent stages include CFU-E until the Baso EB stage. The entire cycle takes about 7 days

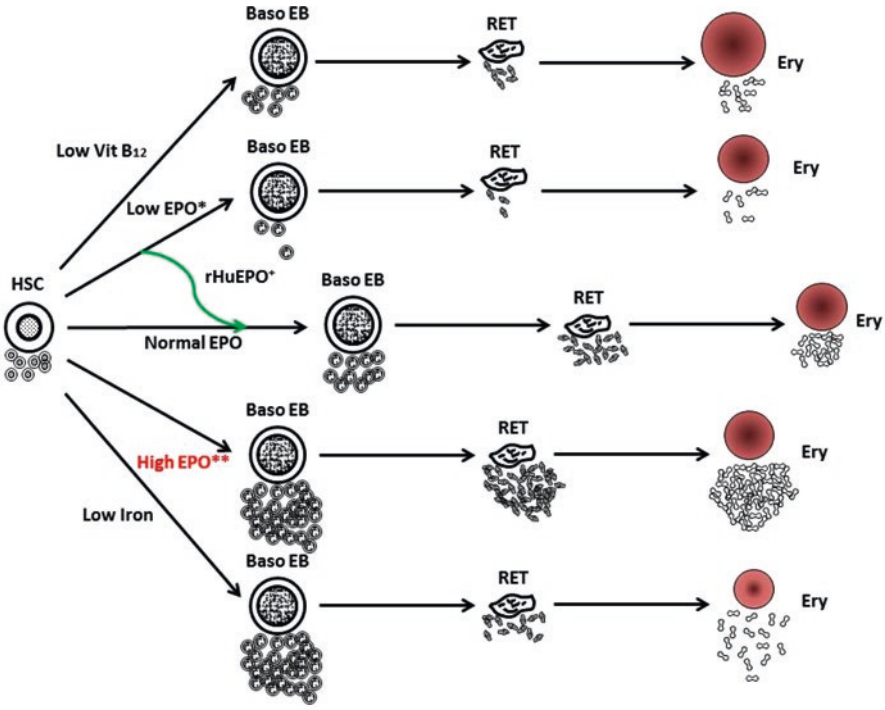

Fig. 5.2 Pathologic erythropoiesis. Normal erythropoiesis leads to less than half survival rate of the basophilic erythroblasts to eventually become erythrocytes (Ery). Under low EPO states, the EPO-dependent cells undergo apoptosis and hence less number of Ery are produced leading to anemia. Giving rHuEPO (*green arrow*) restores erythropoiesis to normalcy provided iron is adequate. The cells themselves are usually normocytic. In certain vitamin deficiencies, there is inefficient erythropoiesis and hence macrocytes are produced. In hemorrhagic or hemolytic states, EPO levels are higher and this leads to an increased Ery production. In iron deficiency with elevated EPO, though the EPO-dependent cells' production is enhanced, during hemoglobin synthesis the inhibited protein synthesis results in microcytes and a lower number of Ery

inner cortex in the kidney [2]. This production is expanded into the outer cortex in response to hypoxia and anemia, a region that is especially susceptible to hypoxia [3]. Indeed, tissue hypoxia is the pivotal factor that increases EPO production as a final step in a signal transduction pathway involving several proteins among which hypoxia-inducible factors (HIFs) play a central role.

Though EPO transcripts have been isolated from several other tissues, they do not contribute significantly to circulating levels; 90% of all EPO produced in the body is produced in the kidneys and about 10% is produced in the liver [4, 5]. EPO receptors (EPO-R) bind circulating EPO and initiate signal transduction within the burst-forming unit-erythroid (BFU-E), the first cell in the erythropoietic cellular lineage to express the EPO-R [6]. The EPO-R exists as a homodimer bound to Janus Kinase-2 (JAK-2) protein and in turn to transferrin receptor 2 (TfR-2) [7]. Upon binding EPO, the EPO-R complex is internalized and undergoes conformational

change leading to a cytoplasmic segment of the complex being phosphorylated and stimulating multiple signaling pathways within the cell leading to gene transcription [4, 8]. The effective half-life of EPO is only about 30–40 min because of dephosphorylation and degradation of both EPO-R and JAK-2 [9].

Due to the central role of the kidneys in EPO production, it is not surprising that anemia is a nearly universal complication with progressive loss of glomerular filtration rate (GFR), developing gradually and increasing in severity as kidney disease progresses [10]. Indeed, anemia becomes a ubiquitous problem by the time patients are nearing the need for renal replacement therapies [10, 11]. The most common reason for the anemia characteristic of chronic kidney disease (CKD) is a relative insufficiency of EPO production [11], although the complex clinical picture of most patients suffering from CKD often includes additional conditions complicating the etiology of anemia, such as inflammation or iron deficiency. Nevertheless, pharmacologic replacement of EPO has revolutionized the approach to anemia in patients with CKD. Until the 1980s, anemia of CKD was managed with regular blood transfusions and obviously, this came with several complications such as sensitization, transfusion reactions, secondary iron overload, etc. [12]. This resulted in a generally accepted trend of lower hemoglobin levels in end-stage renal disease (ESRD) patients. Since its introduction, EPO and newer synthetic forms have formed the cornerstone of anemia management in CKD.

Recombinant Human Erythropoietin and Its Derivatives: Clinical Considerations

Recombinant human erythropoietin (rHuEPO) was developed in the 1980s and was noted to have a surprisingly high efficacy in correcting anemia of CKD [13] (Table 5.1). The extraordinary success of rHuEPO led to the spawning of several derivatives of the original molecule that can be collectively called epoetins or erythropoiesis-stimulating agents (ESAs). rHuEPO and its derivatives are glycosylated proteins and hence are produced from mammalian cell lines and in vivo action is dependent on the degree and nature of glycosylation. The epoetins α and β were the first molecules manufactured and became widely used after supportive evidence from multiple clinical trials. The typical dosing schedule of rHuEPO in routine clinical practice is three times a week, with the medication being administered along with the HD treatment, typically by the dialysis nurse. This schedule also assures a close to perfect adherence with the prescribed regimen, enhancing the effectiveness of the therapy.

Intravenous (IV) administration continues to be the preferred route for rHuEPO in patients receiving maintenance HD, even though the bioavailability of the IV route is inferior compared to the subcutaneous (SQ) route [14]. The half-life of rHuEPO in vivo is only about 4–11 h [15]. SQ administration leads to a more sustained increase in serum rHuEPO levels; the bioavailability is 20–25% and measurable rHuEPO levels can be detected even 4 days after administration [15, 16]. The SQ dose is also ~25% lower for the same clinical effect compared to the IV dose,

Table 5.1 Currently available rHuEPO agents and other EPO-based agents in the US

	Molecule	Brand name	Company(s)	Year introduced/approved	Major trials	Status
1	Epoetin-α	Epogen®, Eprex®, Erypo®	Amgen, Janssen	1989 (Epogen in the US), 1990 (Eprex, Erypo in Europe)	Normal Hct [56], CHOIR [59]	Active
2	Epoetin-β	NeoRecormon®	Boehringer-la Roche	1990 (Europe)	Bennett [205]	Active
3	Darbepoetin-α	Aranesp®	Amgen	2001 (US and Europe)	TREAT [60]	Active
4	Epoetin-δ	Dynepo®	Shire	2005 (outside US)	Martin et al. [206]	Withdrawn
5	CERA	Mircera®	Roche	2007	STRIATA [28], ARCTOS [25]	Active
6	Peginesatide	Omontys®	Affymax	2012	Emerald 1, 2 [82] Pearl 1, 2 [81]	Suspended/restricted [80]
7	HX575 (biosimilar)	Binocrit® (Europe)	Sandoz	2007 (Europe), pending (US)	Monitor-CKD5 [207]	Active/pending
8	SB309 (biosimilar)	Retacrit® (Europe)	Hospira	2007 (Europe), pending (US)	Phase 3 Trial [208]	Active/pending
9	Epoetin- α biosimilar	–	Amgen	Pending	SHADE [209]	Pending

Peginesatide is a peptide without similarity to the EPO molecule but that has action on the EPO receptor (see text). *CERA* continuous erythropoietin receptor activator

thus providing cost benefits [17, 18]. One complication possibly linked to SQ use of certain products is pure red cell aplasia (PRCA) [19]. As a result of this possibly idiosyncratic reaction, the SQ route has become less popular in spite of its multiple other benefits.

Following the successful adoption of rHuEPO, there have been several attempts to pharmacologically manipulate its structure in order to achieve a more favorable therapeutic profile. Darbepoetin-α was the first such agent introduced; due to enhanced glycosylation darbepoetin has a larger size and a much longer half-life compared to rHuEPO [20, 21]. In the case of darbepoetin the route of administration (IV vs. SQ) does not seem to influence efficacy, and less frequent dosing makes it ideal for certain patients such as those on home dialysis therapy. Adoption has been slow due to costs but has been favored once incorporated into center-specific protocols. A conversion factor of 200 units rHuEPO to 1 μg of darbepoetin is typically used for initial dosing. Another newer agent developed to further increase the half-life of rHuEPO was the continuous EPO receptor activator (CERA), created by binding EPO to polyethylene glycol. CERA has a half-life of 5 days irrespective of route of administration [22, 23]. Studies have proven the non-inferiority of CERA [24], which has been used for many years now in other countries, and also recently started being adopted by dialysis providers in the US for anemia management. Introduction of CERA (Mircera®, Roche) into the market was supported by its non-inferiority vs. darbepoetin in a randomized trial in CKD patients [25]. With a very long half-life and extended administration intervals, CERA offered a convenient alternative in non-dialysis CKD [26] and peritoneal dialysis patients whose contact with the dialysis providers is not on a daily basis [27]. IV CERA was pitted against darbepoetin in dialysis patients in a randomized trial and was found to be clinically as effective in maintaining Hgb levels in the target range as darbepoetin [28], thus paving the way for its mainstream use. Though cost-effectiveness is still not proven [29], CERA is a promising addition to the quiver of agents that are currently at our disposal.

Biosimilars and "Copy" Epoetins

Medicines that mimic biologic molecules but are produced artificially, that are clinically similar in action but having minor differences in clinically inactive metabolites are called biosimilars. Biosimilar peptides have been around for several years now and have been successful when used in many different diseases and conditions. A wide variety of drugs are available in this class including hormones, vaccines, growth factors, monoclonal antibody-based agents, etc. In the strictest sense, the advent of biosimilars in the management of anemia occurred with the introduction of darbepoetin. That said, the term "biosimilar" is currently applied to the generic formulations of the patented molecules epoetins-α and β in the US. In Europe, the first few biosimilars licensed were generic manipulations of epoetins-α and ζ (zeta) after their patent expirations [30]. The complex structure of these proteins necessitates an equally complicated manufacturing process, which results in subtle

differences in the end product of different manufacturers. These end products also differ in their pharmacologic actions [31]. Therefore, an identical replication of epoetins-α or ζ is not possible, even though their main biologic actions are compatible with the mechanism of action of rHuEPO; hence the term "biosimilar" is applied to them. Since they are produced in different cell lines, it is possible that there are isoforms in the same batch of products that can alter clinical pharmacology; possibly contributing to serious side effects such as PRCA [32, 33]. One deterrent to the manufacturing of biosimilars was the discovery of several cases of PRCA with one of the epoetin-α biosimilar brands [34]. Another was the ever-decreasing price of the novel ESAs and hence the loss of financial impetus to continue to make newer biosimilars. With patent expiration of the original ESAs, the introduction of several biosimilars of rHuEPO onto the US market is imminent.

In the study on the efficacy of biosimilars, the first trial compared the efficacy of the molecule HX575, the first biosimilar approved in Europe in 2007, to original rHuEPO molecules. Based on the structure of epoetin-α, HX575 was pharmacokinetically equivalent to epoetin-α but patient exposure was different as seen in the area under the curve (AUC) levels [35]. This finding is suggestive of different clinical response to equivalent dosing with biosimilars and hence close monitoring to hematologic indices was recommended [36]. When given to patients as maintenance therapy for anemia, HX575 was deemed clinically equivalent to epoetin-α [37] and as safe as epoetin-α [38]. However, with some evidence of differing clinical efficacy based on manufacturing locations [39], it was not deemed feasible to reliably incorporate HX575 in anemia protocols. SB309 was the second biosimilar agent to be developed based on epoetin-ζ. This molecule's lower bioavailability compared to epoetin-α was attributed to the higher protein content of epoetin-α [40]. SB309 was found to be clinically equivalent to epoetin-α in the initial correction phase study and in two maintenance trials [41–43]. This was further corroborated in a crossover trial with a similar safety profile as epoetin-α [44]. Immunogenicity is a legitimate concern with biosimilars and this was also studied in ESA-naïve CKD patients. Of 337 patients randomized to receive either HX575 or SB309 via the SQ route, two patients developed antibodies and one had PRCA [45]; this was attributed to tungsten exposure during manufacturing [46]. None of the biosimilar studies have reported such antibodies when given IV [47]. Due to the rare nature of this adverse effect, only long-term clinical use will provide robust information about the magnitude of PRCA with biosimilars.

In the US, the first biosimilar agent for any indication was approved under the biosimilar approval pathway Biologics Price Competition and Innovation Act (BPCIA) in 2014 [48]. Under this act, protection for the reference epoetin lasts 4 years from a biosimilar agent [49]. Specifically for management of anemia, there are at least six trials registered using biosimilar agents by the two companies aiming to introduce biosimilars in the US [50, 51] in both dialysis and non-dialysis-dependent CKD patients in different stages of completion. The Food and Drug Administration has a stringent and time-consuming process for biosimilar agents and this may slow down introducing more biosimilars into the US market. This cautious approach is supported by an increase in the prevalence of PRCA in countries

that have used biosimilars for several years [52]. Though production and use have not been as controlled as the original epoetins even in Europe, reassurance can be had from the European experience of almost a decade of use, especially in a highly regulated and stringent environment. That and with a regimented approach paralleling that of the original biologic agents, there is not a strong argument to keep biosimilars out of reach of US nephrologists' use in the near future.

For several years now and in several countries, epoetin-like peptides have been developed without licensing and stringent production standards. They differ dramatically in their structure and hence also in their clinical efficacy and side-effect profile [53]. There is a concern for increased incidence of PRCA with these products due to potentially lower production standards and for various other adverse effects due to unregulated or clandestine use, but due to a mismatch between demand and affordability, these are probably the only means of access to ESAs in many countries [54]. More data are awaited on these "copy" epoetins.

Treatment of Anemia with rHuEPO and Its Derivatives: Efficacy and Safety

The efficacy of rHuEPO in correcting anemia in CKD and ESRD has been successfully established shortly after the manufacturing of the molecule for therapeutic utilization. After rHuEPO administration, a measurable increase in reticulocytes is seen in 7–10 days with an increase in hemoglobin following in 2–3 weeks. A concomitant drop in serum iron indices is evident unless iron is replenished simultaneously (more about the role of iron below). The widespread availability of rHuEPO in dialysis patients has resulted in a rapid uptick in its clinical application, and a broadening of its recipient base from dialysis patients to the much larger group of patients with non-dialysis-dependent CKD, and separately to patients with anemia caused by chemotherapy. The initial enthusiasm surrounding rHuEPO led to a belief that complete therapeutic correction of anemia of CKD and normalization of blood hemoglobin concentrations using this agent was possible and beneficial; hence a series of randomized controlled clinical trials were designed to test this hypothesis.

The approval of ESAs was based on small clinical trials and other observational studies showing clinical benefit along with avoidance of repeated transfusions [55]. Encouraged by these data, the first prospective randomized trial to assess the possibility of benefits in normalizing blood hemoglobin concentration was the US Normal Hematocrit (Hct) trial [56]. The goal of the study was to ascertain if a higher target Hct achieved with ESA therapy was indeed better. Dialysis patients were randomized into two groups with different target Hct (30% vs. 42%), and followed until the development of the composite primary end point of death or first nonfatal myocardial infarction. The study was prematurely halted due to a higher proportion of patients in the higher Hct target group reaching the primary end point, thus obviating the possibility of any benefit in "normalizing" the Hct and suggesting that treatment with rHuEPO to a normal Hct target may in fact be detrimental. In addition to the primary end point, other clinical end points such as vascular access

thromboses also suggested detrimental effects from the normal Hct treatment paradigm. Another study evaluating the change in the left ventricular volume index in dialysis patients with different Hgb targets (9.5–11.5 g/dL vs. 13.5–14.5 g/dL) detected no difference in outcome between the two groups, suggesting a lack of benefit from normalizing Hgb levels to another hypothetical treatment target [57]. Along with the clinical trials conducted in dialysis populations, several trials were designed to test similar hypotheses in anemic patients with non-dialysis-dependent CKD. The Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta (CREATE) trial was an international randomized controlled clinical trial of patients with advanced non-dialysis-dependent CKD, which studied the incidence of cardiovascular events in two groups assigned to different Hgb targets and different timings of epoetin-β initiation [58]. No differences in primary end points were detected and, interestingly, the group initiating epoetin-β earlier experienced a shorter time to dialysis. The Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial was another large randomized controlled clinical trial designed to address the effect of different Hgb treatment targets (11 g/dL vs. 13 g/dL) on clinical events [59]. The group assigned to the higher Hgb target experienced a significantly higher rate of the composite primary end point (congestive heart failure, hospitalizations, stroke, or myocardial infarction) compared to the low target group. Also, there was not any improvement in the quality of life as perceived by the participants in the higher Hgb target. Similar results were reported in the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) trial using darbepoetin, in that the darbepoetin-treated arm experienced a significantly higher incidence of stroke compared to the placebo arm, and without a benefit in the other study end points [60]. Benefits from using ESAs were also examined in trials conducted in non-CKD patients, especially those with heart failure. In the Reduction of Events by Darbepoetin Alfa in Heart Failure (RED-HF) trial, patients with systolic heart failure were administered darbepoetin in an effort to study its effect on a composite primary end point of death or hospitalizations related to heart failure [61]. Interestingly, no differences were seen in the treatment group compared to placebo and a higher incidence of thromboembolic events in the darbepoetin group led to a halt in design of large trials using ESAs in heart failure. This is in contrast to the trials conducted with intravenous iron (IVI) in heart failure (discussed below).

From these clinical trials, it has become clear that normalization of Hgb using treatment with rHuEPO is still fraught with difficulties: it does not result in the expected clinical benefits, it could be detrimental, and last but not least, it is very expensive. Hence, current guidelines suggest maintenance of Hgb in the 10–12 g/dL range with focus on avoiding transfusion, recognizing the benefits of rHuEPO therapy in sparing blood transfusions in patients with severe anemia of CKD, but also acknowledging a lack of high-quality evidence on the ideal Hgb level for these interventions. It also remains unclear if the Hgb treatment targets established in the wake of the rHuEPO trials are valid end points for therapeutic strategies employing agents with distinctly different mechanisms of action (vide infra). Post-hoc analyses have suggested that the adverse effects seen in the high-Hgb target arms of the rHuEPO trials may have been attributable to the higher doses of

rHuEPO and/or the relative iron deficiency induced by this strategy [62]; hence the ideal therapeutic targets for other anemia therapies will have to be evaluated in dedicated clinical trials.

ESA Hyporesponsiveness

One of the common problems with ESA therapy in dialysis patients is hyporesponsiveness to ESAs. Hyporesponsiveness is defined as inability to reach the target hemoglobin level irrespective of the ESA dose and continued need for supra-normal doses of ESAs [63, 64]. This is most commonly due to the chronic state of inflammation seen in dialysis patients as part of the malnutrition-inflammation complex syndrome (MICS) [65–67]. MICS has far-reaching consequences beyond ESA hyporesponsiveness [68]. Dialysis patients have several other exclusive causes (tunneled cuffed catheters, impure dialysate, etc.) that make this clinical predicament common [69, 70]. Apart from MICS, several other causes for ESA hyporesponsiveness have been identified (Table 5.2). A consistent finding is a low serum albumin in many patients with hyporesponsiveness; in fact, serum albumin is a strong predictor of ESA response in these patients [71]. Addressing the etiology of hyporesponsiveness (when possible) is usually curative of the condition.

PRCA

In 2002, a serious side effect, PRCA was reported in patients receiving SQ epoetin-α [19]. This condition is characterized by severe hypo-proliferative anemia caused by the lack of a functional EPO response resulting from an immune response against circulating EPO molecules [72]. Though several causes were proposed (including storage quality lapses and syringe material breakdown) [73], the finding of SQ administration was a stark constant in the reported cases. As mentioned above, PRCA has also been described in conjunction with other ESAs, and remains a dreaded complication of such therapies [19, 74]. At this time, evidence suggests that the development of antibodies against conventional rHuEPO can also neutralize

Table 5.2 Common causes of ESA hyporesponsiveness

Cause	Example
Dialysis-related	Endotoxins and impure dialysate (chloramines) [210], cellulose membranes [210], TCC [69]
	Aluminum toxicity, β2-microglobulin amyloidosis
	Severe secondary hyperparathyroidism [211]
	Inadequate dialysis [212]
Bone marrow disorders	Myelofibrosis, sickle cell anemia, pure red cell aplasia [213], iron restriction/bleeding
Malnutrition	Vitamin B12 deficiency, L-carnitine, folic acid, pyridoxine
Medications	Dapsone, Primaquine, nitrofurantoin, Sirolimus, ACE inhibitors
Inflammation	MICS [68], SLE, failed kidney transplant
	Chronic infections: Osteomyelitis, cellulitis, HIV

TCC tunneled cuffed catheter, *MICS* malnutrition-inflammation complex syndrome, *SLE* systemic lupus erythematosus, *HIV* human immunodeficiency virus, *ACE* angiotensin-converting enzyme

native EPO leading to PRCA, but how this can lead to PRCA in dialysis patients [72], most of whom have minimal native EPO production, remains unanswered. There is evidence that native EPO is not completely lost even in patients with ESRD and can be augmented in acute anemia but not in chronically low Hct states [75, 76]; this "basal" EPO production may even contribute to post-transplant erythrocytosis [77, 78]. Hence, it is possible that this minimal EPO production is essential in avoiding PRCA rather than hemoglobin homeostasis. Treatment of PRCA consists of blood transfusions and immune suppression; a report suggested that an ESA with molecular structure distinctly different from EPO such as peginesatide (vide infra) may be successful in alleviating PRCA [79]. PRCA typically subsides after kidney transplantation, presumably because of the immune suppression received.

Peginesatide

A novel molecule, peginesatide, was developed as an agent that would activate the EPO-R but not have any structural similarity to EPO or its synthetic derivatives [79]. It is a pegylated, dimeric peptide that showed good in vitro results on erythroid progenitor cell lines and with animal studies showing encouraging data, a small group of dialysis patients with previously diagnosed pure red cell aplasia (PRCA) were given peginesatide in an open label trial [80]. This led to three large clinical trials to study the applicability of peginesatide to both CKD and dialysis patients. In the Peginesatide for the Correction of Anemia in Patients with Chronic Renal Failure Not on Dialysis and Not Receiving Treatment with Erythropoiesis-Stimulating Agents) (PEARL 1 and 2) trials, CKD patients were randomized to receive either darbepoetin in the control arm or peginesatide in the treatment arm [81]. Though the efficacy of peginesatide was similar to that of darbepoetin in correcting the Hct, the study was halted due to an unacceptably high incidence of cardiovascular events and death. The Efficacy and Safety of Peginesatide for the Maintenance Treatment of Anemia in Patients with Chronic Renal Failure Who Were Receiving Hemodialysis and Were Previously Treated with Epoetin (EMERALD 1 and 2) trials which was conducted with peginesatide in dialysis patients also reported similar results of efficacy when compared to epoetin [82]. Interestingly, the cardiovascular adverse events and mortality rates in both groups in these trials were similar. This discrepancy in the adverse events with peginesatide in the two sets of trials are yet unexplained. As mentioned above, peginesatide has been sparingly used in patients with anti-erythropoietin antibodies with clinical benefit [80]. That said, at this time, the side-effect profile has relegated peginesatide to an "also-ran" in the race for the next agent for anemia management.

Iron

Iron is a key ingredient in normal erythropoiesis. A typical daily adult diet contains about 15–20 mg of elemental iron; only 10% of this is absorbed, which is usually sufficient to offset iron losses from epithelial desquamation and

sporadic microscopic bleeding. Most of the iron needed for daily erythropoiesis, about 20–30 mg, is reclaimed from senescent RBCs by macrophages. Iron in the diet is absorbed only in the ferrous form (the ferric form has to be converted to the ferrous form and ascorbic acid facilitates this conversion) through the enterocytes via transporters and then into the plasma through ferroportin-1 (FP-1) [83]. Iron is delivered in the ferric form to transferrin [84] that in turn delivers the iron by binding directly to a transferrin receptor (TfR-1) on the surface of the erythroblast which is then internalized into a siderosome [85]. The ferric form is then reduced to the ferrous form and utilized for heme synthesis within the mitochondria and the transferrin is recycled back into the circulation [84–86].

Recently, hepcidin has been identified as a key player in iron metabolism. Hepcidin is produced by the liver in response to various stimuli [85, 87–89] (with inflammation being a key one) and was initially identified as an antimicrobial peptide. Indeed, hepcidin is possibly protective in the setting of a severe infection or bacteremia by restricting the availability of iron for the microorganism's enzymes that use iron as a co-factor. Hepcidin blocks the absorption of iron from the enteral tract or the release of iron from the reticulo-endothelial cells by binding to FP-1 leading to its internalization and degradation [90]. Hepcidin inactivation leading to juvenile hemochromatosis, a particularly severe form of hereditary hemochromatosis and the role of mutation in the coding gene, HJV, has been well described [91–93].

Iron Replacement in Anemia

Iron replenishment is an essential aspect in the management of anemia of various etiologies; mostly when iron deficiency is the main cause of the anemia, but also in the context of other treatments, such as with rHuEPO. In addition to the "usual" iron losses, CKD patients may also lose iron as a result of more frequent gastrointestinal or other bleeding, frequent blood testing or blood losses related to hemodialysis [94]. Coupled with ineffective iron absorption through the gastrointestinal tract in dialysis patients [95], this loss of iron can amount to a negative iron balance of 1.5–3 g per year [96]. Unless at least a similar amount of iron is supplemented, ESA treatment is expected to be ineffective. Also, with rHuEPO administration and enhanced erythropoiesis, there is significant decrease in iron stores and a lack of replenishment leads to suboptimal and eventually arrested response to rHuEPO or its derivatives. The traditional route of administration for iron has been oral. Ferrous sulfate in oral preparations has been effective in patients with iron deficiency who are devoid of chronic inflammation and liver disease, but less so in patients in whom hepcidin prevents effective restoration of iron stores. In such patients intravenous iron (IVI) preparations have gained popularity (Table 5.3), especially in cases where this route is also convenient and practical, such as chronic HD patients and this is supported by evidence of clinical benefit and cost savings [97–99].

Table 5.3 IVI agents available in the US

Brand name	IVI product	Company	Molecular weight (MW)	Maximum one time dose	Test dose
INFeD®	Iron dextran (low molecular weight)	Watson pharmaceuticals	96 kDa	1000 mg[a]	Yes[c]
Dexferrum®	Iron dextran (high molecular weight)	American regent labs	265 kDa	100 mg[b]	Yes[c]
Ferrlecit®	Iron gluconate	Watson pharmaceuticals	38 kDa	125 mg[a]	No
Venofer®	Iron sucrose	American regent labs	43 kDa	500 mg[a,d]	No
				100 mg[b]	
Feraheme®	Ferumoxytol	AMAG pharmaceuticals	750 kDa	510 mg[a]	No[c]
Ferinject®	Ferric Carboxymaltose	Vifor pharmaceuticals	150 kDa	1000 mg[a]	No

kDa kilodaltons
[a]Indicates a slow infusion
[b]Indicates rapid infusion/IV push
[c]Indicates anaphylactoid type reactions described
[d]Not necessary but slow infusion over a minimum of 15 min

Assessment of Iron Stores and Therapeutic Targets

The gold standard method for assessment of iron stores continues to be the invasive semi-quantitative bone marrow staining for iron but this is rarely done [100, 101]. A liver biopsy and staining to assess the liver iron stores is also done only in extreme circumstances, although an indirect assessment via the *Superconducting Quantum Interference Device* (SQUID) may offer an alternative [102]. In clinical practice, iron stores are assessed most often by measuring serum ferritin and transferrin saturation (TSAT) but since inflammation to some degree is present in a large majority of patients, relying on these parameters to initiate IVI is fraught with difficulty as ferritin and TSAT are inversely affected by inflammation [103–105]. Since the lower limits of both ferritin and TSAT have low sensitivities and there is an appreciable response to IVI in patients with normal or even moderately elevated levels of either one [97, 106, 107], their utility in triggering replacement is questionable. Absolute iron deficiency states are consistent with very low ferritin levels, even in the presence of inflammation but this association ceases to regulate ferritin levels once a minimum amount of iron becomes available hence increasing the specificity of a very low ferritin level [108, 109]. Use of ferritin to withhold IVI is again not reliable. Even when IVI replacement guidelines were strictly adhered to, a majority of patients were noted to have iron overload on hepatic imaging [110]. Therefore, dependence on ferritin and TSAT alone is problematic.

Another unique condition seen in patients who are being treated with ESAs is relative iron deficiency. Driven by exogenous ESAs, the bone marrow "strips" the transferrin-bound iron at a rate that exceeds its replenishment. Hence, even though there is not a real body iron deficiency, TSAT can be <20% [111]. In these patients,

using the traditional tests is inaccurate at best. Another issue that has been mentioned above is the inhibitory effect of hepcidin on mobilization of iron stores in inflammatory states. This leads to low TSAT but normal or even high ferritin levels. These two situations aptly summarize the handicap associated with using TSAT and ferritin as markers for iron stores [111].

Other indicators such as reticulocyte hemoglobin content (CHr), percentage of hypochromic red cells, or soluble transferrin receptor test (sTfR) have been evaluated as indices for iron stores. CHr is a test that detects the amount of iron available for hemoglobin production. It does not have the bias of other tests that rely on the entire age spectrum of RBCs, and it has shown better accuracy in response to IVI in dialysis patients when compared to ferritin and TSAT [100]. The latter study also established a cutoff level for CHr to be used to guide IVI. Percentage of hypochromic red cells (PHRC) estimates the concentration of Hgb in the RBCs and has comparable specificity for detecting iron deficiency states [107, 112] but is not useful when blood samples have a long transit time to the central laboratories as is the case in most large dialysis companies [111] in the US. Similarly, using sTfR as a diagnostic tool relies on the correlation of increased sTfR on the surface of erythroblasts in iron-deficient states but this is confounded by ESAs; ESAs also can lead to elevated sTfR levels by virtue of an increased cell mass. As a result, the results of using this test are mixed in studies of ESRD patients [113, 114]. The newer tests for iron estimation have generated low interest thus far and, at this time, we are still reliant on TSAT and ferritin to determine the necessity of IVI in the US.

Optimal target iron levels have been deduced after observational studies have shown that optimal erythropoiesis is seen in dialysis patients when TSAT is 30–50% and ferritin is as high as 1200 ng/mL [115]. Also, ferritin levels <2000 ng/mL may not be associated with iron overload as evidenced by imaging studies of the liver [105]. The predicament with this is the association of high ferritin with increased dialysis morbidity, iron overload [116], and mortality [117], though one study suggested that the association of elevated ferritin with poor outcomes is confounded by markers of inflammation and malnutrition [118]. In summary, current K/DOQI guidelines recommend individualization of IVI administration when serum ferritin is >500 ng/mL [105].

Benefits of IVI

There is no doubt about the hematological benefits of IVI in both non-dialysis and dialysis patients. Not only do patients have a robust increment in Hct levels but there is a lower ESA requirement when IVI is concomitantly given [97]. An important question is whether or not such hematologic benefits extend to improved clinical outcomes.

Cardiovascular benefits of correction of iron deficiency have been well studied and documented. The first study performed in patients with congestive heart failure (CHF) showed improved cardiac function and New York Heart Association (NYHA) classification [119] with SQ EPO. In another study conducted in CHF patients, IVI also improved measures of functional status and quality of life indices along with fewer hospitalizations [120]. Similar results were also found in the ferric carboxymaltose

evaluation on performance in patients with iron deficiency in combination with chronic heart failure (CONFIRM-HF) trial [121]. Compared to a placebo, patients on IVI performed significantly better on subjective scores and had fewer hospitalizations as well; differences between the two groups were seen halfway into the study period and continued going forward.

An additional benefit of iron supplementation is a lowering of ESA requirements. Lower ESA requirements were seen in dialysis patients when given IVI compared to oral iron [122]. A follow-up study to the Dialysis Patients' Response to IVI with Elevated Ferritin (DRIVE) study showed significant reduction in both ESA requirement and cost of anemia management in response to IVI [98, 99]. Whether this translates into better clinical outcomes still needs examination. Cause for skepticism comes from finding poor correlation between higher hemoglobin levels from ESAs' administration and equivocal clinical outcomes in heart failure [61]. Currently, a large trial is underway to study all-cause mortality in dialysis patients receiving IVI; the results are keenly awaited to shed more light on all-cause mortality and non-fatal cardiovascular events of IVI using iron sucrose [123].

Safety Profile of IVI

A major conundrum with IV iron preparations is the non-physiologic nature of this route, resulting in various complications ranging from allergic reactions and anaphylaxis to potential for oxidative stress, kidney damage, and iron overload [124, 125].

Iron overload has been a major issue ever since transfusions were used to treat anemia of CKD with wide ranging cellular effects [126, 127]. With the emergence of IVI as a pivotal tool to tackle anemia of CKD, concerns for iron overload are legitimate. As mentioned earlier, ferritin levels can help with starting IVI but may not be accurate when used to screen for withholding IVI in patients.

Short-term side effects of IVI administration are usually mild and include nausea, hypotension, and/or diarrhea. The more severe reactions such as urticaria, anaphylactic shock, or laryngeal edema are more common with iron dextran preparations due to the dextran component and there is some evidence that the larger dextran component leads to more frequent reactions [128–131]. This necessitates a test dose administration and careful monitoring. Prospective studies to specifically assess adverse effects have been few so far; retrospective analyses of previously conducted large clinical trials will help to study causality and assess safety profiles of IVI. A large double-blinded study noted that hemodialysis patients, when exposed to sodium ferric gluconate, had similar levels of anaphylactic reactions as the placebo group [132]. This, along with a similar safety profile for iron sucrose [133], suggests that non-dextran-based IVI is relatively safe and does not need a test dose.

In contrast to the immediate and/or short-term side effects, studying long-term adverse effects are obviously more difficult. Sparse data exist that are sufficiently robust to indicate a causality link between IVI and adverse effects, though the Ferinject assessment in patients with Iron Deficiency Anemia and Non-Dialysis-Dependent Chronic Kidney Disease (FIND-CKD) found similar event rates in both the low and high ferritin target groups treated with IV ferric carboxymaltose [134]. There are conflicting data on IVI and association with risk of death and mortality

rates [135, 136]. A more recent observational study found a lower risk of death in dialysis patients who were given less than 400 mg IVI when compared to those who were either given more or not given any IVI [118]. IVI has been studied in comparison to oral iron in clinical trials of non-dialysis CKD patients and has been found to be similar in efficacy and in incidence of adverse reactions such as cardiovascular and thromboembolic events [134, 137]; the type of IVI used (ferric carboxymaltose or iron isomaltoside) did not significantly affect adverse events. This is in contrast to a smaller single center clinical trial that showed a higher incidence of cardiovascular events and infections in the IVI group compared to the oral iron group leading to early termination of the trial [138]. To ascertain the true effect on adverse events, much larger trials with IVI are necessary.

An association of interest with IVI is the risk of infection. Iron is a preferred element for several bacteria including Staphylococcus species [139]. Even oral iron has been shown to support bacterial growth in vitro [140], hence this is a big concern especially because of the scale of IVI use in dialysis patients worldwide. A small prospective study did not show any correlation with infections [141], but a much larger observational study showed a small but specific link between iron dosing and infection-related hospitalizations; risk was highest in those individuals who had a high serum ferritin and received a higher dose of IVI [142]. The same study also found an association between infections and bolus dosing. Infections and infection-related hospitalizations were also noted to be higher in patients treated with IVI compared to oral iron in non-dialysis CKD patients in a recent trial [138]. In summary, it is advisable to withhold IVI in patients with systemic/extensive bacterial infections and to have a measured approach in general considering that dialysis patients are vulnerable to infections by nature of their chronic illness.

A long-term potential side effect of IVI is nephrotoxicity due to the cytotoxic effects of iron-induced oxidative stress and mitochondrial injury [143–145], which has been suggested with iron sucrose and ferric gluconate [143]. A head-to-head comparison study noted a higher degree of proteinuria with iron sucrose compared to ferric gluconate [146]. Due to the strong correlation of proteinuria with CKD progression to ESRD and to cardiovascular disease, there is a legitimate concern for long-term IVI safety. When studied prospectively if IVI forms did indeed affect proteinuria upon repeated exposure, iron sucrose was found to have a sustained proteinuric effect in CKD patients when compared to ferric gluconate [147]. Taken together, these findings suggest that IVI has to be used judiciously in non-dialysis-dependent CKD patients.

A new development in the US that might be detrimental to ESA use and increase IVI use has been the introduction of a “bundled” payment for IV iron and ESAs in 2011 by Center for Medicare and Medicaid Services (CMS). Following the introduction of this payment system, the mean Hgb and ESA doses declined whereas a higher rate of transfusions and use of IV iron was noted [148]. Whether the potential for a financially motivated increase in IVI administration in the US is indeed leading to changes in practice trends raises further concerns about safety and requires urgent investigation in dedicated clinical trials [70].

Hypoxia-Inducible Factor (HIF Modulation)

HIFs are proteins that play a pivotal role in signal transduction of EPO production (Fig. 5.3). The HIF complex consists of either HIF-1α or an oxygen-sensitive HIF-2α subunit that is associated with a constitutively expressed HIF-1β subunit. Human renal fibroblasts, under hypoxic conditions, activate only HIF-2α and not HIF-1α [149]; this finding has been supported by evidence of activating mutations in HIF-2α leading to erythrocytosis [150]. HIF-2α, in the absence of hypoxia, is hydroxylated at 2 proline residues by proline hydroxylases [151] (2-oxoglutarate superfamily-dependent dioxygenases [152, 153]); this step marks the HIF-2α for binding by von Hippel Lindau (VHL) protein and subsequently for ubiquitination and proteasomal degradation [154]. Proline hydroxylase 2 (PHD2) has been identified as the key enzyme that regulates HIF-2α stability; PHD2 has also been identified in mutations leading to erythrocytosis [155–157]. HIF-2α, in hypoxic states, is not hydroxylated due to inhibition of proline hydroxylase and hence forms a heterodimer with HIF-1β that translocates to the nucleus and binds to the hormone response element (HRE) of EPO and induces EPO transcription [158]. Ergo, PHD2 inhibitors represent a novel target for therapeutic intervention and this opportunity has been pursued to develop therapeutic agents that can stabilize the HIF complex and hence positively influence EPO production.

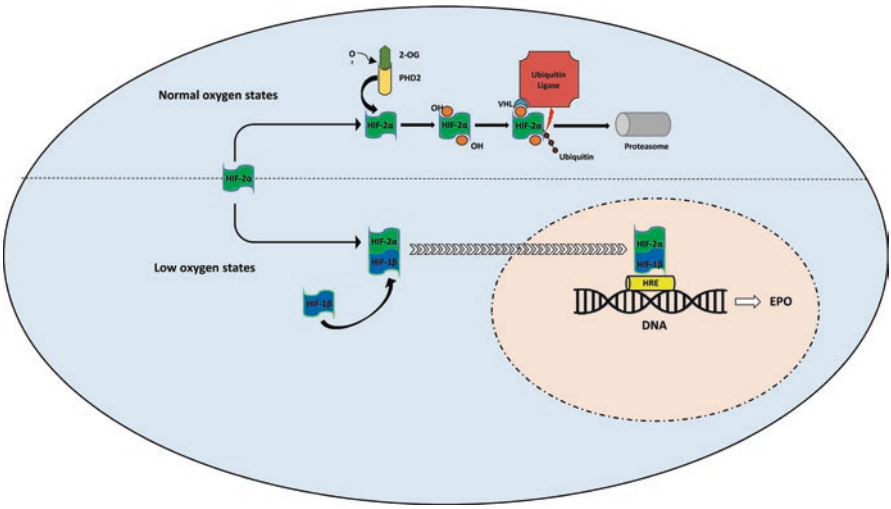

Fig. 5.3 EPO production signaling pathway. Under normoxic conditions, HIF-2α is hydroxylated at 2 proline residues by PHD2 and this marks HIF-2α for VHL protein binding. The VHL protein and its associated ubiquitin ligase attach ubiquitin to HIF-2α and hence relegate it for proteasomal degradation. Under hypoxic conditions, HIF-2α dimerizes with HIF-1β, a constitutively expressed protein, and the heterodimer translocates to the nucleus and binds to the hypoxia response element (HRE) of EPO to increase EPO gene transcription

Table 5.4 Clinical trials currently underway for PHD2 inhibitors and other novel agents

	Drug/molecule	Category	Company	Involved in clinical trials	Patients category
1	FG-2216	PHD2 inhibitor	Fibrogen	Suspended	N/A
2	Roxadustat (FG-4592)	PHD2 inhibitor	Fibrogen	Phase III	CKD, HD
3	Molidustat (BAY-85-3934)	PHD2 inhibitor	Bayer	Phase I	CKD
4	Dapradustat (GSK1278863)	PHD2 inhibitor	Glaxo-SmithKline	Phase IIa/IIb	CKD, HD
5	JTZ-951	PHD2 inhibitor	Japan tobacco Inc	Phase I	N/A
6	AKB-6548	PHD2 inhibitor	Akebia therapeutics	Phase IIa/IIb	CKD, HD
7	Lexaptepid	Hepcidin inhibitor	Noxxon	Phase IIa pilot	Cancer, HD
8	K-7174, K-11706	GATA-2 inhibitor	N/A	Animal studies	N/A
9	Sotatercept	Activin trap	Acceleron/Celgene	Phase IIa	HD

CKD chronic kidney disease, *HD* dialysis, *PHD2* prolyl hydroxylase domain

Proline Hydroxylase (PHD2) Inhibitors

PHD2 enzymes have a wide range of intracellular functions and targets and hence it is important to have highly specific molecules and probes that can target the hypoxia-HIF-2α-EPO pathway without adversely affecting other cellular processes [159]. Such molecules have been developed in the last several years and hold much promise in providing physicians with another arrow in their quiver to manage anemia [160].

Structure-based drug design and high-throughput screening for candidate drug compounds have allowed pharmaceutical companies to identify potential molecules that inhibit PHD2 [161]. From the time two compounds were developed to have potent PHD2 inhibition [162, 163], several proprietary compounds with different structures have emerged [164–167] and are currently being investigated in various stages of clinical trials (Table 5.4). It is imperative that these small molecules meet safety standards before large-scale clinical trials are conducted. Amongst the data that are keenly awaited from the trials is the effect of concomitant use of PHD2 inhibitors and conventional ESAs and the effect on adverse effects *vis-a-vis* ESAs.

Potential Pitfalls of PHD2 Inhibitors

Due to the broad range of potential targets of PHD2 inhibitors, it is conceivable that their use can increase the risk of certain diseases and/or disease progression. Von-Hippel-Lindau disease, a disease due to a germline mutation in the VHL allele, is an obvious threat. With increased HIF activity, the phenotype of the disease is replicated to some degree, especially in clear cell renal cell carcinoma (CCRCC) where there is a

strong linkage between HIF-2α [168–170]. One other oncogenic association is pheochromocytoma [171, 172]. It is logical to surmise that HIF activation can make other cancerous cells aggressive due to its angiogenic capacity, epithelial to mesenchymal transformation and promotion of metastasis [173–176]. Interestingly, the oncogenic potential has not been noted in patients with a form of polycythemia that is due to biallelic inactivating mutations of the VHL gene [157, 177]. That said, these patients have been noted to have higher pulmonary artery pressures raising concerns for pulmonary hypertension [178]. Another ominous observation is reduced life expectancy in these patients, possibly from thromboembolic events [179]. There is conflicting data regarding the effect of HIF activation on CKD progression. One study suggests an accelerated loss of renal function whereas a few others have shown a rather "protective" effect with a slower rate of loss of GFR, interestingly even in diabetic nephropathy [180–182]. In summary, large clinical trials will throw light on these concerns with persistent HIF activation as a promising avenue for anemia management.

Novel/Experimental Agents

Hepcidin Antagonism

Hepcidin antagonism as a possible means to ameliorating anemia in dialysis patients was supported by animal studies using monoclonal antibodies against hepcidin [183]. Recently, a small clinical trial was conducted using an oligoribonucleotide called lexaptepid to study the effect of this molecule on serum iron levels [184]. Clinically significant increases in iron levels were noted in the treatment arm when compared to placebo. This strategy has the potential to open another avenue of agents to tackle anemia. A possible concern with hepcidin antagonism would be an increased risk of infections, considering hepcidin's protective properties. This field is in its infancy and requires more investigation.

GATA Inhibition

GATA sequence is a short sequence of nucleotides that are negative regulatory element for EPO transcription [185]. GATA is essential for maturation of the erythroblasts but has to be turned off to allow for maturation into RBCs [186]. Thus it was postulated that GATA inhibition would allow for increased EPO production. Two GATA inhibitors have been developed that support this hypothesis [187, 188]. Still in its infancy, animal studies have shown promise but this is yet to be translated into human studies.

Erythropoietin Gene Therapy

Early experiments were conducted to test if the EPO gene could be delivered in vivo using transfected dermal cells using adenovirus transfection [189]. Animal studies were promising in their results [189]. This has been taken to the next level when a

small group of peritoneal dialysis patients in Israel showed a sustained response to the treatment in a clinical trial, without needing external EPO supplementation [190]. It remains to be seen if this can indeed become a possible avenue to deliver and have sustained in vivo EPO production.

Activin Traps

Another group of proteins that affect a variety of cellular functions are the small mothers against decapentaplegic proteins (SMADs), particularly SMAD4 [191]. Activins are small dimeric proteins that have an inhibitory effect on several cellular processes and exert their actions via receptors that transduce their signals through SMAD4 [192]. Sotatercept is a chimeric protein that binds to activins and prevents their inhibitory effect [193]. In an initial Phase I trial, it had encouraging effects on bone resorption and reduction of osteoporosis and also on increasing Hgb levels with a low incidence of side effects [194] and is currently being tested in a Phase II trial [195]. Sotatercept has been shown to have multiple actions to increase erythropoiesis; such as angiotensin expression, inhibition of expression of vascular endothelial growth factor and hepcidin inhibition [196, 197]. This is another interesting avenue that needs further inquisition to ascertain the long-term applicability and role in anemia management.

Role of Endogenous Sex Steroids

The male hormone testosterone has been known to have a potent effect on erythropoiesis for a long time now and its replacement has been used just as long for the treatment of anemia [198, 199]. Anemia can be significant in patients who have undergone extensive hormonal ablation and radiotherapy for prostate cancer [200]. Hence, it is possible that an age-related decline in testosterone can contribute to anemia. Aging also leads to progressive decline in renal function and with it, anemia should increase in prevalence. Though older men with CKD have a higher prevalence of anemia than young male CKD patients, younger female CKD patients are noted to have a higher prevalence of anemia than older female CKD patients (likely a result of menstrual blood loss in younger women) [201]. An Italian study examined the association of anemia in elderly subjects; anemia was more prevalent in those with CKD [202] and subsequent re-analysis of the data showed that subjects with low testosterone, irrespective of gender, had a higher prevalence of anemia compared to subjects with normal testosterone [203]. A recently conducted open-label study in patients with hypogonadism showed potent increase in EPO levels when given testosterone improving anemia and other serum parameters that were studied such as total cholesterol [204]. Taken together, it is not unreasonable to consider testosterone as a possible therapeutic option for CKD patients, especially in the elderly. Obviously, much needs to be studied and tested before this becomes a mainstream and viable option.

Conclusion

Anemia, especially "EPO-deficient" anemia, is a major quandary for nephrologists on a daily basis. It is one of the most common yet most important problems that need to be addressed in a timely and thorough manner. Suboptimal response to current anemia management tools is commonplace and with the introduction of newer agents and novel therapeutics, it is possible to have several options available at the disposal of the treating physician. While some components of anemia management are well established, other ideologies can and should be challenged to improve the quality of care that our patients will receive. With more data being generated and analyzed each year, our knowledge and subsequently therapeutics will result in not only better Hgb values but also a better health outcome.

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Anemia of Chronic Kidney Disease

6

Mina El-Kateb and Robert Provenzano

Introduction

Anemia, once the bane of chronic kidney disease with little in the way of management or treatment, was eliminated with the advent of recombinant erythropoietin. The ability to treat anemia using recombinant DNA represents one of the greatest milestones in medicine. Taking this therapy from bench research to clinical medicine and alleviating the scourge of anemia for ESRD and CKD patients has been a journey for researchers, clinicians, and patients. The past 30 years of observational and randomized trials have guided clinicians and regulators to the appropriate application of these therapies. Unlike few areas of medicine, the story surrounding management of anemia continues to unfold. New agents, hypoxia-inducible factor stabilizers (HIF), and oral transcription agents that activate erythropoietin during hypoxic conditions are now in Phase 4 trials and may offer a more physiologic approach to manage anemia. The appropriate application of these new therapies is now being determined, and so the story continues.

Prevalence

Anemia of CKD is common. Hazmi et al. found that almost half of all CKD patients with a creatinine of 2 mg/dL have a hematocrit less than 36% [1] and as many as 90% of patients with estimated GFR less than 30 mL/min per 1.73 m^2 are anemic with most having hemoglobins less than 10 g/dL. Estimated GFR as well as serum creatinine levels have been used clinically as surrogates of renal function; correlation between both of these two markers and hematocrit level have been well

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documented. The direct correlation seen between the level of hematocrit and predicted GFR is relatively strong ($r = 0.49$) as is the inverse correlation between creatinine and hematocrit levels ($r = -0.37$) [1].

Pathophysiology

The development of anemia in chronic kidney disease (CKD) is multifactorial but ultimately presents as a normocytic normochromic anemia. This is the result of a complex interaction between decreased production and increased destruction of RBCs.

Renal parenchymal loss present in advancing CKD is the principal cause of decreased RBC production. The shrinking, scarred kidney results in a decreasing number of type I dendritic cells that are responsible for the production of erythropoietin [2, 3].

Erythropoietin is the main stimulus for RBC production in the bone marrow, referred to as erythropoiesis. Additionally, in the setting of CKD, chronic inflammation results in the sequestration of iron into iron storage cells; these include the duodenal enterocytes, hepatocytes, adipocytes, and the macrophages of the reticuloendothelial system [4, 5]. These cells store and transport iron and are influenced by hepcidin, which drives them to internalize the cell surface iron exchanger, ferroportin. In doing so, the iron remains stored within these cells and cannot reach the bone marrow; consequently, RBC production is diminished.

Shortened RBC survival is the result of the accumulation of uremic toxins and increased free radical production [6, 7]. These same uremic toxins accumulate and result in poor clotting. Under normal circumstance, mild trauma in the GI tract heals and goes unnoticed, but in the setting of uremia and poor clotting, this same trauma results in chronic bleeding and significant blood loss over time.

Decreased RBC production and shortened survival is an oversimplified description of the anemia of CKD. Consequently, the workup, diagnosis, and treatment of this complex topic require a multidisciplinary approach. The evidence for this approach is entrenched in decades of evidence-based medicine, with billions of dollars of government funding and pharmaceutical research geared at minimizing the impact of anemia on the patients of CKD, by way of erythropoiesis-stimulating agents (ESA). Arguably one of the most extensively researched topics in medicine, anemia is but one of several issues encountered in the management of a patient with CKD. It is, therefore, important to always keep in mind the larger framework of the patient as a whole, not simply a summation of his or her list of comorbidities.

Identification

Identifying anemia of chronic kidney disease in a timely fashion is essential in order to minimize cardiovascular and cerebrovascular complications, as well as its impact on the patient's quality of life.

As mentioned, anemia of CKD (ACKD) is normocytic and normochromic (NCNC), specifically, the mean red blood cell (RBC) volume is 80–100 fL (femtoliter or 10^{-15} L) and the central pallor of the RBC, on a peripheral smear, is less than one-third of the average RBC diameter. Diagnosing this anemia requires a complete blood count to identify its existence as well as a peripheral smear to identify its morphology. The remainder of the workup is geared at identifying confounding causes of a NCNC anemia while addressing the coexisting iron deficiency.

Common confounders of normocytic anemia can be classified broadly as endocrine, infectious, malignant, medication-induced, and hemolytic etiologies. While a detailed discussion of these causes may be out of the scope of this chapter, it is important to address the more commonly occurring etiologies of a NCNC anemia in the setting of CKD.

The most important of these confounders is arguably anemia of inflammation, formerly anemia of chronic disease. While most CKD patients exhibit some degree of a chronic inflammatory response, it is important to eliminate chronic infections as the source of this inflammation. Common sources of infection in CKD patients include tunneled dialysis catheters and even arteriovenous grafts, which can result in bloodstream infections, endocarditis, and osteomyelitis [8, 9]. Another important cause of normocytic anemia is bone marrow suppression found many malignancies, the chief being multiple myeloma. These patients typically present later in life with bone pain, anemia, kidney injury, occasionally hypercalcemia, proteinuria, and potentially with lytic lesion on X-ray and large kidneys on ultrasound [10]. Medications are also another common source of a NCNC anemia seen in patients with CKD. Medications can directly suppress the bone marrow or cause hemolysis, both of these mechanisms occur more frequently in patients with CKD due to decreased drug clearance. Less common causes, but unique to the patient with CKD, are pure red cell aplasia and dialysis-induced hemolysis.

The typical workup should include a complete blood count with differential, peripheral smear, comprehensive metabolic panel, reticulocyte count, lactate dehydrogenase, thyroid-stimulating hormone, vitamin B-12, folate levels, and an iron panel. An older CKD patient (>50 years old) with proteinuria and large kidneys, irrespective of their diabetic status, should also be screened for multiple myeloma with serum and urine protein electrophoresis combined with immunofixation [11].

Commonly, patients with CKD have an underlying iron deficiency, superimposed onto ACKD. Often, this is due to gastrointestinal (GI) blood loss in a uremic milieu [12, 13]. Dialysis patient also tends to lose blood from the dialysis process itself. This includes blood loss from the initial cannulation process, blood sampling, dialyzer clotting, blood lost in the dialyzer circuit and post-treatment losses, resulting in as much as 2.5–5 g loss of iron per year [14]. Anemia from blood loss ultimately results in the depletion of iron stores and is referred to as an absolute iron deficiency.

As a result of chronic inflammation, CKD patients also display a functional iron deficiency (FID). Often referred to as iron-restricted erythropoiesis, FID is defined as iron stores that are normal or elevated but with inadequate accessible iron for incorporation into erythroid precursors [15, 16]. Laboratory findings typically

include an elevated ferritin of >100–200 ng/mL, indicative of an elevated iron storage form in conjunction with a reduced transferrin saturation <20%, the available iron form.

Whether functional or absolute, CKD patients tend to experience dramatic iron deficiency, making them more susceptible to developing anemia. Additionally, their comorbid conditions can sometimes confound the etiology of their normocytic, normochromic anemia. Ultimately, in advanced CKD the main culprit of this normocytic, normochromic anemia is the functional renal cell loss leading to a decreased erythropoietin production.

Treatment

The advent of human recombinant erythropoietin, in 1989, revolutionized the management of the anemia in patients with chronic kidney disease. Before then, management of anemia in CKD patients essentially consisted of blood transfusions and androgen therapy. Transfusions improved hemoglobin, leading to improved symptoms and quality of life. Testosterone enanthate was also regularly used in conjunction with blood transfusions in ESRD patients. Its mechanism of action was thought to be the induction of endogenous EPO production, sensitization of progenitor to the effects of EPO, and increased RBC survival [17–20]. Its use was limited due to its side-effect profile, including acne, virilization, peliosis hepatis (the formation of blood-filled cavities in the liver), priapism, sexual dysfunction [21], liver function abnormalities, and the risk of hepatocellular carcinoma.

Complications of blood transfusions are manifold and include, but are not limited to, transmission of infection, both bacterial and viral, immune and allergic transfusion reactions, including transfusion-related acute lung injury and anaphylaxis, iron overload, volume overload, and hyperkalemia [22–27]. The last two, as expected, are more frequently encountered in the CKD and ESRD population. This was highlighted in a recent prospective study following 7829 patients with CKD 4 and 5, non-HD patients hospitalized with a diagnosis of hyperkalemia or heart failure. Transfusions increased the risk of hyperkalemia sixfold and heart failure by nearly fourfold [28]. Blood transfusions also have a negative impact on sensitization to new antigens, ultimately resulting in a prolonged wait on the transplant list for a renal allograft—a life-prolonging therapy! [29] Based on data from the 2010 United States Renal Data System, transfused patients were three times more likely to be highly sensitized than those who never received a transfusion. These data translate into a dramatically longer wait time, with those unexposed having about a 2.5-year wait, while those highly sensitized had a wait that was greater than 5 years [30].

Iron Therapy

Iron replacement is a key component of anemia management in CKD. Over 30 years ago, it was recognized that iron depletion was an important contributor of anemia in

Table 6.1 Comparing oral to intravenous iron formulations [37–41]

	Oral agents	Intravenous agents
Most common agents	Ferrous fumarate	Ferric gluconate
	Ferrous gluconate	Ferumoxytol
	Ferrous sulfate	Iron sucrose
	Polysaccharide-iron complex	
Side effects	Gastrointestinal (metallic taste, flatulence, constipation, diarrhea, abdominal pain, green stool, etc.)	Hypersensitivity reaction (mostly at injection site, rarely anaphylaxis)
	Itching	Dizziness
		Headaches
		Itching
Comparison	Inexpensive	Costly
	Frequent dosing	Infrequent dosing
	Poor compliance	Better compliance
	Safer	More efficacious and reliable

patients with CKD. Supplementing iron to these patients' medical therapy often improved, if not corrected their anemia [18, 31–33]. Furthermore, insuring that iron stores are replete is essential for the proper response to erythrocyte-stimulating agents [34]. Safety, cost, and ease of administration of oral iron may make it superior to the intravenous formulations; however, efficacy and tolerability of IV iron currently make it the mainstay for iron replacement in CKD (Table 6.1) [35, 36].

Currently, one of the major limitations to the use of IV iron is the theoretical potential for exacerbating bacterial infections and/or bacteremia. This hypothetical risk stems from the fact that iron excess in vitro catalyzes multiple essential steps in bacterial cell metabolism, growth, DNA/RNA replication, and protection from free radical killing, as with inhibiting the immune system [42–44]. States of iron overload, either primary or secondary, have been associated with higher rates of bacterial infections and bacteremia [45]. Recent data looking directly at the impact of iron infusion on the infectious rates of dialysis patients suggest it may be safe in the setting of active bacterial infection [46, 47]. Nevertheless, the data compiled thus far is retrospective and correlational and no randomized controlled data exist looking directly at the impact of infusing IV iron in the setting of culture-proven bacteremia, in patient who may otherwise benefit from iron supplementation.

Pharmaceutical Therapy

In its original phase III trial in 1989, recombinant human erythropoietin (rHuEPO) demonstrated an impressive ability to improve hematocrit, raising hematocrit from 22.3 to 35% in 12 weeks in greater than 97% of hemodialysis patients, eliminating the need for transfusions, and in patients with iron overload, decreased serum ferritin by nearly 40% [48]. Consequently, there was a dramatic increase in the average hemoglobin of all dialysis patients. Transfusions decreased by greater than two

Table 6.2 Comparison of different ESAs

Formulation	Half-life (h)	Dosing (days)	Modification to epoetin alfa	Disadvantage
Epoetin Alfa	7–8	2–3	–	Frequent dosing
Darbepoetin	25	7–14	Hyperglycosylated	
C.E.R.A.	130	14–28	Pegylation	
Peginesatide	25	28	NOT epo-based	Hypersensitivity

times from 1992 to 2005, with most of the drop observed in the first 5 years after the introduction of rHuEPO, nearly eliminating severe anemia as a major cause of death in ESRD patients [49]. Encouraged by these data, physicians began using suprath-erapeutic doses, in an attempt to normalize hemoglobin, the consequences of which are discussed later. Currently, there are multiple formulations of ESAs (Table 6.2).

The history of the development of rHuEPO deserves some focus, as the process was unique in its time. In 1985, after isolating and cloning the gene for human erythropoietin, researchers were able to introduce this gene into Chinese hamster ovary cells, and consequently produce biologically active human erythropoietin [50]. The gene encodes a glycoprotein consisting of a 165-amino-acid sequence with a glycosylated portion essential for its safeguard against enzymatic degradation [51]. In 1989, epoetin alfa was created and separately marketed by the pharmaceutical companies Johnson & Johnson and Amgen, who labeled their products Procrit™ and Epogen™, respectively. In 1991, epoetin beta was introduced, and while having an identical amino acid sequence to epoetin alfa, its carbohydrate moiety differs in configuration and consequently its half-life [52, 53]. Despite its longer half-life, it has not gained widespread use in the United States. Pure red cell aplasia is an uncommon disorder associated with the absence of red cell precursors, low reticulocyte count, and severe anemia. This disease is generally idiopathic but in a few rare situations, it has been associated with the formation of autoantibodies to erythropoietin in the setting of ESA use. The vast majority of these cases were in association with epoetin beta, and the European formulation of epoetin alfa, Eprex™ manufactured by Janssen-Cilag.

One widely accepted, extended release formulation is darbepoetin alfa, which is a hyperglycosylated epoetin alfa [54, 55]. In fact, with its 25.3 h half-life and once-weekly schedule, it has become the predominant ESA for the treatment of CKD patients in the outpatient setting [56]. Darbepoetin alfa is marketed by Amgen under the trade name Aranesp™.

Methoxy polyethylene glycol-epoetin beta, a continuous erythropoietin receptor activator (Micera™), was created with an even longer half-life of over 130 h, by the adding polyethylene glycol to epoetin beta [57]. With a once-monthly dosing, Micera™ can have a significant cost reduction of 59% [58].

All ESAs mentioned thus far have relied on adding varying types of carbohydrate molecules to the parent peptide in order to deliver the original erythropoietin molecule in a stable vehicle. Peginesatide, developed in March of 2012, is a peptide ESA that has no sequence homology with erythropoietin but a similar intracellular

signaling cascade [59, 60]. Its efficacy was confirmed in CKD patients in the PEARL 1 and 2 trials, but these trials demonstrated significantly higher cardiovascular end points as compared to darbepoetin, consequently this medication was approved only for use in dialysis patients [61]. Its lack of sequence homology with erythropoietin made it an ideal agent in the setting of anemia due to erythropoietin-induced antibody-mediated pure red cell aplasia, and in fact, peginesatide was used on 14 of these patients with positive results [62]. Unfortunately, post-market safety surveillance indicated a high rate of hypersensitivity reactions with the initial administration of the drug, including anaphylaxis and death. In 2013, peginesatide was withdrawn from the market [63].

With all the above available erythropoiesis-stimulating agents, as well as biosimilar formulations, the principal ESAs in use in the U.S. remains epoetin alfa and its hyperglycosylated counterpart darbepoetin alfa.

There remains an ongoing debate as to which target hemoglobin should these agents be used to achieve. There is no doubt that these agents can reliably and effectively raise hemoglobin, the open question is; where is the balance between clinical benefit and safety. If, for example, raising hemoglobin enables the patient to avoid the ischemic cardiac penumbra and the side effects of blood transfusion, then in theory, normalizing hemoglobin should be the goal. Indeed, this was the predominant belief in the late 1990s and early 2000s.

But, initial doubts about normalizing hemoglobin were the first cast in 1998 with the Normal Hematocrit Study [64]. This trial looked at 1233 hemodialysis patients with cardiac disease who were randomized to either partial or complete correction of hemoglobin, with epoetin alfa. Surprising at the time, the study was terminated early due to safety signals in the normalized hemoglobin group. While there were more events in the normal hematocrit group ($N = 202$ vs. 164 in the partial correction group), the difference was not clinically significant. However, this was the first trial to suggest harm at higher hemoglobin targets. Although this study was conducted on dialysis patients, one may, inaccurately, be tempted to generalize these results to the patient with CKD.

The first direct evidence that higher hemoglobin targets may be detrimental to CKD patients came from the CREATE and CHOIR trials [65, 66]. In the CREATE trial, 603 CKD patients were randomized to either a target hemoglobin of 10.5–11.5 g/dL or 13–15 g/dL, using epoetin beta. After a 3-year follow-up, the need for dialysis was significantly greater in the patient with the higher hemoglobin target. However, there was also a significant improvement in the quality of life measure in the same.

The CHOIR trial, similarly conducted, looked at the frequency of cardiovascular events in 1432 CKD patients randomized to either a full or partial correction of hemoglobin, using epoetin alfa. The target hemoglobin was 13.5 g/dL in the full correction group as compared to 11.3 g/dL for the partial correction cohort. At 16 months, the trial was stopped prematurely when significantly more patients ($N = 125$) in the complete anemia correction group had more cardiovascular events as compared to the partial correction group ($N = 97$).

Finally, further confirmation of serious safety signals appeared in the TREAT trial [67]. This was an international effort to accurately and concisely answer the question whether normalizing hemoglobin with ESAs was harmful to CKD patients or beneficial. With the largest cohort to date, 4038 CKD patients with diabetes were randomized to either hemoglobin of 13 g/dL or placebo, using darbepoetin alfa. This placebo group received "rescue" darbepoetin when hemoglobin dropped below 9 g/dL. With a 29-month follow-up, there was no difference in the frequency of mortality or cardiovascular event rate in both groups. There was, however, a significantly higher rate of cerebral vascular events in the higher hemoglobin cohort as compared to the lower hemoglobin group. There was also a higher rate of venous thrombo-embolic events (deep vein thrombosis and pulmonary emboli) in the higher hemoglobin group and a trend toward higher mortality in patients with a history of malignancy. This trial ended any debate as to whether higher hemoglobin goal (>11 g/dL) achieved with ESAs was safer than rescue ESA use when hemoglobin dropped below 9 g/dL.

ESA Side Effects

One of the most commonly encountered side effects of ESA is hypertension. An increase of over 10 mmHg in diastolic blood pressure was encountered in as much as 35% of the patients enrolled in the original phase III trial of erythropoietin. Pooled data suggest that hypertension develops in about 20–30% of patients as a consequence of erythropoietin use [68]. There are many mechanisms by which ESAs can elevate blood pressure. These mechanisms include: the direct effect increasing hematocrit, changes in production or sensitivity to endogenous vasopressors, alterations in vascular smooth-muscle ionic milieu, dysregulation of production or responsiveness to endogenous vasodilatory factors, a direct vasopressor action of EPO, and finally arterial remodeling through stimulation of vascular cell growth [69]. The dominant mechanism seems to be an EPO-induced atrial hypertension with a dose-dependent increase in hypertension rate with the administration of EPO [70, 71].

Another commonly encountered adverse effect related to the use of ESAs is thrombosis. The etiology is mostly due to increasing viscosity brought on by increased hematocrit but also through a hematocrit-independent induction of platelet activation [72]. In dialysis patients, this translates into higher rates of cerebral vascular events, myocardial infarction, and graft thrombosis [67, 73]. Broadly, the rate of thrombosis was found to be approximately six times more frequent in patients receiving rHuEPO than those not receiving this therapy [74].

An additional untoward effect of ESA agents is the impact on vascular endothelium. Erythropoietin is associated with a direct vasoconstrictive effect on both the renal and mesenteric blood vessels. Additionally, stimulation of the V2 receptors can lead to an enhanced cell proliferation, and induction of proto-oncogenes [75, 76].

Dosing and Administration

Erythropoiesis-stimulating agents can be administered intravenously or subcutaneously, with equal efficacy. Most CKD patients receive the subcutaneous route due to ease of administration and a slightly longer half-life.

Current FDA regulations recommend ESA use only as a rescue treatment for hemoglobin <9 g/dL, predominantly in order to avoid complications related to transfusion and help alleviate anemia-induced clinical symptoms. Due to the substantial body of literature demonstrating significant morbidity and mortality in using these agents at higher hemoglobin targets (>11 g/dL) and an abundance of side effects.

The KDIGO anemia guidelines released in 2012 recommend tailoring treatment with ESAs for each patient by balancing the potential benefit of reducing transfusions and anemia-related clinical symptoms against the potential risk associated with ESA use (e.g., cerebral and cardiovascular events, vascular access loss, hypertension) [77]. Those guidelines refer to a large body of literature with a moderate quality of evidence suggesting that a modest hemoglobin target between 9 and 11 g/dL avoids the risk of repeat blood transfusion and poor quality of life while minimizing the reported risks of higher hemoglobin.

For all CKD patients with anemia requiring iron supplementation, a goal of transferrin saturation (TSAT) no greater than 30% and ferritin level no greater than 500 ng/mL should be achieved. Although most patients with >100 ng/mL (or TSAT >20%) will have adequate bone marrow stores, supplementing those patients may result in either improved hemoglobin level or decrease in ESA dose. There is evidence to suggest that patients with ferritin level as high as 1200 ng/mL can still benefit from IV Iron, but the KDIGO guidelines do not recommend it [78]. They also give a non-graded recommendation to avoid giving IV iron to patients with active infections.

Newer Agents

Much has been done to explore novel therapies that will correct anemia and avoid cardiovascular, cerebrovascular, and malignant side effects experienced with the use of ESAs. Several agents are in the process of evaluation which include hypoxia-inducible factors stabilizers, hepcidin inhibitors, and GATA-2 inhibitors.

Hypoxia-inducible factors (HIF) are transcription factors that upregulate the expression of the erythropoietin gene. Under normal conditions, propyl hydroxylase (PHD) uses oxygen as a cofactor to hydroxylate HIF-α. This enables Von Hippel Lindau (VHL) tumor suppressor protein to target HIF-α and deliver it for proteasomal degradation. HIF propyl hydroxylase inhibitors prevent HIF from being hydroxylated and ultimately enzymatically degraded, which enables HIF to travel to the nucleus and induce transcription of the EPO gene (Fig. 6.1). In July 2015, the results of phase 2 trials with Roxadustat™, an oral HIF propyl hydroxylase inhibitor, were published. Ninety-six patients were compared to placebo; at 1.5 and 2 mg/

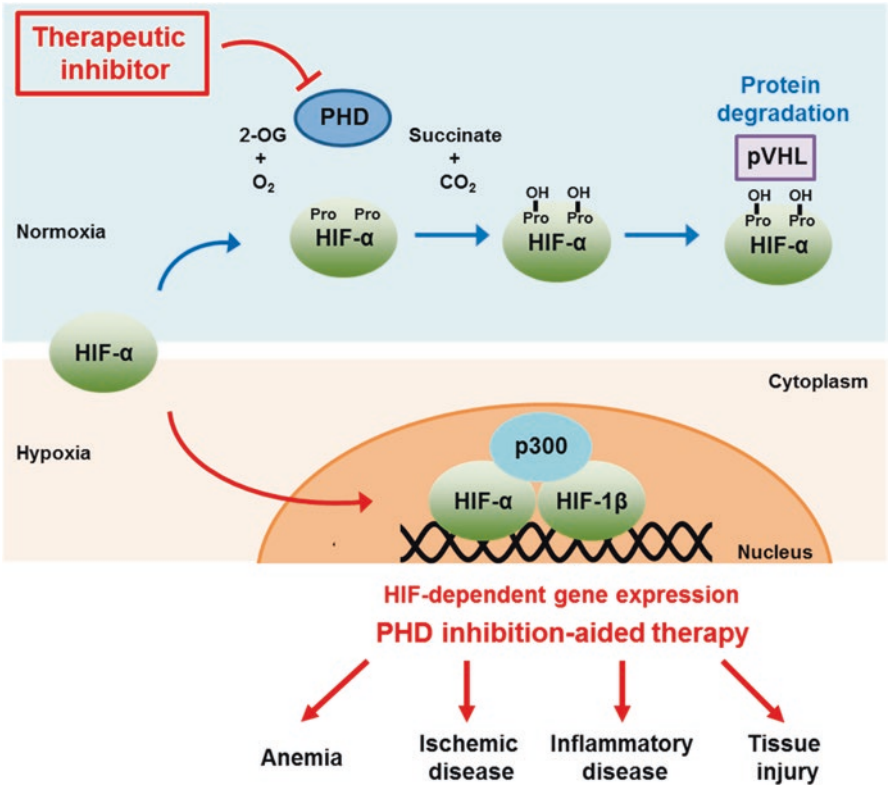

Fig. 6.1 Mechanism of action of HIF stabilizers (also referred to as PHD inhibitors). Inhibition of propyl hydroxylase leads to stable hypoxia-inducible factors which can ultimately travel to the nucleus and induce epoetin production. *PHD* propyl hydroxylase, *HIF* hypoxia-inducible factor, *VHL* Von Hippel Lindau. (Reprinted from Kim SY, Yang EG. Recent advances in developing inhibitors for hypoxia-inducible factor prolyl hydroxylases and their therapeutic implications. Molecules. 2015;20(11):20,551–20,568. With permission from MDPI AG (Basel, Switzerland). Under the Creative Commons License: https://creativecommons.org/licenses/by/4.0/

kg dosing, Roxadustat™ induced a transient elevation in erythropoietin and reduced hepcidin expression, translating into a dose-dependent hemoglobin rise. The side-effect profile was similar to placebo [79]. One potential disadvantage of this therapy is in the induction of a multitude of other hypoxia-induced genes, such as those of vascular endothelial growth factor which may potentially lead to tumor growth and worsening diabetic proliferative retinopathy, although to date no evidence has been of this [80, 81].

Additionally, hepcidin is the major regulator of iron storage in the setting of active inflammation. Interleukin-6 induces its (mostly) hepatic production. Hepcidin regulates the amount of iron absorption by the duodenal enterocytes [82]. It also controls iron release from these and other cells including hepatocytes and macrophages in the reticuloendothelial system [83]. Hepcidin binds to ferroportin (a

transmembrane iron exporter) and internalizes it, thereby preventing the iron release from these cells [84]. While still in early experimental phase, hepcidin antagonism as an anemia treatment has been researched. Monoclonal antibodies against hepcidin as well as direct mRNA-based antagonists of hepcidin have been developed [85]. Concerns about this modality over potential infectious risk are appropriately placed since hepcidin has an innate antimicrobial effect.

The GATA (guanine-adenine-thymine-adenine) transcription factors are a family of transcription factors characterized by their ability to inhibit EPO gene expression through binding the GATA DNA sequence. Of particular importance is the GATA-2 transcription factor that has experimentally been inhibited by two experimental inhibitors K-7174 and K11706, leading to improved anemia markers in experimental animal models [86, 87]. Compound K-7174 s mechanism of action has been linked to inhibition of hepcidin [88].

With multiple experimental and novel therapeutic agents, only the HIF stabilizer has made a significant leap into applicable medicine with the phase 4 trials currently underway.

Regulatory Impact on Anemia Management

While this chapter stresses the importance of evidence in driving decisions to manage CKD patients with anemia, the impact of funding and policy on actual medical practice cannot be ignored. It is important to explore how policy has ultimately had such an invasive but integral regulatory impact on medical practice. In 1967, the Gottschalk report argued the need for Medicare reimbursement for dialysis and transplant as established treatments and not. It was not until October 30th of 1972 that President Richard Nixon signed an amendment to social security payment extending Medicare coverage to those with chronic kidney disease. Finally, these patients could put their concerns about the financial burdens of their treatment behind them. With the advent of epoetin, in 1989, there was concern that, as a separately billable pharmaceutical, that there may a conflict between the financial benefit to maximize its use and known clinical benefit(s). In 1994, Medicare expenditure for epoetin therapy alone exceeded 700 million dollars [89]. However, the increasing expansion in ESA dosing did not translate into higher hemoglobin levels, and in 1993, concerned about the increase in financial expenditure, the Centers for Medicare and Medicaid Services (CMS) initiated the National Anemia Cooperative Project [90]. Published in 1996, this was an expert opinion-based anemia treatment algorithm that emphasized target hematocrit >30% with emphasis on iron replacement [91, 92]. In 1997, the National Kidney Foundation (NKF) published the first evidence-based guideline—Dialysis Outcomes Quality Initiative (DOQI) anemia guideline.

In 2007, following the publication of both the CHOIR and CREATE trials, the food and drug administration (FDA) issued a black box warning for ESAs further limiting its use and consequently cost. The FDA advised health care professionals to use the lowest dose that will gradually increase the hemoglobin concentration to

the lowest level sufficient to avoid the need for red blood cell transfusions. The FDA also warns that ESAs increased the risk for death and for serious cardiovascular events when administered to target hemoglobin of greater than 12 g/dL.

Considering regulatory impact and the evidence against driving higher hemoglobin levels with erythropoietin, the current trend in the management of CKD patients with anemia is essentially geared at minimizing transfusions with target hemoglobin above 9 g/dL. The ultimate goal is always to tailor treatment for each patient. This translates into minimizing the impact of low hemoglobin on the patient's quality of life (i.e., improving dyspnea, fatigue, sleep disturbances, and cognitive decline) while concomitantly, avoiding the use of exaggerated or supratherapeutic doses of ESAs in order to achieve an idealized hemoglobin level.

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Anemia in Liver Disease

7

Yuval A. Patel, Matthew R. Kappus, and Andrew J. Muir

Introduction

A wide spectrum of different types and causes of anemia are associated with liver disease (Table 7.1). Etiologies include portal hypertension-related acute and chronic gastrointestinal hemorrhage, which is predisposed by impairment in blood coagulation seen in liver disorders. Liver disease as well is associated with alterations in red blood cell membranes and hypersplenism, which contribute to hemolysis. Particular types of liver afflictions are associated with anemia, such as hemolytic anemia in Wilson's disease, viral hepatitis-associated aplastic anemia, anemia secondary to multiple etiologies in alcoholic liver disease, and ribavirin-induced hemolysis in the setting of treatment for hepatitis C. Lastly, nutritional deficiencies related to liver disease are also noted to play a key role in defective erythropoiesis, such as folic acid deficiency in alcoholic liver disease. Figure 7.1 provides a flowchart to help guide evaluation of anemia in patients with liver disease.

Portal Hypertension-Related Anemia

The development of portal hypertension in liver disease predisposes to both acute and chronic gastrointestinal bleeding. In cirrhosis, or end-stage liver disease, portal hypertension may develop as a consequence of increased resistance to blood flow through the liver secondary to architectural distortion from fibrous tissue and regenerative nodules, and is defined by a hepatic venous pressure gradient (HVPG) >5 mmHg [1]. Active intrahepatic vasoconstriction also plays a role, contributing 20–30% of intrahepatic resistance, mostly due to reduced endogenous production of

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Table 7.1 Etiologies of anemia in chronic liver disease

Acute blood loss anemia (portal hypertension-related)
Esophageal varices
Gastric varices
Rectal varices
Chronic blood loss anemia
Portal hypertensive gastropathy
Portal hypertensive enteropathy
Portal hypertensive colopathy
Gastric-antral vascular ectasia
Acute hemolytic anemia
Diffuse intravascular coagulation
Drug-induced hemolysis
Chronic hemolytic anemia
Splenic sequestration
Hemolytic anemia associated with copper accumulation
Bone marrow insufficiency
Chronic alcohol abuse
Viral hepatitis-induced aplastic anemia
Drug-induced
Pegylated interferon therapy
Ribavirin therapy
Micronutrient and vitamin deficiencies
Iron deficiency
B12 deficiency
Folate deficiency
Vitamin K deficiency, leading to coagulopathy

nitric oxide [1]. Portosystemic collaterals, most commonly gastroesophageal varices, are formed in an attempt to decompress the elevated pressures in the portal system. This collateralization is insufficient given a concurrent increase in portal venous inflow from splanchnic arteriolar vasodilation as well as higher resistance noted in the collaterals [2]. In effect, portal hypertension is thought to occur as a result of both an increase in portal resistance as well as portal inflow.

Gastroesophageal varices are the clinically most relevant portosystemic collaterals as their rupture leads to acute anemia through variceal hemorrhage, the most common fatal complication of cirrhosis [1]. These collaterals are present in about 50% of patients with cirrhosis, and correlate with increased severity of liver disease in regards to prevalence and risk of hemorrhage [1]. Variceal hemorrhage occurs at an annual rate of 5–15% with the most important predictor being the size of the varices, with large varices carrying the highest risk at 15% [1]. Acute hemorrhage may cause severe hypovolemia and subsequent secondary iron-deficiency anemia,

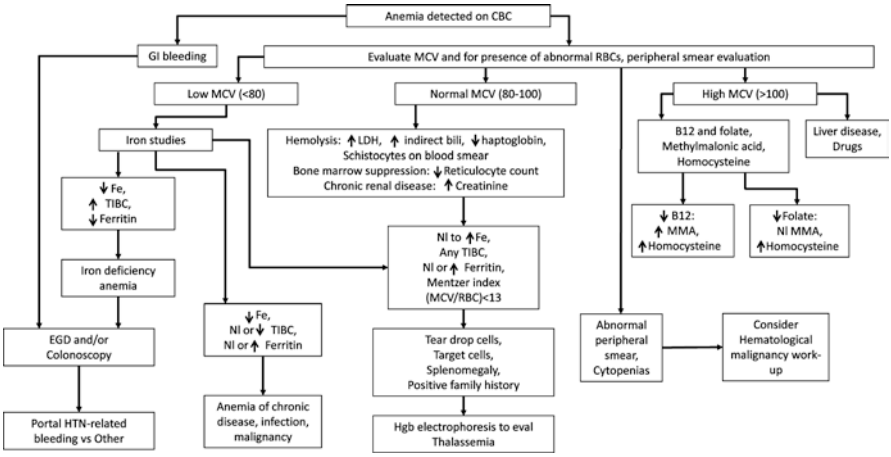

Fig. 7.1 Anemia algorithm

especially in recurrent bleeding due to depletion of iron stores [3]. Variceal hemorrhage is an emergency and requires prompt volume resuscitation and blood transfusion to maintain a hemoglobin of ~8 g/dL to correct the acute anemia [1]. Medical therapy includes administration of vasoactive drugs such as terlipressin or octreotide [3]. Endoscopic therapy includes band ligation of esophageal varices as well as tissue adhesive obturation of gastric varices [1]. Details regarding the management and treatment outcomes of varices are beyond the scope of this chapter.

Patients with cirrhosis may develop chronic bleeding throughout the gastrointestinal tract from portal hypertensive gastropathy (PHG), portal hypertensive enteropathy, portal hypertensive colopathy, or gastrointestinal vascular ectasia (GAVE). Chronic gastrointestinal bleeding refers to a reduction in hemoglobin of 2 g/dL within a 6-month period without evidence of acute bleeding or non-steroidal anti-inflammatory drug use [4], though other definitions including the presence of iron-deficiency anemia with a positive fecal occult blood test have also been used [5]. These patients with chronic gastrointestinal blood loss may similarly develop secondary iron-deficiency anemia. Among these etiologies, PHG is the most common, and is recognized endoscopically as a mosaic-like pattern called "snake-skin mucosa" with or without red spots [6]. Portal hypertensive enteropathy and colopathy describe similar changes in the small bowel and colonic mucosa, respectively. PHG is most commonly found in the proximal stomach (fundus and body) [6]. The prevalence of PHG in patients with cirrhosis ranges from 20 to 98%, with this variation in the literature secondary to multiple factors including lack of uniform diagnostic criteria and classification, as well as different patient populations [4]. Several studies demonstrate a higher prevalence of PHG in patients with more severe liver disease and other complications of portal hypertension, such as esophageal varices [4]. Some studies suggest that the prevalence of PHG may increase once esophageal varices are obliterated, though this is controversial [6].

Many patients with PHG are asymptomatic, though up to 60% exhibit symptoms related to chronic gastrointestinal bleeding [4]. Classification of PHG is done based on severity, with most experts recommending a two-category system [7]. Mild is defined as only noting the snakeskin mosaic pattern; severe is defined as snakeskin mosaic pattern with flat or bulging red or black spots, or related to acute GI bleeding [7]. Acute GI bleeding is fairly uncommon in PHG, ranging from 2 to 12%, and is identified endoscopically [4].

Treatment options are targeted according to the presentation of the patient in terms of the rate of bleeding and related symptoms. It is not uncommon to identify asymptomatic incidental PHG during esophageal varices screening in patients with cirrhosis. At this time, primary prophylaxis of GI bleeding in patients with PHG has not been assessed thoroughly and is not recommended routinely. However, in patients with severe PHG but without varices or active bleeding, prophylaxis with non-selective β-blockers may be considered, though this approach is controversial [4]. For patients where chronic blood loss is of concern, such as those that present with PHG and iron-deficiency anemia, it is important to exclude other causes of iron-deficiency anemia. Iron replacement therapy should be started in all patients with iron-deficiency anemia caused by PHG, either oral preparation or intravenous dependent on the iron deficit [4]. The use of nonselective β-blockers may reduce blood losses in patients with a concern of chronic bleeding secondary to PHG, and therefore should be implemented in this circumstance [5]. For patients with acute GI bleeding secondary to PHG, again, it is essential to exclude other causes of bleeding. Early and aggressive generalized support is essential including blood transfusion to a hemoglobin goal of ~8 g/dL, antibiotics such as IV quinolone or third-generation cephalosporin for spontaneous bacterial peritonitis prophylaxis, and vasoconstrictor therapy with terlipressin, somatostatin, or somatostatin analog such as octreotide [4]. Once the diagnosis of actively bleeding PHG is confirmed endoscopically, argon plasma coagulation (APC) may be applied through an endoscope in situations where a single or a limited number of culprit oozing lesions are apparent on a patient by patient basis [4]. Initiation of non-selective β-blockade should also be considered given evidence of control of acute bleeding within 3 days in 13 (93%) of 14 patients in one small study [8]. Vasoconstriction with octreotide infusion for 48 h has also demonstrated efficacy to stop bleeding [9]. Patients with refractory chronic bleeding due to PHG include those with the disease that remain transfusion dependent despite iron therapy and β-blockade [4]. Patients with refractory acute bleeding due to PHG include those that have recurrent hematemesis despite treatment, such as with vasoactive medications, and/or a 3 g hemoglobin decline without transfusion or an inadequate hemoglobin rise after transfusion [10]. In these cases, rescue therapies such as shunt surgery or TIPS may be considered, as both have been shown to be effective [10]. Although repeated application of APC appears as an attractive option, there are currently insufficient data to recommend it, though this can be considered on a patient by patient basis [4].

One of the major differential diagnoses for acute or chronic GI bleeding that may appear similar to PHG is GAVE, or “watermelon stomach” [4]. GAVE also appears as flat red spots, but in streaks of erythema that appear to be emanating from the

pylorus [4]. GAVE is thus located in the distal stomach (antrum), whereas PHG is located in the proximal stomach (fundus and body), which may help distinguish the disorders [4]. GAVE similarly typically presents with chronic GI blood loss and iron deficiency and is a relatively rare cause of acute hemorrhage in patients with cirrhosis [4]. It is also seen in other diseases such as in patients with chronic renal disease, bone marrow transplantation, as well as autoimmune disease such as scleroderma [4]. Interestingly, there does not seem to be a distinct relationship between GAVE and either portal hypertension or cirrhosis, but it is encountered in both clinical situations [4]. The differentiation between GAVE and PHG is important given the different treatment paradigms. GAVE is treated with endoscopic thermoablation with APC as the method of choice, as this has been shown to be effective across multiple studies [11]. Large case series using APC have reported efficacy >90% with no further need for blood transfusions with repeated sessions every 2–6 weeks until adequate eradication [11]. Despite the efficacy of APC in the short term, approximately 30–60% of cases may have recurrent bleeding in the long-term, as thermal treatment effects the superficial epithelium while GAVE commonly involves deeper structures such as in the submucosa that likely predispose to recurrent bleeding [11]. Band ligation has also been used to treat GAVE, as this method may more reliably eradicate the deeper vascular structures, and in small studies has been shown to be at least as effective as APC [11]. More recent studies have also evaluated radiofrequency ablation for the treatment of GAVE through paddle applicators with promising results [11]. Given the nature of the method, uniform tissue penetration is easier to obtain with the RFA paddle catheter and operator-dependent effects are minimized. Cryotherapy with nitrous oxide has also been successfully utilized to treat GAVE; a study by Cho and colleagues evaluated 12 patients, eight of whom had failed prior APC treatment, and achieved complete or partial response in all patients without complications [12]. As a last resort, surgical antrectomy has also been shown to be effective and safe for refractory GAVE for otherwise well-compensated cirrhotic patients that fail repeated endoscopic management [11].

Hypersplenism resulting from portal hypertension represents another mechanism of anemia in liver patients. This portal hypertension may be secondary to cirrhosis, or liver disease complicated by portal vein or splenic vein thrombosis. Extravascular hemolysis occurs in the spleen due to macrophages in the setting of red blood cell stasis and trapping [13]. Interestingly, it is the spleen size and not the degree of portal hypertension that determines the extent of erythrocyte sequestration [13]. The degree of anemia that results from this hemolysis mechanism is variable, and is dependent on the ability to counterbalance red blood cell destruction with an increase in production. The main treatment for anemia secondary to hypersplenism depends on the underlying cause. For hypersplenism secondary to cirrhosis with adequate hepatocellular reserve and splenic vein thrombosis, splenectomy may be considered in severe cases after medical efforts are exhausted, though caution is necessary given significant surgical risk [14]. In addition, partial or complete splenic artery embolization has been utilized to treat hypersplenism causing severe thrombocytopenia, and is generally a safer option [15].

Alterations of Red Blood Cell Membrane

Acquired alterations of the red blood cell membrane due to liver disease are associated with variable degrees of hemolysis. The four major variant red blood cell types that result from chronic liver disease include acanthocytes (spur cells), codocytes (target cells), echinocytes (burr cells), and stomatocytes. These alterations of red blood cell membrane occur due to changes in the concentration and ratio of cholesterol and phospholipids in the cell membrane [16]. Of these, acanthocytes are typically associated with the greatest degree of hemolysis. When occurring in abundance this is referred to as spur cell anemia [17]. This occurs because the lipid composition of the red blood cell membrane of these cells renders them more susceptible to splenic entrapment and destruction, and is typically found in alcoholic cirrhosis, though has been noted in other etiologies of cirrhosis and in neonatal hepatitis [17]. The early splenic consumption of red blood cells can be quite significant; a study by Jandl in 1955 found that red blood cell lifespan was diminished by about 50% in patients with cirrhosis [18]. On the other hand, target cells do not appear to have a diminished lifespan, and may actually be protected from the otherwise splenic conditioning process that leads to consumption [16]. Echinocytes have serrated edges over their entire cell surface and develop this distinct shape due to abnormal high-density lipoprotein binding on membrane receptors; these cells are associated with hemolysis to less of a degree [19]. Stomatocytes, produced by bending or folding of the plasma membrane inwards secondary to membrane compositional changes, appear with mouth-shaped areas of central pallor [20]. This red blood cell variant is also implicated in hemolysis to varying degrees. The resulting hemolytic process associated with many of these acquired alterations of red blood cell membrane in liver disease depend both upon the extent of membrane metabolic changes as well as the degree of hypersplenism and splenic macrophage activity.

Viral Hepatitis-Associated Aplastic Anemia

Aplastic anemia is a rare disorder characterized by pancytopenia from a loss of pluripotent hematopoietic stems cells in the bone marrow in the absence of infiltrative disease [21]. Among causes of aplastic anemia, viral hepatitis is an uncommon cause, encompassing 2–5% of documented aplastic anemia cases in the West and a slightly higher proportion in the Far East [22]. This disease typically afflicts young men and adolescent boys within 2–3 months of acute hepatitis and is usually fatal if left untreated due to rapid and severe marrow failure [22]. Viral hepatitis-associated aplastic anemia cases secondary to hepatitis A, hepatitis B, hepatitis C, hepatitis E, cytomegalovirus, Epstein-Barr virus, parvovirus B19, echovirus, GB virus C, transfusion transmitted virus, and SEN virus have been reported; however, the etiology for most cases remains a mystery [23]. In this patient group without identified virus most also lack toxins, drugs, or blood transfusions exposure [24]. The suggested pathogenesis is due to the induction and activation of T-lymphocytes from viruses and/or antigens within the bone marrow of afflicted patients through the release of

interferon-ɣ and potentially other cytokines, ultimately resulting in the apoptotic death of marrow progenitor cells [21]. Furthermore, the development of aplastic anemia after liver transplantation for fulminant non-A, non-B, non-C hepatitis in young patients is not uncommon; the cause of fulminant seronegative hepatitis in such cases is unknown [25].

The clinical presentation of viral hepatitis-associated aplastic anemia is variable, including symptoms and signs related to pancytopenia, such as fatigue and pallor due to anemia, hemorrhagic complications due to thrombocytopenia, and infectious complications due to neutropenia. The diagnosis is suggested by laboratories including the complete blood count revealing pancytopenia and absolute reticulocytopenia. Bone marrow biopsies typically clinch the diagnosis, revealing marked hypocellularity of precursor cells with residual cells appearing normal, as well as absence of malignant infiltrates [26].

Treatment for the condition requires urgent evaluation and hematology consultation. Blood and platelet transfusions should be utilized selectively to limit sensitization, and require irradiation, CMV negativity, and should not be from family members. The two major therapeutic options include hematopoietic stem cell transplantation and immunosuppressive therapy, with hematopoietic stem cell transplantation from an HLA-matched sibling donor being the treatment of choice [23]. A recent meta-analysis found that the mean survival at 5 years for hematopoietic stem cell transplantation was 82% [23]. The same meta-analysis calculated the mean response rate to immunosuppressive therapy at 70% [23]. The details regarding both treatment modalities are beyond the scope of this chapter.

Alcoholic Liver Disease and Anemia

Alcohol consumption is implicated in the development of chronic liver disease and may contribute to anemia through a myriad of effects in the liver patient. Excessive alcohol use may predispose to hemorrhagic gastritis or peptic ulcer disease, and in liver patients that develop portal hypertension from severe alcoholic hepatitis or from alcoholic cirrhosis, may cause portal hypertension related blood loss from bleeding mechanisms described earlier in this chapter. Anemia secondary to vitamin B12 deficiency and/or folate deficiency often found in alcoholic liver disease is described later in the nutrient deficiency portion of this chapter. Of note, the macrocytosis seen in excessive alcohol use is not always related to folate and vitamin B12 deficiency, as this phenomenon is also noted in the absence of these deficiencies [27]. The treatment of alcohol-induced macrocytosis is abstinence. Alcohol is also known to have a direct toxic effect on the bone marrow, resulting in diminished erythropoiesis, which is a further burden of anemia in the liver disease patient [28]. Hemolysis may occur in patients with advanced chronic liver disease due to alcohol through portal-hypertension-related hypersplenism discussed earlier in this chapter. Spur cell anemia has also been noted in patients with alcoholic cirrhosis; this type of hemolytic anemia described previously is caused by a red blood cell membrane defect resulting in spiculated erythrocytes leading to shortened survival [29]. Zieve's

syndrome is an uncommon form of acute Coombs' negative hemolytic anemia that was first described as a triad of jaundice, hyperlipidemia, and alcoholic steatohepatitis in the alcoholic liver disease patient [30]. This acute syndrome is a relatively rare phenomenon first described by Leslie Zieve in 1957 [30]. Patients are noted to have right upper quadrant pain, low-grade fever, and loose stools in addition [30]. Zieve postulated that hyperlipidemia, particularly lipid lysolecithin, was responsible for the membrane instability that resulted in hemolysis [30]. At this time, the exact underlying mechanism for Zieve's syndrome is unknown.

Wilson's Disease and Hemolysis

Wilson's disease is a rare autosomal recessive disorder of copper metabolism that can cause severe liver disease secondary to hepatic copper accumulation and can also more rarely trigger hemolytic anemia [31]. Severe spherocytic Coombs-negative hemolytic anemia is an uncommon manifestation of the disease, occurring in about 10–15% of cases [32]. The hemolysis in Wilson's disease is due to excessive inorganic copper in the blood circulation resulting in oxidative damage to red blood cells in the setting of ceruloplasmin deficiency [31]. Acute hemolysis and acute liver failure due to Wilson's disease often concurrently occur as the initial manifestations of the disease [33]. The hemolytic anemia is less common in isolation, but may also occur as a single acute episode, recurrently in episodes, or chronically, with varying degrees of severity [34]. Management of Wilson's disease is aimed at removing accumulated tissue copper and preventing reaccumulation with lifelong use of chelator therapy, most commonly D-penicillamine [35]. Liver transplantation may be lifesaving in Wilson's disease patients with acute liver failure and is indicated in decompensated cirrhosis patients that fail medical management. Liver transplantation corrects the metabolic defect in Wilson's disease and has demonstrated excellent survival [36].

Anemia Secondary to Treatment of Viral Hepatitis

A common side effect of treatment of HCV with ribavirin and pegylated interferon is anemia. Although currently the treatment paradigms for HCV infection are rapidly changing with the expansion of direct-acting antivirals, ribavirin remains a component of the regimen for many patients. Pegylated interferon is recommended for a small number of patients at this time in countries where direct-acting antivirals are available. Ribavirin causes hemolytic anemia that is dose-dependent. Ribavirin is renally cleared with a greater risk of anemia in patients with chronic kidney disease [37]. Interferon causes bone marrow suppression and has also been associated with autoimmune hemolytic anemia [38]. In order to evaluate for the development of treatment-associated anemia, current practice dictates checking the complete blood count routinely through the course of therapy, with particularly close monitoring in patients with advanced liver disease and/or impaired renal function.

Primary management of anemia in this setting (hemoglobin <10 g/dL) is ribavirin dose reduction. Erythropoietic growth factors have also been used to limited ribavirin dose reduction and to prevent discontinuation [39]. In the evolving age of direct-acting antivirals, few studies exist that evaluate the burden of anemia in combination therapy. Of note, there are no genotypes of HCV that should be treated solely with pegylated interferon and/or ribavirin; when these medications are recommended in regimens they are used in concert with a direct-acting antiviral(s). The FISSION trial, a phase III randomized open-labeled non-inferiority study evaluating sofosbuvir plus ribavirin for 12 weeks versus peginterferon alfa-2a plus ribavirin for 24 weeks for patients with HCV genotype 2 and 3, provides evidence regarding treatment-associated anemia in the new age of direct-acting antivirals. In this study, treatment-associated anemia was more commonly listed as an adverse event in the peginterferon alfa-2a plus ribavirin group (12%) compared to the sofosbuvir plus ribavirin group (8%) [40]. For patients receiving sofosbuvir and ribavirin, 9% had a hemoglobin <10 g/dL and <1% had hemoglobin <8.5 g/dL during treatment. In comparison, for patients receiving peginterferon alfa-2a and ribavirin, 14% had Hgb <10 g/dL and 2% had Hgb <8.5 g/dL. This suggests that regimens utilizing direct-acting antivirals and ribavirin are less likely to suffer from anemia as an adverse event as compared to the older regimens of pegylated interferon and ribavirin.

Anemia Due to Nutrient Deficiencies

Muscle wasting, weight loss, and nutritional deficiencies commonly occur in settings of chronic liver disease. The underlying mechanisms include poor dietary intake (due to anorexia, altered taste sensation, nausea, and emesis), maldigestion, and malabsorption. Malabsorption can be related to concomitant pancreatic and bile acid insufficiency, bacterial overgrowth due to reduced motility, loss of protein secondary to portal hypertension, variable energy utilization, insulin resistance, and impaired protein synthesis due to cytokine-induced inflammatory responses [41, 42]. No difference in prevalence and severity of malnutrition has been observed between alcohol induced and viral-induced liver disease [43], and it is likely that there is similar nutritional risk between all etiologies of chronic liver disease. Due to the role malnutrition plays in the comorbidity of patients with chronic liver disease, the American Association for the Study of Liver Disease guidelines recommends that all patients with liver disease be screened for both protein-calorie deficiency and specific micronutrient deficiencies (i.e., vitamin and mineral deficiencies) [44]. Based on these premises, it is important to consider nutrient deficiencies in relation to anemia in liver disease.

Micronutrient Deficiency

Patients with the chronic liver disease develop some form of malnutrition that becomes more recognizable as the liver disease progresses. Cases of malnutrition can be seen in 65–100% of liver transplantation candidates [45, 46]. The liver contributes to the synthesis, storage, or breakdown of the major macronutrients groups

such as fats, carbohydrates, and proteins, as well as the major micronutrient groups including electrolytes, trace elements, and vitamins [47]. Specific micronutrient deficiencies are increasingly being recognized as manifestations of chronic liver disease, although less obvious than other signs of malnutrition such as protein wasting and sarcopenia. Aside from those micronutrient deficiencies which lead to anemia, patients with the chronic liver disease are at risk for depletion of various fat-soluble and water-soluble vitamins which produce a myriad of symptoms, signs, and complications [42]. The most recognized micronutrients which contribute most directly to anemia would be vitamin B12 (cyanocobalamin) and folate deficiencies. Indirectly contributing to anemia would be the malabsorption of the fat-soluble vitamin, vitamin K. This is used in the synthesis of clotting factors, and vitamin K deficiency usually manifests as bleeding. It is suggested that in those patients with elevated prothrombin time, vitamin K supplementation can be considered.

B12 Deficiency

Vitamin B12 deficiency is a known etiology of megaloblastic anemia. Due to the hepatic activation and transport of water-soluble vitamins like vitamin B12, cyanocobalamin (B12) has been reported in chronic liver disease. A vitamin B12 deficiency increases the concentration of total plasma homocysteine (tHcy) and methylmalonic acid (MMA). These levels have been heralded as the most sensitive and early indicators for vitamin B12 and folate status. In liver disease states ranging from non-alcoholic fatty liver disease to alcoholic liver disease, the serum tHcy levels are routinely elevated [48]. In the setting of chronic liver disease, patients have both decreased intake of vitamin B12 as well as disruption in activation and transport. While vitamin B12 deficiency must be considered, falsely elevated B12 levels have been reported in alcoholic liver disease due to the inclusion of endogenous metabolically inactive forms of cobalamin. In these cases, it may be less helpful to measure a serum B12 level. Holotranscobalamin, which is the active form of cobalamin, has been reported to be more reliable as an early marker of vitamin B12 deficiency in alcoholics [49]. In all patients with decompensated chronic liver disease and megaloblastic anemia, vitamin B12 deficiency should be promptly treated to avoid complications.

Folate Deficiency

Folate deficiency is typically associated with alcoholic liver disease due to reduced dietary intake, intestinal malabsorption, reduced liver uptake and storage, and increased urinary excretion [50]. These mechanisms of folate deficiency are common among all causes of chronic liver disease where intestinal malabsorption and reduced dietary intake are known. Certainly in patients with the alcohol-related liver disease, it is known that rates of folate deficiency are as high at 80%, and that degree of deficiency is not directly related to the severity of disease [51]. It is suspected that due to folate fortification of grain in the United States, these rates are lower today,

with rates of deficiency as low at 19% in patients with all stages of alcohol-related illness [52]. Dietary and endogenous folate plays a key role in the hepatic methionine metabolism, regulating homocysteine levels, antioxidant defense, DNA assembly, and methylation reactions contributing to gene regulation [50]. The final metabolite of folate is S-adenosylmethionine (SAM), and is processed in the liver. SAM is the methyl donor for all reactions involving methylation of DNA, histones, and proteins. In the setting of hepatic impairment, this ability to metabolize available folate is reduced. Hepatic stores of folate are also reduced. In animal studies, those animals fed large quantities of alcohol had reduced folic acid transport at the level of the jejunal brush border [53, 54]. In addition, hepatic metabolism and storage of folate within the liver is significantly reduced by alcohol consumption as evidenced in patients fed folate depleted diets who regularly consumed alcohol versus those who were alcohol-free [55]. Not only does folate deficiency play a role in chronic megaloblastic anemia, it may play an important role in increasing the risk for hepatocellular carcinoma and liver injury due to decreased methylation [56].

Other Causes of Anemia

Lastly, it is important to consider other forms of anemia that may not be related to the patient's primary liver disease. These include primary anemias that are familial or genetically inherited disorders such as alpha or beta thalassemia and sickle cell disease. One must also consider primary hematologic malignancies and dysplasias such as leukemia, lymphoma, or myeloma. Other chronic illnesses such as longstanding renal or heart disease, and human immunodeficiency virus can also lead to suppression of the bone marrow. As these are not the focus of this chapter, they are only mentioned here.

Conclusion

A large spectrum of different types and causes of anemia are associated with liver disease. Patients with liver disease are at risk for portal hypertension-related acute and chronic gastrointestinal hemorrhage and the primary cause of liver disease itself to malnutrition and micronutrient deficiencies. Patients may have greater than one etiology of anemia, and different etiologies may develop at various times in the course of the disease. Clinicians should consider the range of disorders and understand these competing etiologies in order to adequately address the anemia for each patient with the chronic liver disease.

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Anemia in the Patient with Chronic Lung Disease

8

Tim J. McMahon and Ann C. Prybylowski

Overview

In the patient with anemia of chronic disease (ACD), the treatment of choice is to treat the underlying disease. Unfortunately, effective treatments for common lung diseases like COPD/emphysema are not available, and the current state-of-the-art approach is to slow disease progression while treating symptoms. Anemia can cause symptoms that overlap with or exacerbate those in chronic lung disease, can worsen quality of life, and decreases survival; thus, a thorough search for other causes of anemia that are more easily treated is especially important. In particular, iron deficiency anemia, which can coexist with ACD, should be sought and corrected. Vigilance for and treatment of intercurrent infections, which can drive the ACD in these patients, is also warranted.

Epidemiology, Costs, and Morbidity

Anemia in COPD

Chronic obstructive pulmonary disease (COPD) is a prevalent and costly disease. Principally, but not exclusively, caused by the sequelae of cigarette smoking, COPD is increasingly recognized as a systemic disease characterized by inflammation, and not only by a single-organ problem marked by airflow limitation. A number of studies have documented convincingly that hemoglobin and hematocrit levels in COPD

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vary widely along a spectrum from extreme erythrocytosis that is linked to severe hypoxia on the one hand to anemia on the other hand. In COPD and other chronic lung diseases, the polycythemic response whereby erythropoietin induced by marked hypoxia leads to accelerated RBC production is considered to be adaptive by acting to move O_2 delivery toward a normal range. Namely, what is lacking in PaO_2 (dissolved O_2 content in the blood), with the potential to incompletely saturate hemoglobin with O_2, is compensated for by increased O_2-carrying capacity (more hemoglobin), so that O_2 delivery tends toward the normal range. The less frequently, or at least not classically, recognized anemia in COPD is accordingly poorly tolerated in general.

Many studies have attempted to determine the prevalence of anemia in COPD patients but very few agree on the actual prevalence. In a systematic review of seven studies, Yohannes and colleagues found that the prevalence of anemia in COPD ranged from 7.5 to 34% [1]. In general, Yohannes saw a relationship between anemia and increased healthcare utilization, impaired quality of life, premature mortality, and increased risk of hospitalization.

In a query of the Medicare claims database, Halpern and colleagues sought to estimate the prevalence of anemia among patients with COPD and its influence on mortality and morbidity. Among a large study population of Medicare enrollees (nearly all 65 years of age or older), anemia was present among 21%, based on ICD-9 codes or receipt of transfusions. Mortality and morbidity were substantially increased in the patients with anemia, including COPD-related indices of morbidity such as the incidence of acute exacerbations of chronic bronchitis, and the need for mechanical ventilation; and more general indices such as oxygen-dependence and rates of hospitalization. Patients with anemia secondary to nutritional or hereditary causes were excluded. They also estimated the effect of comorbid anemia on costs and health care utilization in COPD. Both claims and reimbursement, reflecting health care costs, were increased significantly by comorbid anemia in each of the several major categories including inpatient and outpatient services, durable medical equipment, nursing home or home health care, and physician services. Interestingly, the increased claims were accompanied in only a small minority of cases by "anemia" as the claim-associated diagnosis. This suggests that the contribution of the anemia to morbidity and costs may not have been recognized.

The economic costs of COPD were studied by Mannino and coworkers using a retrospective observational study of data from the Medicare Supplemental and private insurer databases. The average all-cause total healthcare (HC) costs including Emergency Department (ED) visits, hospitalizations, office visits, outpatient visits, and prescription drugs for 360 days after an official diagnosis of COPD was $38,870 for a patient with anemia vs. $12,978 for a patient with no comorbidities [2]. The reason for the higher HC costs for those COPD patients with comorbid anemia might be that these patients are more likely to be admitted to the hospital during an ED visit.

Chambellan analyzed longitudinal data from a large national registry in France of 2524 COPD patients receiving continuous oxygen therapy, examining relationships between hematocrit at the time of qualification for home O_2 therapy and

clinical outcomes including mortality and hospitalizations [3]. Using WHO definition criteria, anemia was present in 12.6% of men and 8.2% of women in this cohort with advanced COPD (mean forced expiratory volume in 1 s (FEV_1) = 0.92 (SD: 0.37) L, men; 0.68 (0.23) L in women). Perhaps not surprisingly, there was an inverse correlation between hematocrit and age. Hematocrit correlated inversely with FEV_1 (and with the FEV_1/VC (vital capacity) ratio). The researchers interpreted the findings as a possible association between COPD severity and comorbid anemia, and possibly COPD progression; in fact, no diagnostic or serological data were available to estimate the fraction of anemic COPD patients with an identifiable cause of their anemia. In a Cox multivariate model of prognostic factors, hematocrit was second to age in strength, and was prognostically significant independent of age. From hematocrit <35 to >55%, each 5% increase in stratum was associated with improved survival. Across the Hct range, a similar relationship was observed with respect to the frequency of hospitalization and mean number of hospital days per year. The apparent adverse prognostic value of anemia in COPD is particularly strong for patients experiencing gastrointestinal bleeding or after elective repair of an aneurysm [4, 5].

Although the incremental prognostic value of anemia was not present in the landmark analysis by Celli and colleagues that led to the widely used BODE index (body mass index, airflow obstruction, dyspnea, and exercise capacity), in COPD the discrepancy probably reflects the greater COPD severity in the French database analyzed by Chambellan and colleagues [3].

Not only is anemia associated with greater COPD morbidity and mortality, it is also associated with reduced exercise capacity and lower quality of life. Ferrari and colleagues explored the association between anemia, exercise capacity by 6-min walk distance (6 MWD) and peak VO_2, and health-related quality of life through a retrospective analysis of patients at the researchers' outpatient clinic. Anemic patients had more severe COPD based on FEV_1 (0.9 vs. 1.4 L; $p < 0.0001$). The anemic patients had lower 6MWD (267.9 m in anemic vs. 373 m in non-anemic patients; $p = 0.001$), VO_2 max (1.2 L/min anemic vs. 0.9 L/min; $p = 0.011$), and worse quality of life in all categories of the St. George's Respiratory Questionnaire (symptoms, activity, impact, and total).

While anemia is a negative prognostic factor, low-normal hemoglobin (Hgb) is also associated with lower survival. Kollert and colleagues determined that the 58th percentile for Hgb (14.3 g/dL in females; 15.1 g/dL in males) performed best for predicting survival, and this cutoff is above the threshold for defining anemia [6]. Therefore, using the WHO definition of anemia may exclude some patients with a worse prognosis in spite of normal Hgb levels. It is unknown whether aggressive correction of anemia improves outcomes in COPD patients.

Anemia and Asthma

Asthma is an increasingly prevalent and heterogeneous disease. When current guidelines are followed, the majority of patients are responsive to therapy when

used along with avoidance of allergens or other relevant triggers. Among patients with severe and/or refractory asthma, no compelling case has been made in published form of a preponderance of anemia among asthmatics.

Of interest is work by Drury and colleagues who investigated whether atopic disease, including asthma, was associated with an increased risk of childhood anemia through a cross-sectional survey with data from the National Health Interview Survey and the Nutrition Examination Survey. History of asthma was associated with higher odds of anemia (1.31; 95% CI 1.14–1.51), and particularly microcytic anemia, in multivariable models controlling for age, sex, and race/ethnicity [7]. The mechanism of the association is unknown; however, the association with microcytic anemia suggests that iron deficiency anemia or anemia of chronic disease may be present.

Cystic Fibrosis (CF) and Anemia

Each of several mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel lead to the phenotype of cystic fibrosis, a progressive and debilitating lung disease of children and young adults. The consequent abnormalities in airway liquid and solute handling promote impaired mucociliary clearance, among other secondary pathophysiological mechanisms, contributing to the propensity for frequent and chronic respiratory infection in CF, in turn fueling a chronic inflammatory state. Anemia in CF thus shares underlying inflammatory pathways with COPD and other chronic diseases, and is further predisposed or exacerbated by nutritional deficiencies arising from not only the chronic cytokine exposure but also defective enteral absorption of iron and other nutrients because of disturbed mucosal fluid exchange induced by the mutant CFTR. Indeed, evidence suggests that in CF, erythrocytosis is unusual whereas anemia, according to one study, was documented in 20% (12 of 60) of patients [8]. The very low incidence of erythrocytosis among CF patients, even those who are hypoxemic, distinguishes CF from COPD. Fischer and colleagues conducted a careful, serial observational study of 60 CF patients with the aim of characterizing possible determinants of their red blood cell mass (or, in the minority, frank anemia) [8]. Iron deficiency was rare (although about half the population were receiving supplemental iron, 100 mg daily), and the serological profile was consistent with anemia of chronic disease. No significant correlation between the arterial PO_2 (PaO_2) and the hemoglobin (Hgb) concentration was present, again consistent with what has been called a "relative anemia," in which the expected increase in red cell mass in response to hypoxemia is not observed. In a multivariate regression analysis examining relationships between various potential determinants and (Hgb)/hematocrit, CRP (C-reactive protein) emerged as independently associated with (Hgb); additionally, all patients were colonized with either *Pseudomonas aeruginosa (PA)* or *Staphylococcus aureus*. Thus, the chronic inflammatory state in CF is associated with chronic airway colonization and in many, intercurrent infections/exacerbations.

Von Drygalski tested for an association between anemia and decreased lung function in cystic fibrosis patients in a retrospective study. Anemic patients (by WHO criteria) had lower FEV_1 (51.6% anemic vs. 69.7% non-anemic) and FVC (82.5% anemic vs. 95% non-anemic). However, there was no correlation between the degree of anemia and the severity of lung impairment. Of note, some of the non-anemic patients with poor lung function had evidence of iron deficiency. This iron deficiency may limit the expected erythrocytosis that usually accompanies hypoxia. Unfortunately, treating iron deficiency is not always harmless in CF patients. In one case series of CF patients with iron deficiency and chronic *PA* colonization, intravenous iron administration during an admission for exacerbation of CF was followed 3–5 days later in a majority of patients by respiratory deterioration [9].

Anemia in Pulmonary Arterial Hypertension (PAH)

Important for symptoms and morbidity in patients with chronic lung disease, iron homeostasis is also important for the maintenance of red blood cell mass. Iron metabolism is also critical for the efficient turnover of myoglobin and the mitochondrial enzymes of oxidative phosphorylation in the skeletal and cardiac muscles. Ruiter and coworkers examined the prevalence of iron deficiency, with and without anemia, in a cohort of patients treated in a university clinic for idiopathic PAH (i.e., PAH not associated with a systemic disorder). They documented a 43% prevalence of iron deficiency in the 70 patients studied, and found an association of iron deficiency with decreased 6-min walk distance (6MWD, a conventional measure of exercise capacity in PAH) [10]. The 6MWD depression was similar between anemic ($n = 12$) and non-anemic ($n = 18$) subgroups of the iron-deficient patients. Together with the finding that PAH patients with vs. without iron-deficiency did not differ with respect to hemodynamic or demographic characteristics, this raised the possibility that the iron deficiency contributed to the efficiency of peripheral (skeletal muscle) oxygen utilization. Although idiopathic PAH tends to be a disease of younger adult females, there was no obvious gender preponderance of the iron deficiency in their cohort, as might be expected if related to menstrual blood losses. But with only four males in the iron-deficient group, the study was under-powered to detect such an association with gender.

Iron deficiency was shown to provoke PAH in a rat model. Cotroneo and colleagues fed rats an iron-deficient diet. By 3 weeks, the PA pressures were significantly elevated in iron-deficient rats compared to controls [11]. The iron-deficient rats showed pulmonary vascular remodeling with medial hypertrophy and perivascular inflammatory cell infiltration. These differences were shown to improve with iron supplementation. The influence of iron supplementation in PAH patients with iron deficiency is unknown.

Lung Cancer and Anemia

Complicating the paradigm for treating the anemic patient with lung cancer is the frequent comorbidity of lung cancer and COPD in this smoking-related disease. Among patients with solid malignancies, those with lung cancer have the highest incidence of anemia (up to 60% after 6 cycles of chemotherapy) and rates of RBC transfusion (up to 40%) [12]. Therapy-related anemia in lung cancer is most strongly linked to platinum-based regimens, but is also seen more broadly. The symptoms of anemia may overlap or combine with those of cancer as well as any other underlying lung disease (COPD, especially). However, erythropoietin administration should be avoided in anemic patients with solid tumors because of the potential for accelerated progression [13].

Waters and colleagues completed an observational study that suggested that raising Hgb to >12 g/dL improved survival in non-small cell lung cancer (12 months vs. 9 months). Anemia may be a prognostic factor or may actually be a cause of decreased survival [14]. Anemia is frequently present at the time of lung cancer diagnosis before the initiation of chemotherapy, which can also cause/contribute to anemia. Holgersson and colleagues studied patients at the time of their non-small cell lung cancer (NSCLC) diagnosis to see how anemia affects survival. The median survival for anemic patients (<11 g/dL) was 3 months shorter than the median survival for normocytic patients (11.2 vs. 14.5 months) [15]. In patients with anemia, leukocytosis, and thrombocytosis, the median survival was half that of patients with normal values (8 vs. 16 months). Thus anemia is a candidate index that could inform decisions about who needs more intensive care or who would benefit from palliative care.

Sickle Cell Anemia and Lung Disease

Sickle cell disease (SCD) is a hereditary anemia resulting from homozygous point mutation of the hemoglobin beta-globin chain, producing hemoglobin S (HbS). SCD is among the most common autosomal recessive disorders globally. SCD can be regarded as belonging to sickle cell anemia, a family of anemic disorders, also including thalassemia, sickle cell trait (heterozygosity for the sickle mutation), and their combination(s). Although the underlying lesion is specific, even (homozygous) SCD itself is characterized by great heterogeneity among patients in their clinical expression. Polymerization of HbS upon deoxygenation causes the RBC to assume a sickle shape that gives SCD its name. Sickled RBCs deform poorly, impairing the normal flow of blood in microvessels and promoting end-organ injury. Contributing to the vasculopathy of SCD, and probably also to its clinical heterogeneity, are adhesive and other interactions among leukocytes, the sickle RBC, and an inflamed vascular endothelium. These patients are at high risk for disease complications affecting multiple organ systems, and pulmonary consequences of the disease are among the most common. These include asthma (or an asthma-like syndrome) and pulmonary arterial hypertension. Gladwin and coworkers reported a high prevalence of pulmonary arterial hypertension among SCD patients studied at the NIH intramural Clinical Center [16], and reports from other centers or consortia [17] have yielded similar findings.

SCD patients are frequently transfused, but transfusions only have demonstrated efficacy for secondary prevention of stroke or silent cerebral infarction. DeBaun and colleagues found that in children with SCD and history of silent cerebral infarct, those who received regular monthly transfusions had a 58% relative risk reduction of strokes and new or enlarged silent cerebral infarcts over 3 years [18]. The pathogenesis of silent cerebral infarcts may involve anemia such that the blood supply cannot meet with demand. Additionally, Beverung and colleagues found that parents reported better health than the year prior in children who received monthly transfusions [19]. Whether SCD-related pulmonary complications can be prevented or ameliorated with such a transfusion strategy is untested.

Treatment

Treatment of Anemia in the Patient with Chronic Lung Disease

The treatment of anemia in the patient with chronic lung disease consists of treating the underlying cause (summarized in Fig. 8.1). Table 8.1 relates hematological and

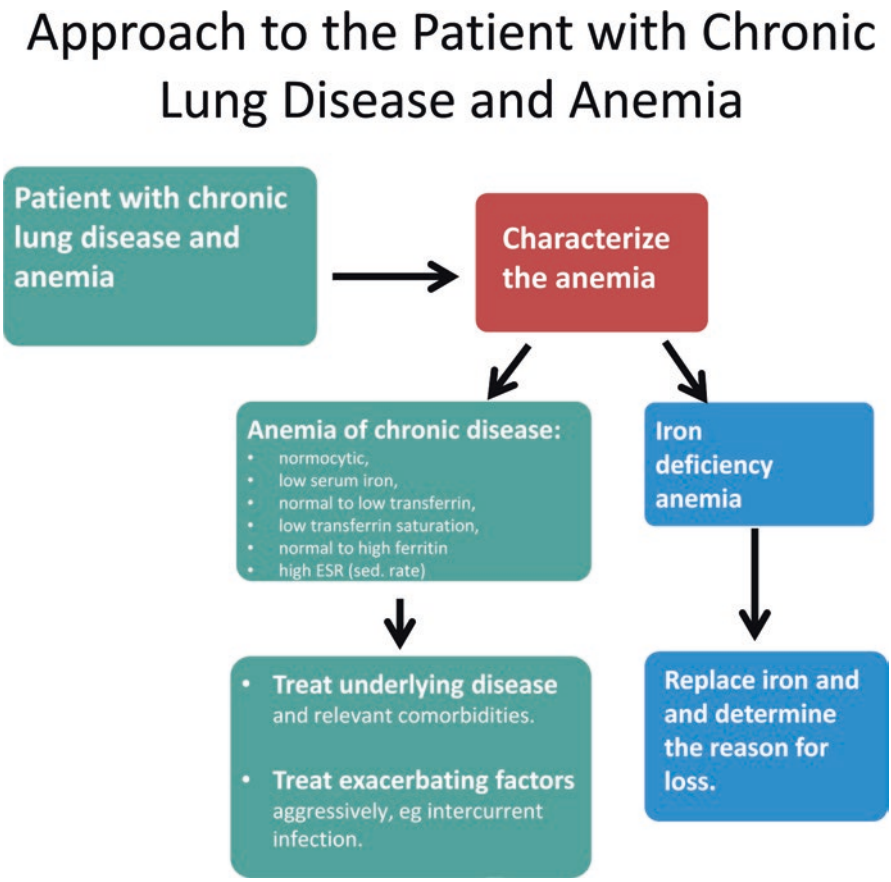

Fig. 8.1 General algorithm for the approach to a patient with both chronic lung disease and anemia. Often iron-deficiency anemia or the anemia of chronic disease, or both, can be present.

Table 8.1 Evaluating the etiology of anemia in the patient with chronic lung disease

Categories	Specific tests	"Usual normal" ranges	Comments
Red cell parameters	MCV (mean corpuscular volume)	82–103 fL	• As in other settings, anemia of chronic disease (ACD) is a normochromic, normocytic anemia.
	MCHC (mean corpuscular hemoglobin concentration)	30–37 g/dL	• Consider other causes of anemia, especially iron-deficiency anemia (IDA), if these parameters are outside of their normal ranges
	MCH (mean corpuscular hemoglobin)	26–34 pg/cell	
Iron indices	Transferrin saturation	15-45%[a]	• IDA (iron deficiency anemia) is relatively common among persons with ACD and chronic lung disease. This is a treatable anemia
	Ferritin	100 ng/mL[a]	• Ferritin is an acute phase reactant. Among persons with chronic lung disease, acute or chronic inflammatory processes may elevate the serum ferritin, even in the co-presence of iron deficiency.
	Content of hemoglobin in reticulocytes (CHR)	>29 pg/cell[a]	• Important to reassess periodically
			• Assesses internal distribution of iron (i.e., "availability" for incorporation into Hgb and reticulocytes)
Reticulocyte response	Absolute reticulocyte count	3–7%	• Reticulocyte index = observed absolute reticulocyte count/ normal absolute reticulocyte count.
Vitamin deficiencies	Vitamin B12, Folate	200–600 pg/ mL	• Manifests as a macrocytosis

[a]For iron stores, current clinical practice guidelines provide thresholds to consider as goals for therapy or thresholds below which supplementation should be considered rather than defining what is normal.

serological indices in anemia of chronic disease and iron-deficiency anemia, the two most common causes in this patient group, and which frequently coexist.

Coexistence of iron-deficiency anemia (IDA) and ACD is relatively common. For cases where there is diagnostic uncertainty, there are two reasonable approaches. Direct staining of a bone marrow aspirate reveals features of ACD, IDA, or both. Iron supplementation (see Table 8.1) is recommended for both simple IDA and IDA in combination with ACD, and should be followed by repeat evaluation of the hematological profile. A positive response to a 4-week trial of iron supplementation

therapy is diagnostically useful. However, lack of a response is possible when IDA coexists with ACD because ACD involves abnormal iron absorption due to deficient export by ferroportin in the duodenal enterocytes. If a patient has an unsatisfactory response to oral iron therapy, a clinician should consider intravenous iron therapy.

Transfusing the Anemic Patient with Lung Disease

The evidence is generally lacking to guide the clinician in decision-making regarding RBC transfusion triggers or thresholds for the patient with acute or chronic lung disease. Consideration should be given to whether the anemia is functionally limiting or whether the lung disease is so severe that transfusions will have very little impact on quality of life. Some insight into transfusion threshold in the acute setting comes from the seminal TRICC (Transfusion Requirements in Critical Care) trial, which demonstrated no benefit of a liberal RBC transfusion strategy (threshold of Hgb 10 g/dL) as compared to a restrictive (Hgb 7 g/dL) RBC transfusion threshold in nonbleeding adults with critical illness [20]. The study population in this randomized trial included a broad cross-section of ICU patients, including those with acute respiratory insufficiency. Significantly, in subgroup analyses, younger patients (<55 years) and those with lower severity of illness (APACHE <20) fared significantly worse when assigned to the liberal-transfusion arm, and mortality and *pulmonary* morbidity were among the events represented excessively in this group.

More broadly, pulmonary morbidity has been documented in a number of clinical trials of "when to transfuse" in the acute care setting, or the RBC transfusion threshold [21]. A clinically important question, recently asked anew with increasing intensity, is whether the duration of RBC storage influences clinical outcomes in potentially vulnerable transfused patients such as critically ill patients and those with acute coronary syndromes. Koch and colleagues' retrospective analysis of patients undergoing cardiac surgery at the Cleveland Clinic demonstrated increased mortality among patients receiving RBC units stored greater than 14 days, as compared with patients receiving exclusively RBC units stored less than 14 days [22]. Again, adverse pulmonary events, such as number of ventilator days, were among the morbidity excessively seen in patients receiving the older RBC units [23]. Despite a carefully conducted multivariable regression upholding the findings, the retrospective nature of this study limits drawing inferences about causality, let alone calling for changes in the pattern of transfusion practice. But this study and others have led to sufficient concern over the consequences of transfusing older RBCs that randomized clinical trials were completed to compare clinical outcomes in patients assigned to "older" vs. "fresher" RBC-transfusion strategies [24, 25]. Lacroix and colleagues performed a randomized, blinded trial of critically ill adults receiving transfusions of either fresh RBCs (<8 days; stored 6.1 ± 4.9 days (mean ± SD)) or standard-issue RBCs (stored 22.0 ± 8.4 days). The 90-day mortality rate was similar for both groups (37% fresh vs. 35.3% standard) [26]. Other complications like ARDS were similar between the two groups (5.7% fresh, 6.6% standard). Additionally, the fraction of patients who required invasive mechanical ventilation

was comparable (97.5% in the fresh-RBC arm vs. 97.3% in the standard-issue RBC arm). Fergusson and colleagues performed a randomized, double-blind controlled trial in premature infants who were transfused either fresh RBCs (<7 days) or standard-issue ("dedicated donor") RBCs. Fresh and standard RBC transfusion strategies were associated with similar rates of neonatal morbidities and mortality [27]. Specifically, pulmonary complications like bronchopulmonary dysplasia occurred at similar rates in the two groups (31.9% fresh vs. 33.3% standard). Steiner and colleagues completed a randomized controlled trial that compared outcomes after the transfusion of fresher (<10 days) vs. older RBCs (>21 days) in patients undergoing complex cardiac surgery. The rates of mortality and adverse events were comparable between the two groups [28]. At this time, the use of fresh RBCs to reduce morbidity and mortality is not justified.

Transfusing for Anemia in COPD

Interest in specifically detecting and treating anemia in the ill COPD patient was intensified by a frequently cited early report that RBC transfusion led to significant reductions in the work of breathing and minute ventilation in 20 anemic, COPD patients in an intensive care setting [29, 30]. In a smaller group of five ventilator-dependent COPD patients with anemia, the same authors reported successfully extubating the patients after raising the Hgb levels to 12 g/dL by transfusion [30]. However, these results have not been replicated in a large, randomized controlled trial. Currently, there is no specific recommended RBC transfusion threshold in COPD, but rather it is recommended that symptoms attributable to the anemia should prompt transfusion if the clinician believes there is insufficient time to await other measures (e.g., iron replacement or EPO) [31].

Erythropoietin for Anemia in Chronic Lung Disease

There is no role for the routine use of erythropoiesis-stimulating agents (ESAs) in the typical patient with the chronic lung disease and ACD. Use of an ESA should in fact be avoided in patients whose anemia is related to concomitant lung cancer or other solid malignancy and its treatment (myelosuppressive chemotherapy) [13]. The reader is referred to the corresponding chapters of this handbook for discussion and treatment algorithms.

Anemia as an Adaptive Response

The disappointing clinical outcomes in studies of RBC transfusion of anemic patients have generally (and appropriately) raised the question of whether RBCs stored for transfusion may be inferior to native RBCs. A case has also been made that anemia in chronic pulmonary and other diseases represents an adaptive state or

response. Specifically, it has been argued that anemia benefits those with chronic illness via the following mechanisms: (a) the sequestration of iron in ACD serves to limit iron availability to microorganisms that require it for growth and proliferation in vivo; (b) minimizing the availability of free iron means avoiding toxic chemistry associated with oxidant responses typical of a chronic inflammatory state [32]; (c) the lower blood viscosity associated with anemia places a smaller load on the vulnerable cardiovascular system of the ACD/COPD patient.

It is argued that this cardiovascular adaptation does not compromise O_2 delivery because of the "reserve" typically inherent to the O_2-delivery/O_2 consumption balance (only 25% or so of the O_2 delivered is actually offloaded under normal circumstances). While plausibly beneficial in any anemic patient with chronic disease, these favorable potential consequences of anemia could be particularly protective in the COPD patient or chronic lung-disease patient, who is at particularly elevated risk for acute and chronic/recurrent infection and cardiovascular sequelae of the underlying disorder, such as right-heart failure.

Relevant Clinical Practice Guidelines

Practice guidelines specific to the evaluation and management of anemia in the patient with chronic lung disease are lacking, reflecting a sparse evidence base. However, extrapolation from general guidelines concerning the management of ACD to the patient with CLD and ACD are very reasonable.

Summary

In COPD and other chronic lung diseases, anemia is not uncommon, and is typically consistent with the anemia of chronic disease (ACD).

A thorough evaluation to exclude other causes of anemia is essential, and in particular iron-deficiency anemia (IDA) together with ACD should be recognized and the IDA treated.

Treatment of ACD, as in other settings, consists of treating the underlying disease. Particular attention to detection and treatment of incident infection is warranted.

Red blood cell transfusion or use of an ESA should be reserved for the CLD patient with significant symptoms likely to be attributable to the anemia. Decision-making in this scenario depends in part on an individualized assessment of the known risks and benefits of therapy.

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Anemia in Malignancy

9

Daniel Lebovic

Introduction

Anemia is frequently encountered in patients with malignancies. It is more common in those with hematological cancers but is often found in solid tumor patients, as well [1, 2]. The etiology of malignancy-associated anemia is generally multifactorial, involving cancer itself, treatment, comorbidities, altered immune function, bleeding, and mechanical destruction of red blood cells [3]. While some of these factors are amenable to treatment, others are irreversible, resulting in persistent anemia in many cancer patients.

Aside from the classical symptoms associated with anemia such as fatigue and decreased exercise tolerance, anemia in malignant disease also impairs response to anti-neoplastic therapies, including chemotherapy and radiotherapy [4]. Decreased hemoglobin is also thought to drive tumor growth and promote the development of treatment-resistant cellular clones [5, 6]. Patients with moderate to severe anemia frequently have difficulty tolerating cancer therapy and often require dose reductions or an abrogated course of treatment. Finally, the deleterious effects of anemia on quality of life are particularly pronounced in cancer patients, and the impact on daily life can be debilitating [7].

An improved understanding of the mechanisms and process of erythropoiesis has led to a paradigm shift in the treatment of malignancy-associated anemia. Whereas in the past, cancer patients with low hemoglobin were generally managed with chemotherapy dose modifications and packed red blood cell transfusions, they are increasingly being treated with erythropoietin-stimulating agents. While there has been some controversy about the relative safety of these agents in such patients,

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Table 9.1 Grading of anemia according to the National Cancer Institute classification

Grade	Symptom severity	Hemoglobin values
0	Within normal limits	12.0–16.0 g/dL for women and 14.0–18.0 g/dL for men
1	Mild	10 g/dL to levels within normal limits
2	Moderate	8.0–10.0 g/dL
3	Serious/severe	6.5–7.9 g/dL
4	Life threatening	<6.5 g/dL

national guidelines support their use along with careful dosing and regular monitoring of blood counts [8].

Definition and Epidemiology

Anemia is generally defined as hemoglobin <14 g/dL in men and hemoglobin <12 g/dL in women [3]. However, from both a symptomatic and prognostic perspective, it is not simply the presence of low hemoglobin but the degree of anemia that is important. The National Cancer Institute has established a grading system to characterize the severity of anemia in cancer patients (Table 9.1) [9].

The reported prevalence of anemia in patients with malignancies varies considerably according to different studies [10–14]. These discrepancies are related to the definition of anemia (any hemoglobin less than normal vs. moderate/severe), disease stage, and cancer subtype. Broadly, speaking, approximately 50% of patients with solid tumors have low hemoglobin, and at least 70% of patients with hematologic malignancies are anemic [14]. In some cancer subtypes, these numbers increase to about 90% [3]. The addition of cytotoxic chemotherapy further increases the risk of anemia [10]. Regardless of its precise frequency, anemia is clearly a very real issue that the majority of cancer patients—and their health care providers—will be forced to contend with at some point in the course of this illness.

Risk Factors

Using data from the prospective European Cancer Anaemia Survey (ECAS), Barrett-Lee et al. analyzed data from 13,628 cancer patients initiating chemotherapy [15]. They identified five pre-treatment factors significantly associated with developing anemia during chemotherapy: initial Hgb ≤12.9 g/dL in females and ≤13.4 g/dL in males; presence of lung or gynecologic malignancy vs. gastrointestinal cancers; cancer at any site other than GI tract and female gender. In addition, platinum-based chemotherapy was associated with a higher incidence of treatment-related anemia [15].

Using the same data set, investigators specifically looked at patients with multiple myeloma and lymphoma (both Hodgkin's and non-Hodgkin's) [2]. They found that the following variables were associated with an increased likelihood of anemia:

initial Hgb ≤13.3 g/dL in males and Hgb ≤12.7 in females; the presence of persistent or recurrent disease; female gender and use of a platinum agent for chemotherapy. Taken together, these results can aid practitioners in identifying patients at increased risk for anemia, facilitating careful monitoring and enabling the initiation of preventative therapies where appropriate.

Symptoms and Consequences of Anemia in Cancer Patients

Fatigue is the most ubiquitous symptom of anemia. Compared to the general population, patients with malignancies are more likely to be fatigued even in the absence of anemia [7].

The presence of anemia in cancer patients results in more severe fatigue compared to both the general population and their non-anemic counterparts [1]. In addition, cancer-related fatigue produces a greater impairment of everyday functioning than the equivalent degree of non-cancer-related fatigue. This means that anemic cancer patients are expected to have a substantially poorer quality of life [14, 16–18]. In fact, several studies have demonstrated a correlation between Hgb levels and quality of life [10].

As anemia becomes more severe, additional signs and symptoms develop, including headache, palpitations, shortness of breath, and tachycardia. Severe anemia may lead to heart failure, pulmonary edema, and cognitive impairment [10]. In the most extreme circumstances, critically low hemoglobin levels can lead to patient death, as may be the case in end-stage acute leukemia, aplastic anemia, and myelodysplastic syndrome [19]. Thus, persistent anemia results in both increased morbidity and increased mortality for cancer patients.

Physiologically, decreased hemoglobin levels may also promote tumor growth, impair response to treatment, and contribute to the selection of treatment-resistant clones. In a number of cancer subtypes, including cervical, head and neck, prostate, bladder, lung and lymphoma, the presence of anemia has been correlated with a worse outcome [20].

Up to 50–60% of advanced solid tumors contain regions of hypoxic tissue [21]. In large part, this occurs because tumors tend to be relatively poorly vascularized and frequently outgrow their blood supply [14]. However, in about a third of cases, tumor hypoxia is facilitated by decreased blood oxygen-carrying capacity due to anemia. Unlike normal tissue, the anomalous tumor vasculature is unable to accommodate for low hemoglobin levels by increasing local blood flow [21]. Thus, anemia both exacerbates and causes tumor hypoxia.

In an analysis of more than 100 patients with locally advanced cervical cancer, the presence of tumor hypoxia was significantly associated with increased extracervical extension and resistance to treatment [22]. Both disease-free and overall survival were reduced in patients with hypoxic tumors. Likewise, in a population of patients with advanced head and neck cancer, disease-free survival was 78% in patients with non-hypoxic tumors, compared to 22% in those with hypoxic tumors ($p = 0.009$) [23].

Although the mechanism by which a poorly oxygenated tumor environment results in more aggressive disease has not been fully elucidated, several important observations have been made. Firstly, hypoxia leads to upregulation of HIF-1∝, a key transcription factor that regulates the expression of more than 30 genes implicated in tumor progression [24]. Increased levels of HIF-1∝ result in angiogenesis via the upregulation of multiple growth factors including vascular endothelial growth factor (VEGF), platelet-derived growth factor-β (PDGF-β), and transforming growth factor β(TGF-β) among others [24–26]. It also causes downregulation of a number of angiogenesis inhibitors. The resulting increase in tumor vascularization is associated with increased tumor aggressiveness, tumor survival, and metastasis [25, 26].

Extreme hypoxia generates genomic instability, giving rise to genomic changes and clonal selection. These conditions generally result in a highly aggressive, multiresistant phenotype [25, 26]. In particular, hypoxia appears to select for the highly deleterious p53 mutation [1, 5, 24]. The ensuing tumor expansion results in outgrowth of the local blood supply, leading to additional hypoxia in a self-perpetuating cycle.

Radiotherapy is an integral part of the treatment of many malignancies. Ionizing radiation causes the formation of DNA-damaging free radicals within tumor cells. Oxygen is a necessary component of the chemical reactions that enable free radicals to cause injury to DNA [27]. It has clearly been shown that a hypoxic tumor environment substantially reduces the effectiveness of radiation [27, 28]. In studies of patients with cervical cancer and patients with head and neck cancer, diminished tumor oxygen levels were associated with increased resistance to radiotherapy [28]. Conversely, increasing tumor oxygen levels both in vitro and in vivo is associated with an increase in tumor radiosensitization [27].

In addition to radiotherapy resistance, hypoxic tumor cells have also been shown to be less susceptible to chemotherapy-induced cell death [14, 26]. It is likely that HIF-1 ∝ upregulation plays an important role in chemotherapy resistance. It is believed that cytotoxic agents cause death of neoplastic cells via apoptosis. Induction of HIF-1 ∝ is associated with decreased levels of the pro-apoptotic proteins Bid, Bad, and Bax [24, 26]. HIF-1 inhibitors restore chemosensitivity in renal cell carcinoma, glioma, and pancreatic cancer cell lines [26].

As a means of chemoresistance, tumors frequently employ drug efflux pumps. These are membrane glycoproteins that propel foreign molecules out the cell interior. Overexpression of key efflux pumps such as MDR1 and MRP2 lead to the removal of chemotherapeutic agents from the tumor cells, resulting in subtherapeutic drug concentrations [29]. HIF-1 ∝ has been shown to upregulate both MDR1 and MRP2. Thus, through a host of mechanisms, hypoxic cell conditions lead to chemotherapy resistance [30].

Causes of Anemia in Malignancy

The mechanisms leading to and maintaining normal erythropoiesis are extremely complex [3, 19], and beyond the scope of this review. Broadly speaking, erythropoiesis requires appropriate nutrients, including iron, B12, and folate. Iron needs to be available to cells for hemoglobin synthesis rather than sequestered inside of storage cells such as macrophages. The bone marrow integrity must be intact and functional. Finally, the appropriate signaling mechanisms must be present in order to drive the process of red blood cell production within the bone marrow [19]. In cancer patients, any number of these factors can be impaired [1, 3, 19].

Patients with malignancies frequently have decreased serum iron levels [31]. This may be a direct result of the neoplasm itself, as in gastric or colorectal cancers that cause GI blood loss. Alternatively, the malignancy may interfere with the patient's nutritional intake, as in esophageal and head and neck cancer that compromise the ability to eat. In other cancer types, anti-neoplastic treatment frequently causes mucositis, nausea, or constipation, making it difficult for patients to consume an adequate diet. In addition to decreased iron, these factors can also result in vitamin deficiencies such as B12 and folate [10]. Myelosuppressive treatment can lead to thrombocytopenia which predisposes the patient to blood loss. In some cases, frequent blood draws for laboratory testing further reduce the patient's iron stores [1, 32].

While true iron deficiency may be a factor in the anemia of malignancy, functional iron deficiency (also called relative iron deficiency) is thought to play an even greater and more ubiquitous role [32, 33]. Hepcidin is the key hormone involved in iron trafficking [14, 32, 33]. This 25-amino acid polypetide is produced in the liver and, to a lesser extent, in monocytes. Hepcidin blocks the iron export channel, ferroportin, causing sequestration of iron within its target cells. Increased hepcidin levels result in decreased iron absorption from the gut, as well as iron trapping inside of reticuloendothelial cells. As a consequence, serum iron levels decrease and ferritin levels rise. While the patient may have adequate storage iron, the effect of elevated hepcidin is to significantly limit the availability of that iron for erythropoiesis [32]. Interleukin-6 (IL-6) levels are commonly increased in inflammatory and malignant states, and this cytokine has been shown to directly upregulate hepcidin expression [34]. Thus, IL-6 is felt to be a key mediator of functional iron deficiency. Investigators found that in ovarian cancer patients, IL-6 levels were inversely correlated with hemoglobin levels [35].

In cancer patients, the normal molecular events that control erythropoiesis are disrupted. Under routine conditions, red blood cell synthesis is regulated by physiologic stimuli that result in the production of erythropoietin (EPO), a hormone that is primarily manufactured in the kidney, as well as smaller amounts in the liver [10]. EPO regulates the differentiation and survival of red blood cell precursors inside the bone marrow [32]. A primary downstream mediator of EPO is the GATA1 transcription factor that regulates expression of EPO-specific genes, such as the globin genes [36]. GATA1 effects are inhibited by GATA2 and NFκB [37] (Fig. 9.1).

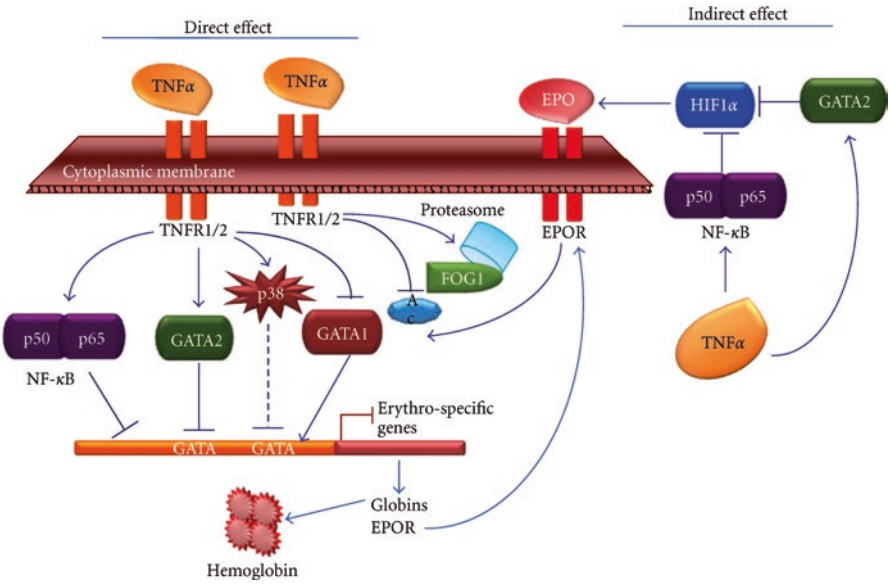

Fig. 9.1 TNF α inhibits erythropoiesis by direct and indirect effects. In the indirect effect, TNFα activates the transcription factors NF-B and GATA-2, which were also reported as involved in Epo production inhibition by blocking HIF1α in vitro. Low level of Epo decreases the EpoR-mediated signaling pathways resulting among others, in the downregulation of GATA-1, and consequently in a possible deregulation of EpoR expression. TNFα was also reported as activating GATA-2 whose overexpression is known to prohibit erythropoiesis. The combined effects of TNFα result in the decrease in erythro-specific genes expression and hemoglobin production. (Reprinted from Morceau F, Dicato M, Diederich M. Pro-inflammatory cytokine mediated anemia: regarding molecular mechanisms of erythropoiesis. Mediators of Inflammation. 2009; (epub ahead of print). http://dx.doi.org/10.1155/2009/405016. Used with permission under the Creative Commons License: https://creativecommons.org/licenses/by/3.0/)

In the malignant state, the production of various inflammatory cytokines impedes the normal role of EPO on multiple levels. Tumor necrosis factor (TNF) ∝ is a key player in this inflammatory process [38, 39]. By upregulating GATA2, TNF∝ causes inhibition of HIF-1∝, which in turn results in decreased EPO synthesis. GATA2 also blocks the transcription of multiple EPO-specific genes. Among these genes is that of the EPO receptor itself, further limiting the bone marrow's ability for erythropoiesis. TNF∝ also stimulates NFκB that further enhances the inhibitory effects on the expression of EPO-specific genes, leading to an additional decrease in red blood cell production.

EPO also assumes an integral role in erythropoiesis by enabling the survival of the red blood cell precursors, colony forming unit-E (CFU-E), and proerythroblasts [19]. These cells express large amounts of Fas receptors. When Fas binds to Fas ligand (Fas-L), apoptosis is triggered. EPO downregulates Fas receptors on CFU-E and proerythroblasts thereby promoting their survival. However, in the pro-inflammatory cancer environment, the balance is tipped toward apoptosis due to

decreased EPO production [1, 19]. Furthermore, TNFα, as well as other pro-inflammatory cytokines directly induce apoptosis in CFU-E and proerythroblasts, overcoming the effects of endogenous EPO [19, 38]. Thus, in cancer patients, various cytokines both impair EPO production and counteract the physiologic effects of EPO within the bone marrow. This sequence of events is thought to be especially pronounced in multiple myeloma, wherein the incidence of anemia at diagnosis is approximately 75% [2]. Multiple myeloma cells express Fas-L which is thought to bind to Fas receptors on red cell precursors, leading to apoptosis and a concomitant lack of erythrocyte differentiation [40].

In addition to these numerous impediments to red blood cell production, neoplasia can also be associated with increased erythrocyte destruction [1]. Immune system dysregulation may lead to hemolytic anemia. This can either be related to warm auto-antibody production (e.g., chronic lymphocytic leukemia) [41] or cold agglutinins (e.g., Waldentrom's macroglobulinemia) [42]. Pure red cell aplasia, linked to thymoma and some lymphoid malignancies, is an autoimmune disorder involving the destruction of erythrocyte precursors in the bone marrow [43]. A rare but life-threatening example of immune dysregulation is hemophagocytic lymphohistiocytosis (HLH) whereby macrophages devour (phagocytose) erythrocytes, leukocytes, and/or platelets. This condition is most commonly associated with lymphoid malignancies but has been described in myeloid neoplasms and solid tumors, as well [44].

In cancer patients, direct bone marrow interference is another potential mechanism of anemia. Most commonly, this occurs via myelosuppressive or myelotoxic antineoplastic treatments such as chemotherapy and radiotherapy [10]. In some cases, the malignancy itself may occupy the bone marrow to a large enough extent that normal hematopoiesis is not feasible. This is often true of malignancies that "reside" in the bone marrow like leukemia and multiple myeloma. However, solid tumor metastasis to the bone marrow results in similar consequences [45].

Patient Evaluation

The primary focus of the anemia workup in a patient with a known malignancy is to determine, first, whether any component of the anemia is related to non-malignancy factors and second if any of these factors are reversible. This latter consideration is particularly important for maximizing patient quality of life and can also be helpful with determining the ability to tolerate intensive anti-neoplastic therapies.

Iron studies, including serum iron, total iron binding capacity (TIBC), transferrin saturation, and ferritin should be checked. Vitamin B12 and folate levels should be assessed, as well. A thorough history and physical examination is critical to identify the possibility of ongoing blood loss. Examination of the peripheral blood smear, reticulocyte count, lactate dehydrogenase (LDH) levels, Coomb's testing, and haptoglobin can aid in diagnosing hemolytic anemia. Renal function should be assessed, as renal impairment is a frequent cause of hypoproliferative anemia in cancer patients, particularly the elderly [1].

Nutritional deficiencies should be addressed. Functional iron deficiency is typically manifested by low serum iron, normal or low TIBC, low transferrin saturation, and elevated ferritin [14]. Treatment of functional iron deficiency is discussed in the next section. In some instances, malignancy-associated autoimmune hemolysis may respond to standard treatments such as high-dose glucocorticoids [41]. However, relapses are common and, eventually treatment of the underlying malignancy may be required.

Management of Anemia in Malignancy

For many years, red blood cell transfusion was the only means available to increase hemoglobin in anemic cancer patients [1]. There are a number of risks associated with the administration red blood cell transfusions. Many of these concerns are not specific to patients with malignancy. These include viral and bacterial infections, acute and delayed hemolytic transfusion reactions, febrile nonhemolytic transfusion reactions, transfusion-associated lung injury (TRALI), volume overload, post-transfusional purpura, iron overload, and anaphylaxis [1]. In general, however, transfusions are considered reasonable and safe when administered with appropriate procedural techniques and close patient monitoring [46].

Interestingly, an association has been suggested between the receipt of multiple red blood cell transfusions and cancer recurrence [47]. Prospective studies have shown a correlation between the number of red blood cell transfusions and colorectal cancer recurrence [1, 47]. This is thought to be on the basis of weakened immune responsiveness as a consequence of depressed cytotoxic T-cell, natural killer (NK) cell, and monocyte function [48]. It would appear that this immunosuppression is related to the presence of leukocytes in the transfused products. The use of leukoreduction techniques significantly lowers the number of donor white blood cells per unit of blood by 99.99%. This substantially reduces the immunological issues associated with leukocytes [49]. In the United States, more than 80% of institutions dispensing blood products have a policy of universal leukoreduction [50].

When transfusing patients with malignancy, the optimal target hemoglobin remains undefined. Studies in the critical care setting have suggested equivalent and possibly improved outcomes with more restrictive transfusion thresholds wherein patients are given blood if Hgb is <7 g/dL, and the target Hgb is 7–9 g/dL [51]. However, it is difficult to generalize these findings to the more chronic anemia associated with malignancy where ongoing, outpatient quality of life becomes an important concern. At the same time, as noted above, more frequent transfusions imply an added risk of complications and possibly an adverse effect on cancer itself. Therefore, national guidelines generally recommend individualizing the decision to transfuse for Hgb between 7 and 10 g/dL according to the particular patient and the specific clinical circumstances [52].

Recombinant human EPO or erythropoietin-stimulating agents (ESAs) were first introduced for the treatment of anemia in patients with end-stage renal disease [10]. ESA use in these patients resulted in a significant reduction in the number of red

blood cell transfusions [53]. These impressive data led to the investigation of ESAs in malignancy-associated anemia. A number of large, randomized trials conducted during the 1990s through the early 2000s demonstrated a significant reduction in transfusion requirements for cancer patients undergoing chemotherapy who were treated with ESAs [10]. A meta-analysis of 25 randomized controlled trials (RCTs) comprising 3069 patients confirmed that ESA use reduced the relative risk of red blood cell transfusion in patients with malignancy-associated anemia undergoing chemotherapy (RR 0.67, 95% CI 0.62–0.73) [54]. Of interest, the benefit was more marked for patients with solid tumors compared to hematologic malignancies, possibly owing to the increased likelihood of bone marrow involvement in the latter group [54].

The impact of ESA treatment on quality of life (QOL) in anemic cancer patients has been studied, as well. In a prospective, RCT, Littlewood et al. [55] examined the impact of ESA use on quality of life in patients with solid tumors or non-myeloid hematologic malignancies treated with non-platinum-based chemotherapy. They found significant improvements in QOL scores for patients receiving ESAs vs. placebo. However, a similar study in patients with advanced breast and lung cancer did not demonstrate a clear overall QOL benefit in those who received an ESA, although there was a correlation between increased Hgb and decreased fatigue [56]. The authors concluded that overall QOL in cancer patients is determined by many factors (pain, cancer activity, psychological state, etc.) of which fatigue is only one. Therefore, the use of ESAs cancer patients may be more accurately thought of as a means to abrogate patient fatigue rather than enhance global quality of life.

These early studies focused primarily on reduction of transfusion requirements, increase in Hgb, and improvement in QOL. Though it was not designed to assess overall or disease-specific survival, the Littlewood study showed a trend toward improved overall survival (17 months vs. 11 months; $P = 0.13$) [55]. Similarly, a meta-analysis of 19 RCTs, including 2865 patients, also demonstrated a survival benefit [57]. Of note, the ESAs used in these trials were either epoetin alpha or epoetin beta, with the dosing frequency generally borrowed from ESRD patients, i.e., three times weekly. Subsequently, investigators began to use higher doses with once-weekly administration, as an enhanced convenience to the patient [10].

In 2003, the results of two large RCTs designed to directly evaluate ESA impact on overall survival in cancer patients were published. Henke et al. [58] randomized head and neck cancer patients undergoing radiotherapy to receive epoetin beta 300 IU/kg three times per week or placebo. Target Hgb was ≥14 g/dL in woman and ≥15 g/dL in men. Patients in the treatment group had significantly lower survival ($P = 0.02$). More patients in this group died of cardiac disorders, and there was a higher incidence of hypertension, hemorrhage, and thrombosis, including pulmonary embolism. In addition, there was an increased rate of loco-regional tumor progression in patients receiving an ESA.

In the BEST study [59], 939 women with metastatic breast cancer receiving chemotherapy or radiotherapy were randomized to epoetin alpha 40,000 IU weekly or placebo. An independent data safety monitoring committee terminated the study early due to a significant survival advantage in the placebo group (76% vs. 70%

alive at 12 months; $P = 0.01$). Deaths due to fatal thrombotic events and disease progression were significantly higher in the treatment group. Interestingly, the increased mortality in the group receiving an ESA occurred within the first 4 months; by 19 months, the survival curves had converged [60].

Two other trials evaluating ESA use in cancer patients not receiving chemotherapy, the EPO-CAN-20 non-small cell lung cancer study and the Amgen-103 study, likewise demonstrated poorer overall survival in patients receiving an ESA compared to placebo [1]. A Cochrane meta-analysis of relevant studies confirmed that cancer patients receiving an ESA had a shorter survival time compared to their untreated counterparts [61].

These results are surprising; in theory, increasing Hgb levels should lead to enhanced tumor oxygenation. This, in turn, would be expected to decrease tumor aggressiveness and increase sensitivity to anti-neoplastic therapy. Moreover, non-anemic patients are expected to have a better tolerance for chemotherapy, enabling them to receive more complete treatment. Both of these factors should result in improved survival [10].

Several hypotheses have been proposed to explain the unexpected impact on survival seen in these ESA trials. Preclinical studies have reportedly shown high levels of EPO and EPO receptors on breast cancer cells, as well as other cancer subtypes [62, 63]. This has raised the possibility that EPO may stimulate tumor growth, and in fact, some preclinical studies appear to support this contention [64]. Nevertheless, it is generally agreed by investigators that recombinant EPO is very unlikely to promote tumor growth to any clinically relevant extent. Even if tumor cells do express EPO receptors, their numbers are miniscule compared to the quantity of EPO receptors expressed on erythroid cells [1, 65]. Furthermore, EPO receptor-ligand affinity is so much greater on erythroid cells compared to non-erythroid cells that the likelihood of tumors outcompeting erythroid cells for recombinant EPO binding is virtually zero [1, 66].

A more likely explanation for the decreased survival in ESA-treated patients is the increased risk of thromboembolic and cardiovascular events seen in this population [66, 67]. By stimulating erythropoiesis, EPO generates an increased RBC mass. It has been shown that elevated RBC mass is associated with hypertension, hyperviscosity, increased risk of thrombosis, vasoconstriction, and decreased renal and cerebral blood flow [1]. It is important to note that standard lab values for RBC, Hgb, and hematocrit include plasma volume, and therefore do not directly measure RBC mass. Because of the body's tendency to maintain a constant blood volume, an increase in RBC mass caused by recombinant EPO may result in a concomitant reduction in plasma volume, leading to hyperviscosity and hypercoagulability [1]. In addition, EPO itself may have thrombogenic properties. In vitro studies have shown enhanced platelet reactivity and endothelial activation in the presence of EPO stimulation [1].

Further analysis of those studies showing a negative impact on survival for patients receiving ESAs found that subjects in the treatment arms tended to have relatively high Hgb levels (13–15 g/dL) [68]. This likely resulted in a significantly elevated RBC mass, potentially predisposing to the increased incidence of

thromboembolic and cardiovascular events. In fact, the earlier studies that suggested a survival benefit for ESA treatment in cancer patients (albeit without survival being their primary outcome) generally treated patients to more modest Hgb levels [54]. It was therefore proposed by some investigators (as well as the FDA) that ESA therapy is safe in cancer patients as long as the treatment goal is simply to correct anemia, i.e., achieve Hgb no greater than 12 g/dL [69]. However, subsequent meta-analyses have not demonstrated any correlation between target Hgb levels and mortality. Even taking into account achieved Hgb concentrations, there was no association between higher Hgb levels and risk of death [8, 70]. Therefore, an unequivocally safe target Hgb level has not been identified, and it is plausible that this target level may vary from patient to patient.

As a result of increased hepcidin, many patients with a malignancy will have functional iron deficiency [32, 33]. Several prospective RCTs have evaluated the provision of iron supplementation when these patients are receiving ESA therapy [71, 72]. Patients who were given intravenous iron supplementation demonstrated improved Hgb response and decreased transfusion requirements compared to those who received oral iron supplementation or no iron supplementation. As such, patients receiving ESA treatment should undergo regular monitoring of iron, TIBC, transferrin saturation, and ferritin levels with intravenous iron supplementation provided as needed [8].

In 2007, the American Society of Clinical Oncology and the American Society of Hematology (ASCO/ASH) issued a joint practice guideline for the use of ESAs in cancer patients [69]. The guideline was updated in 2010 [8]. The recommendations take into account the somewhat conflicting findings of the multiple studies described above. On the one hand, it is clear that ESA use decreases transfusion dependence and improves symptoms of fatigue. At least to some extent, it also improves the quality of life. Conversely, it appears to increase the risk of thrombotic and cardiovascular events, leading to decreased survival. Navigating these opposing outcomes is an example of the very difficult choice that many cancer patients are ultimately forced to make between longevity and quality of life. Indeed, the FDA currently limits the indication for ESA therapy to patients receiving chemotherapy for palliative rather than curative intent [8].

The ASCO/ASH guideline [8] recommends the use of ESAs in cancer patients with anemia undergoing chemotherapy. The guideline does not distinguish based on whether the intent of the chemotherapy is curative or palliative. It is recommended that ESA therapy be initiated when Hgb falls to <10 g/dL. For patients with Hgb between 10 and 12 g/dL, consideration can be given to starting ESA therapy depending on individual patient circumstances. While the 2007 guideline recommended Hgb target at or near 12 g/dL, the 2010 update recognizes that a conclusively acceptable target level has yet to be identified. The guideline now suggests increasing Hgb to the lowest concentration needed to avoid transfusions. ASCO/ASH also recommend careful assessment of the risk of thromboembolism in each patient, as well as thoroughly counseling the patient regarding the risks and benefits of ESA therapy. In anemic cancer patients not receiving chemotherapy, ESA treatment is not recommended.

Conclusion

In patients with malignancies, anemia is a highly prevalent—and in some cancer subtypes, a nearly universal—condition. Anemia negatively impacts the quality of life, impairs response to treatment, and may generate a more aggressive tumor phenotype. While a great deal of progress has been made in elucidating the cellular processes underlying the evolution of anemia in cancer patients, much work is left to be done.

Treatment of malignancy-associated anemia has advanced beyond the transfusion of red blood cells. ESA therapy is now commonplace. However, questions regarding its safety, appropriate patient selection, and ideal target Hgb remain unanswered. Additional prospective, randomized trials are needed to address these important issues. It is hoped that as science gains a better understanding of the process of erythropoiesis, as well as the physiologic response to inflammation and malignancy, more targeted, effective—and above all—safer treatments will be developed.

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The Complex Relationships Between Iron Regulation, Obesity, and Anemia

10

Stephen Z. Fadem

Is Anemia Associated with Obesity in the Normal Population?

Obesity has become more common in the past several decades, and has had considerable influence upon health from both a quality of life and an economic perspective. The World Health Organization estimates that there are more than 1.9 billion adults who were overweight, of which over 600 million were obese. It was also estimated that 41 million children under the age of 5 years old are overweight or obese. By 2025, it is estimated that obesity will affect 18% of men and 21% of women worldwide [1]. It is a public health problem, associated with diabetes and cardiovascular disease. With its attendant comorbidities, it is a number one health concern.

Iron is essential for normal cellular function, but in excess can be toxic. Iron-deficiency anemia is the most common form of anemia worldwide, and affects 1.6 billion persons [2]. Obesity is related to the clinical picture of iron deficiency, but this may relate to inflammation rather than nutrition. This chapter will look specifically at obesity from the perspective of its relationship to inflammation and iron regulation.

In observational studies looking closely at the association between anemia and obesity, a clear relationship has yet to be identified. In the third National Health and Nutrition Examination Survey (NHANES) BMI was stratified into normal (<25 kg/m^2 n = 6059), overweight (25 to <30 kg/m^2 n = 5108), mildly obese (30 to <35 kg/m^2 n = 2366), moderate (35 to <40 kg/m^2 n = 850), and severe (≥40 kg/m^2 n = 465). When data were adjusted for age, gender, menstrual periods, race, ethnicity, alcohol use, education, smoking, and dietary iron ingestion, those in the higher weight group did not have a lower hemoglobin. However, the iron stores were lower in the

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severely obese vs. the normal group (odds ratio (OR) 2.4; 95% CI 1.5–3.7) and the odds ratio for inflammation as measured by the C-reactive protein was higher (OR 10.5; 95% CI 6.6–16.6) [3].

The relationships between obesity and anemia are not direct, nor straightforward. In an observational study from Taiwan, the risk for iron-deficiency anemia was compared with dietary intake in 1274 females 19 years of age and older between the years 2005 and 2008. In the normal BMI group vs. the group of 24% who were overweight vs. the group of 25.3% who were obese, the incidence of anemia was 19.5, 8.6, and 6.2% respectively. Anemia was observed to be less in the group presenting with the highest BMI. What was noticed, however, was that the risk of iron-deficiency anemia rose in obese persons eating a higher fat and lower carbohydrate diet (OR 10.119; 95% CI 1.267–80.79) [4]. The relationship between iron status and obesity has been extensively reviewed in 15 studies dating from 1962 through 2011, and confirm that regardless of iron supplementation or other dietary factors, obesity is associated with iron-deficiency markers. Furthermore, iron absorption has been shown to be affected by obesity [5].

Paradoxically, in a Colombia nutritional survey, obesity was associated with a lower incidence of anemia. The survey investigated 3267 females between the ages 13 and 49. The group was divided into adolescent women, ages 13–17 and adult women, ages 18–49. Patients who were pregnant, who exhibited a high C-reactive protein level (>0.12 g/L) or who presented with a low plasma ferritin level (plasma ferritin <12.0 μg/L) were excluded. The anemia was altitude-adjusted. 29.2% of subjects were overweight and 13.1% were obese. 16.1% of the women had iron deficiency and 32.5% were anemic. Both being overweight or being obese were associated with a 20% lower incidence of being anemic than having a normal weight ($P < 0.05$). BMI was not significantly associated with iron deficiency, yet iron deficiency was present in 6.3% of women and anemia present in 12.8% of women who were overweight or obese. Iron deficiency (13.5%) and anemia (21%) were more prevalent in the older population of women [6].

Despite the lack of a clear relationship, it becomes apparent after reviewing these clinical nutrition survey findings that there is an association between iron deficiency and obesity that may be exacerbated by age or a high-fat diet. The next step is to determine what the proposed mechanisms can be and what lessons we can learn from a clinical perspective.

Proposed Mechanisms Linking Iron Regulation with Obesity

Iron transport and its relationship to anemia have been reviewed elsewhere in this book. This discussion will therefore be limited to the relationship between iron transport and obesity. First, we must have a clear understanding of what obesity is, and how it impacts the body with respect to inflammation.

Body Mass Index (BMI)

We can define obesity as an energy imbalance, and for purposes of this chapter base it upon the body mass index, a representation of the body weight or mass adjusted for height. BMI is measured as the ratio of kilograms per meter squared (kg/m^2). The calculation of ones BMI is relatively simple and several tools are available on the web. Here is one that was programmed by this author (http://touchcalc.com/bmi). A BMI of greater than 25 kg/m^2 is considered overweight, and if over 30 kg/m^2 obese. The concern lies in strong observational data that links an elevated BMI to both cardiovascular disease and type 2 diabetes, but what is even more concerning comes from long-term follow-up studies of adolescents with high normal BMI ranges. An Israeli study demonstrated those in the high normal 50th–74th BMI percentiles had had a graded risk for death. The hazard ratio (HR) for persons in the 95th BMI percentile when compared with those in the 5th–24th percentiles was 4.1 (95% CI 3.1–5.4) for death from coronary artery disease during a follow-up period of 30–40 years [7]. There are two forms of obesity, truncal and visceral. In truncal obesity, adipocytes store free fatty acids and become engorged. In visceral obesity, however the fatty acids are first accumulated by the liver and then stored in the abdominal area. Visceral obesity has a higher risk of causing cardiovascular disease.

Obesity and Inflammation

Inflammation, in its broadest sense, is a mechanism the body uses to respond to an "attack" and to initiate repair, particularly from what it considers a foreign body such as a bacteria or a virus. Inflammation can also be triggered by obesity, and in this circumstance, is a low-grade insidious process. The inflammatory process associated with obesity is widespread, affecting the liver, pancreas, heart, brain, and muscle, in addition to adipocytes. In many disease states, the insidious inflammatory response leads to further harm in these tissues. The story of obesity and inflammation starts with the adipocyte (fat cell), which in addition for being a repository for free fatty acids, shares in the control of several metabolic processes, playing roles in the regulation of inflammatory mediators, iron, the appetite, lipids, and sugar. Overexpression of inflammatory and macrophage genes occurs when adipocytes are exposed to obesity and undergo a remodeling process. The presence of obesity recruits and then stimulates the macrophages interspersed with the adipocytes to release of inflammatory cytokines. The fat cell also releases adipokines that play key roles in an autocrine, paracrine, and endocrine fashion. The two most studied adipokines are adiponectin and leptin.

Adiponectin

Adiponectin is a 224-amino-acid collagen-like protein that is related to complement factor C1q and its C-terminal domain is similar to tumor necrosis factor alpha (TNF-α). It is often referred to in the literature as Acrp30. It is secreted from adipocytes, particularly in anorexia, and circulates in plasma, increases the oxidation of free fatty acids (FFA) in many tissues, especially skeletal muscle where it interacts via adiponectin receptor 1 and the liver where it interacts through adiponectin receptor 2. It leads to accumulation of free fatty acids inside the adipocyte, and clears free fatty acids from the circulation. Fat oxidation is mediated by adiponectin activation of AMP-activated protein kinase. This enzyme increases glucose transport into muscle and can be activated by exercise. It improves insulin resistance through the PPAR-α (peroxisome proliferator-activated receptor alpha) pathway. Exercise training may increase adiponectin receptors [8, 9].

Adiponectin increases insulin sensitivity and low adiponectin levels are associated with obesity, type 2 diabetes, and decreased FFA entering the subcutaneous adipocyte. The metabolic disease occurs as excess FFAs are deposited in other locations. Adiponectin levels fall in truncal obesity, and are also low in association with visceral obesity. As the levels fall, insulin resistance increases as do markers of inflammation. Lower adiponectin levels have been implicated in the relationship between macrophages and atherosclerosis. Thus, lowered levels of adiponectin are associated with cardiovascular disease and myocardial infarction [8]. Low adiponectin levels are correlated with elevated C-reactive protein, IL-6, the production of TNF-α and the inhibition of Nuclear factor-κB (NF-κB) signaling. High levels of adiponectin promote IL-10 which blocks inflammation.

The pathogenesis of this relationship is just becoming clearer and a strong relation between adiponectin and metabolic disorders such as type 2 diabetes with macrophage regulation and chronic low-grade inflammation are apparent. Monocytes from the bone marrow are recruited to adipose tissue by adipokines and differentiate into macrophages. There are two phenotypes of macrophages, M1 and M2, and their respond to different cytokines can change their phenotype. M1 macrophages are anti-inflammatory, while M2 type are proinflammatory. Macrophages infiltrate into adipose tissue and when polarized into a proinflammatory M2 phenotype reduce adiponectin receptor 1 and 2 expression, but when proinflammatory stimuli are altered and the M1 phenotype expressed, the adiponectin receptor is upregulated [10].

Adiponectin and Iron

The presence of iron inside the adipocyte decreases adiponectin production. In type 2 diabetes, a low adiponectin state, adiponectin is inversely related to serum ferritin. An increase in iron inside the adipocyte is associated with decreased adiponectin mRNA and regulates adiponectin transcription.

Like the macrophage and the enterocyte, adipocytes have an iron exporter, ferroportin. Ferroportin is responsible for shuttling iron out of the cell. When ferroportin decreases, cells such as adipocytes, macrophages, and enterocytes load with iron. In the adipocyte, this iron loading has been demonstrated to decrease adiponectin [11]. The amount of iron in the adipocyte also regulates leptin and food intake.

Leptin

Leptin is a member of helical cytokines, and its membrane receptors are structurally like the receptors on cytokines. IL-6 and leptin receptors have some subunits in common. It was the first adipokine discovered and has a direct effect on the hypothalamus to reduce appetite and control food intake. In obesity, leptin concentrations increase, but target cells become resistant to its action [8].

Leptin and Iron

Increasing dietary iron intake enhances the appetite and lowers serum leptin levels. This has been shown independent of inflammation and was demonstrated in a study of 75 subjects who had serum leptin levels that were inversely associated with serum ferritin (Pearson's $r = -0.396$, $P = 0.0004$). Ferritin is an intercellular protein capable of storing and binding iron. Small amounts secreted serum function as an iron carrier.

Dietary iron overload increased iron accumulation in adipocytes by 115% in mice, and in turn decreased leptin mRNA. Serum leptin levels decreased with iron loading (Pearson's $r = -0.26$, $P = 0.001$). Iron increased phosphorylation of the cAMP-Responsive Element Binding Protein (CREB) and decreased activity in leptin promoter region. Through this dual action, feeding effects on metabolism were exhibited; iron influenced mice eating behavior. After a 9–12-week period, mice fed a high iron chow ate more during each 15-min feeding interval over a 3-day period when studied in a metabolic chamber ($P < 0.0001$) [12].

Hepcidin and Ferroportin

The linkage between obesity, the ensuing inflammatory response, and subsequent dysregulation of iron lend insights into mechanisms behind type 2 diabetes and cardiovascular disease progression. Understanding this linkage is relevant from a clinical perspective because of its potential to improve management strategies and health outcomes.

Hepcidin is a 25-amino acid peptide, synthesized by the liver. It is discussed extensively elsewhere in this book. In summary, it functions as mammalian iron-exporter inhibitor, mainly expressed in macrophages and in enterocytes, playing a

key role in plasma iron turnover. Increasing liver cell hepcidin interferes with iron release from reticuloendothelial macrophages, and blocks iron absorption by trapping iron in duodenal enterocytes [13]. Ferroportin resides in the cell membrane of enterocytes, macrophages and hepatocytes and is inhibited by hepcidin within 3–6 h [11]. Hepcidin binds ferroportin, internalizing and degrading it [14]. The inhibition by hepcidin locks iron into cells and prevents its entry from enterocytes to macrophages. It also locks it in adipocytes promoting a decrease in adiponectin. In iron deficiency, hepcidin synthesis is downregulated, allowing the ferroportin release of iron from enterocytes and macrophages. Leptin, on the other hand, directly increases liver cell hepcidin. Adipose tissue expression of hepcidin does not appear to respond to low levels of iron [15].

Bacterial lipopolysaccharides and inflammatory cytokines such as interleukin-6 can cause overexpression of the hepcidin gene, and mediate the synthesis and release of hepcidin. On the other hand, hypoxia, ineffective erythropoiesis and anemia both decrease hepcidin synthesis, permitting iron release from enterocytes and macrophages to take part in erythropoiesis [16].

Hepcidin and Obesity

In a case-controlled study, 70 children with iron-deficiency anemia, 35 of whom obese and 35 normal BMI, were compared with 30 age-matched controls without anemia. Hepcidin levels were lowest in anemic children of normal weight ($P < 0.01$) and highest in anemic children who were obese ($P < 0.01$). The hepcidin levels rose in the anemic children who were normal weight after iron therapy, but did not change with the addition of iron therapy in obese children initially anemic even though the serum ferritin increased. This would suggest that hepcidin was also controlled by other factors besides iron deficiency. The C-reactive protein levels were also higher in the obese children than age-matched controls. The relationship with obesity and elevated hepcidin levels could be associated with inflammation [17].

In obesity, hepcidin mRNA expression is increased in adipocytes and correlates with the inflammatory cytokine interleukin-6. This may explain that the low transferrin saturation ratio that is observed in obese patients may well reflect that the fat cell also contributes hepcidin in response to interleukin 6.

Obesity, Iron Regulation, and Inflammation in the Elderly

Obesity may place an added burden on the elderly, particularly with respect to iron status, nutrition, and the immune response. It is estimated that older adults are becoming increasingly obese. The prevalence of obesity in Americans over 65 years of age was 34.6% in 2010 [18]. Being overweight or obese may have a negative impact on mortality in the elderly, and although the elderly have a substantial decline in the immune response, the added impact of obesity on inflammation is not clear. Studies looking at anemia in the elderly are retrospective and observational,

hence do not account for concomitant diseases. Anemia in the elderly may be nutritional, related to poor oral intake, impaired iron absorption, or increased gastrointestinal blood loss. The role of hepcidin in the elderly is not clear and further investigation is needed [5].

Obesity and Hepatic Oxidative Stress

Hepcidin synthesis in the liver is downregulated by decreased iron stores and a low transferrin saturation ratio. Hepcidin production by liver cells increases in response to leptin, as has been demonstrated in hepatoma cells cultured with leptin. The hepcidin promoter has been cloned in these cells, and it has been shown that incubation with leptin activates the signaling pathway, increases hepcidin mRNA, increased activity of the promoter, and increased transcription [13].

Increased liver iron storage in obesity may play a role in nonalcoholic steatohepatitis. This was studied in a genetic mouse model of leptin deficiency obesity, and it was shown that mice fed iron-rich chow for 8 weeks developed hepatic iron overload, principally in the reticuloendothelial system. This was associated with liver cell degeneration and death. TNFα and interleukin-6 were elevated. Inflammatory markers in macrophages were also increased compared with controls. In this model, the combination of iron overload and obesity produced hepatocellular injury [19]. Serum ferritin as well as hepcidin increases in obesity. Furthermore, hepatic iron content measured using magnetic resonance imaging, also is increased in obese subjects. When controlling for BMI, hepatic iron and serum ferritin contribute independently to circulating hepcidin levels. In children with the nonalcoholic fatty liver disease, hepcidin levels are significantly higher than obese children without the disease [20]. Dietary weight loss decreases serum hepcidin ($p = 0.01$), serum ferritin levels ($p = 0.01$), and hepatic iron concentrations ($p = 0.002$) [21].

Maternal Obesity and Hepcidin

Maternal obesity is also associated with a heightened inflammatory response. This can impact the fetus because of the relationships between myelination, iron regulation, and inflammation on neurodevelopment. Hepcidin levels associated with obesity may play a role in decreasing iron availability for myelination. It was demonstrated in animal studies that a maternal high-fat diet prior and during pregnancy induced altered iron regulation in the brain of male offspring [22]. This suggested that obesity and inflammation did not override iron-mediated hepcidin production during pregnancy. It has further been shown that maternal cord blood hepcidin and C-reactive protein was higher while cord blood iron was lower in obese females [23]. But, this is controversial. An analysis of 230 pregnant adolescents between ages 13 and 18 years were studied with respect to obesity, maternal iron status, including hepcidin and inflammatory markers such as interleukin-6. Forty percent of the adolescents were obese, and as predicted had elevated hepcidin

levels ($p = 0.02$) and a positive interleukin-6 association with hepcidin ($P = 0.0001$) at delivery, although not during midgestation [24].

Weight Loss—Bariatric Surgery

Weight loss after obesity has been associated with several positive events, such as an increase in transferrin saturation rate and lowering of the C-reactive protein. In cases where diet alone is not successful, bariatric surgery may be required. The three most common procedures are laparoscopic adjustable gastric banding, Roux-en Y gastric bypass, and laparoscopic sleeve gastrectomy. They are designed to limit stomach capacity and induce early satiety. The presence of small intestine bacterial overgrowth results in the malabsorption of vitamin B12 and thiamine, and occurs in 25–40% of patients regardless of the procedure. Protein malnutrition can occur following the Roux-en Y procedure, but is generally managed with oral supplements. Nevertheless, patients having bariatric surgery need to be monitored with respect to protein, vitamin, and essential mineral malabsorption. They need to be assessed regarding drug absorption and bioavailability. Gastric pH, gastric emptying changes, and altered intestinal surface area can affect drug absorption. Altered intestine anatomy can affect proteins such as cytochrome P450 3A4 and P-glycoprotein, and can change the rate of absorption and the pharmacokinetic profile of medications commonly used. Clinicians must be mindful of these changes when patients are undergoing an organ transplantation, and the profiles for drugs such as cyclosporine and tacrolimus may be changed [25].

Chronic Kidney Disease

Obesity is one of the strongest risk factors for chronic kidney disease. It has been proposed that being overweight or obese increases the risk of developing ESRD by a factor of 2–7 compared to those who have a normal BMI [1]. Both chronic kidney disease and obesity are associated with a heightened inflammatory state regardless of anemia status.

Anemia is an important predictor of all-cause mortality in obese patients with high risk for cardiovascular disease and normal kidney function. In an evaluation of 9697 overweight/obese patients with high risk for cardiovascular disease, mild (HR 1.50 95% CI 1.17–1.93)(Confidence Interval), and moderate to severe (HR 1.61 95% CI 1.04–2.51) anemia were associated with an increased risk of a primary cardiovascular or cerebral vascular events. When adjusted for the presence of kidney disease, anemia is no longer of independent prognostic importance [26].

In patients undergoing hemodialysis, hepcidin synthesis is upregulated, but clinical studies demonstrate that in the dialysis population, this is not influenced by obesity [27]. In a study of 37 obese and 37 normal-BMI hemodialysis patients, hepcidin levels were not significantly different (118.3 ± 67.7 vs. 119.3 ± 78.0 ng/mL; $p = 0.95$). However, hepcidin levels can be lowered in dialysis patients. A small

clinical study in 38 dialysis patients demonstrated that flaxseed oil, already known to reduce inflammatory markers in dialysis patients, reduces hepcidin and increases hemoglobin, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration when compared with medium chain triglycerides. This suggests that in the dialysis population, hepcidin elevation is chiefly a function of inflammation [28]. Hepcidin modulators such as lexaptepid pegol (NOX-H94) are in the safety/efficacy phase (Phase 2a) of testing in dialysis patients with ESA-hyporesponsive anemia (https://www.clinicaltrials.gov/ct2/show/NCT02079896 accessed Jan 1, 2017).

The factors contributing to the anemia of chronic kidney disease do not appear influenced by obesity in this population. They include (1) ineffective erythropoiesis secondary to reduced erythropoietin formation, (2) chronic blood loss related to dialysis, platelet dysfunction and heparin requirements, (3) the anemia of chronic disease related to chronic inflammation and hepcidin, and (4) shortened red blood cell survival that is independent of dialysis. The requirements for recombinant human erythropoietin and intravenous iron are unchanged regardless of BMI, and it is becoming clear that intravenous iron plays a role in perpetuating chronic inflammation regardless of BMI [27]. The consequence of intravenous iron on cytokine activation, oxidative stress, and reactive oxygen species generation in hemodialysis patients and age and sex-matched controls were compared. The area under the serum concentration–time curve for free iron (non-transferrin bound iron) was threefold higher with iron sucrose compared to iron dextran (202 ± 53 vs. 74 ± 23 μM min/L, $P = 0.04$). IL-6 is elevated 2.1-fold with iron dextran and 2.6-fold with iron sucrose but not in controls ($P < 0.05$). Mitochondrial membrane potential decreased in 50% healthy controls and 100% hemodialysis patients (100% vs. 50%) [29]. A liberal vs. conservative approach to iron sucrose therapy in hemodialysis is currently being studied (Proactive IV Iron in Hemodialysis Patients—PIVOTAL Trial—http://www.kidneyresearchuk.org/pivotal).

Conclusion

Obesity is a key risk factor for several disorders—the metabolic syndrome, type 2 diabetes, cardiovascular disease, gestational diabetes, and nonalcoholic steatohepatitis. While obesity is not directly related to anemia, it plays a key role in iron regulation. This regulation, while clandestine, helps potentiate the adverse consequences of obesity. Unraveling the reasons why iron regulation is affected by obesity lends insight into how the metabolic pathways between obesity, inflammation, and cardiovascular disease are related.

The adipocyte is a major cell that interacts with the dietary iron intake. It releases adiponectin, a hormone that helps transport glucose into the cell with exercise and clears free fatty acids from the circulation. Decreased adiponectin is associated with central obesity, type 2 diabetes, and cardiovascular disease. Iron and inflammation play a role in the fat cell release of adiponectin.

Iron and inflammation also stimulate the blockage of leptin, resulting in an increased appetite and potentiating further obesity.

Hepcidin is an important factor that sequesters iron inside the reticuloendothelial system, adipocytes, and the intestinal enterocytes. It blocks intestinal absorption of iron. It is reduced in iron-deficiency anemia, but increased in iron overload, inflammation, and dialysis. Its role is to decrease circulating free iron in the presence of inflammation and to limit iron as a nutrient to invading bacteria.

Obesity is a risk factor for kidney disease, but does not play a role in anemia management in this population. In kidney disease, hepcidin levels are elevated because of inflammation, but intravenous iron sucrose has also been shown to increase interleukin-6 levels.

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Post-Transplant Anemia

11

Venkat Ramanathan and Sreedhar Mandayam

Anemia, as defined by a reduction in hemoglobin or hematocrit value below the lower limit of normal for gender and age, is very common following successful renal allograft placement. This is hardly surprising considering that even well-managed end-stage renal disease (ESRD) patients have an average hemoglobin of 10–11 g/dL in the pre-transplant setting. Some surveys have reported the prevalence of post-transplant anemia (PTA) to be close to 40% at one-year post-transplant follow-up [1–3].

In the early/immediate post-transplant period, anemia is universal due to pre-existing anemia while on dialysis, surgical blood loss, phlebotomy, and aggressive volume resuscitation during surgery. Anemia is seen despite rapid improvement in erythropoietin (EPO) production in the functional allograft and resolution of the uremic milieu occurring post-transplant. Erythropoietin production increases by day 2 and often reaches 4–5-fold by the third post-transplant week [4, 5]. Early erythropoietin surges may not be efficient in correcting anemia due to the persistent uremic milieu. In addition, the endocrine functions of the kidney do not always parallel the excretory functions due to damage to peritubular interstitial cells [6].

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Impact on Patient and Graft Survival

PTA is associated with poor graft survival [7–11]. While this is strictly an association as causality has not been proven, it is possible that anemia may upregulate several inflammatory genes. This upregulation, however, has not been shown to result in acute rejections. Some studies have shown an association between PTA and multiple rejection episodes. Again, these data do not support that PTA predisposes a transplant patient to acute rejection. On the contrary, PTA may be a consequence of acute rejection and its treatment. Patients who have multiple rejection episodes often require more potent immunosuppressive medications that have myelosuppressive properties. In addition, multiple rejections can result in poor allograft function and result in erythropoietin deficiency. In native kidneys, anemia and associated kidney hypoxia favor the development of fibrosis, angiogenesis, and inflammation [12, 13]. But it is unclear if we can extend this observation to renal allografts. Also, the degree of anemia and hypoxia that is needed to induce these fibrotic changes in the allograft is unknown.

PTA is also associated with poor patient survival. In patients with chronic kidney disease (CKD), anemia is associated with congestive heart failure, left ventricular hypertrophy, and increased risk of morbidity, hospitalization, and mortality. However, in kidney transplant patients, these data are less convincing. While there are studies that show a higher risk for mortality [7, 8, 14, 15], other studies have failed to show any association [9, 16]. Again, it begs the question whether anemia and poor patient survival following a renal allograft is merely an association or causal. Similar to observations in dialysis patients, PTA may reflect underlying comorbidities and systemic inflammation, and observed poor outcome may not be related to anemia per se. Clarification of any association will require large randomized controlled studies.

Causes of Anemia

Post-transplant anemia may be related to blood loss, decreased RBC production, or due to increased RBC destruction (Table 11.1). While iron deficiency, folate deficiency, and other vitamin deficiencies cause anemia in all patients, the following sections will primarily address causes of anemia that are unique to kidney transplant patients.

Blood Loss Anemia

Blood loss is a common cause of anemia in kidney transplant patients. In the immediate post-transplant period, intraoperative blood loss and blood loss related to frequent phlebotomies are common causes of anemia. In the first few weeks after transplantation, patients frequently get multiple vials of blood drawn to monitor kidney function, screen for viral infections and also to adjust the doses of

Table 11.1 Causes of post-transplant anemia

(a) Blood loss anemia
Intraoperative blood loss
Repeated blood draws—phlebotomy
Gastrointestinal
Menstrual blood losses
(b) Decreased RBC production
Decreased effective production
Iron deficiency—absolute or functional
Folate deficiency—medications
Pure red cell aplasia—parvo virus, medications
Bone marrow suppression—medications (Mycophenolate, Imuran), infections (viral)
Poor allograft function—erythropoietin deficiency
Ineffective erythropoiesis
Folate deficiency
Sideroblastic anemia—INH and other drugs
(c) Increased RBC destruction
Microangiopathic hemolytic anemia—calcineurin inhibitors, Sirolimus
G6PD deficiency—sulfa medications

immunosuppression medications. In one study, average blood loss related to operative and phlebotomy losses in the first 12 weeks after transplantation was approximately 755–860 mL [17]. This can quickly result in profound anemia, especially if there is preexisting iron deficiency at the time of kidney transplantation or if allograft function is poor. Fortunately, in most patients, brisk production of erythropoietin from the new allograft negates the drop in hemoglobin, despite blood draws.

Later in the post-transplant period, causes of anemia due to blood loss change and include gastrointestinal bleeding and menstrual blood losses. Common causes of gastrointestinal bleeding include gastritis, peptic ulcer disease, diverticulosis, and arterio-venous malformations [18, 19]. While transplant medications, including steroids and mycophenolate, can cause gastritis and significant gastric erosions, infectious etiologies including cytomegalovirus can also play a role. Other rare causes of bleeding include post-transplant lymphoma in GI tract and Kaposi sarcoma.

In women, abnormal uterine bleeding is a common cause of anemia. When on dialysis, hypothalamic–pituitary–ovarian axis dysfunction can lead to menstrual irregularities, anovulation, and infertility [20, 21]. After kidney transplantation, ovarian axis dysfunction may improve resulting in regular menstruation cycles.

Similarly, fertility can also be restored with normal kidney function. But in some transplant patients, despite normal allograft function, luteal insufficiency may persist and premature ovarian failure can occur. It is not uncommon to see menorrhagia worsen after kidney transplantation. Unless specifically asked, women often do not report excessive menstrual blood losses to the transplant physician. The slow decline in hemoglobin and mean corpuscular volume (MCV) levels should prompt the physician to inquire about menstrual losses. If confirmed, patients should be referred early to a gynecologist. It is also important to rule out other structural lesions, including endometrial carcinoma and uterine fibroids. In women with significant menstrual losses, parenteral iron may be frequently necessary to keep up with ongoing losses. When indicated, hysteroscopic endometrial ablation is a safe and effective procedure in transplant patients [22]. However, in some women, hysterectomy may be needed. Kidney transplant patients who undergo hysterectomy have higher incidence of perioperative complications, but surgery does not affect graft outcome [23].

Transplant Medications and Anemia

The majority of medications used in kidney transplant recipients to prevent acute rejections and opportunistic infections can cause PTA. While anemia related to myelosuppression is common, there are unique aspects that deserve special mention.

Calcineurin Inhibitors and Anemia

Calcineurin inhibitors (CNI), tacrolimus and cyclosporine, are associated with microangiopathic hemolytic anemia (MHA) [24, 25]. CNIs exhibit their immunosuppressive properties by inhibiting the cytoplasmic dephosphorylation of NFAT (nuclear factor of activated T-lymphocytes), thereby preventing it from entering the cell nucleus and triggering downstream cytokine production. Similarly, CNI inhibitors also inhibit protein phosphatase 2B in vascular endothelium [26]. This phosphatase inhibition leads to excess secretion of unusually large von Willebrand factor (ULVWF) multimers from vascular endothelial cells, and the anchorage of these long strings of ULVWF to endothelium leads to platelet adhesion and aggregation. Under normal secretory conditions, circulating ADAMTS-13, a vWF cleaving metalloproteinase, will quickly cleave these long strings and avoid platelet thrombi. But CNI inhibitor-induced excess secretion of ULVWF strings appears to overwhelm the capacity of ADAMTS-13 activity, thereby leading to platelet thrombi and TTP. There are also data that CNI inhibitors may mediate Weibel-Palade body exocytosis. WPBs are endothelial granules that store coiled vWF multimers. All these factors play a role in CNI inhibitors causing MHA.

Sirolimus and Anemia

Depending on the anemia definition used, the prevalence of anemia associated with sirolimus (SRL) has been reported to be present in 16–56% of patients [27]. Multiple

factors appear to play a role, including the dose of SRL, other transplant medicines used concomitantly, level of allograft function, and time when assessed post-transplant. Evidence for any direct link between SRL and anemia is best exemplified in CNI withdrawal studies. When patients with CNI toxicity or chronic fibrosis were switched from CNI to SRL-based regimen, a significant drop in hemoglobin was noted, despite improvement in allograft function [28]. Some studies have extended this observation to show improvement in hemoglobin after discontinuation of SRL.

Interestingly, the data in children are less clear. A study from the ESPN/ERA-EDTA registry failed to identify SRL as an independent risk factor for anemia in children. But it is important to note that only 2% of children were maintained on SRL-based regimen in the registry.

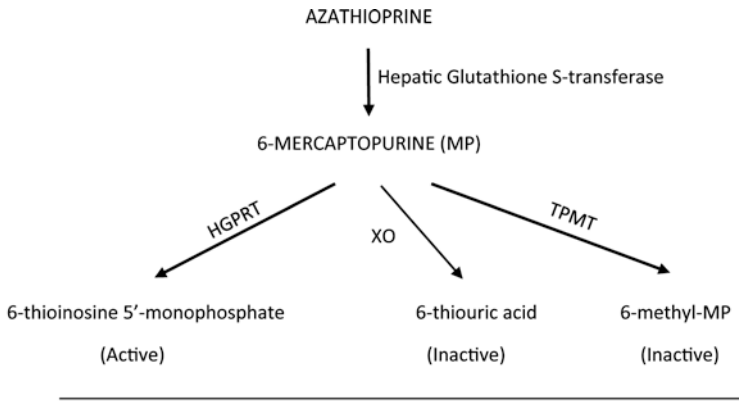
Sirolimus-induced anemia is associated with low mean corpuscular volume (MCV), microcytosis. While it is tempting to attribute microcytosis to absolute iron deficiency [29], or functional iron deficiency resulting from inflammation and reticuloendothelial blockade [28], observed microcytosis may be independent of iron homeostasis. In some patients, administration of parenteral IV iron improved microcytosis [30], but the role of iron's contribution to microcytosis is not convincingly proven. Microcytosis may be iron-independent and be related to direct mTOR inhibition affecting protein translation, cell growth, and cell cycle progression [31, 32]. SRL may affect erythroid progenitor differentiation and erythropoietin receptor-mediated proliferation [32, 33]. It is unclear if SRL induces an erythropoietin-resistant state and if this resistance can be overcome with higher level of endogenous or exogenous erythropoietin.

In SRL-treated patients, anemia appears to be less commonly observed than the observation of a low MCV. Some studies suggest that anemia develops only when there is associated EPO or iron deficiency. When the mTOR pathway is inhibited in healthy rats with normal kidney function, microcytosis is induced, but not anemia [34]. But, in humans with allograft dysfunction, SRL often leads to microcytic anemia rather than isolated microcytosis.

There are also reports of microangiopathic hemolytic anemia associated with sirolimus and everolimus use. However, the exact mechanism of MHA associated with mTOR inhibitors is currently unknown.

Azathioprine and Anemia

With the advent of mycophenolate, there has been a significant decline in the use of AZA in kidney transplant patients. Thus the use of AZA is now limited to patients who are unable to tolerate mycophenolate or sirolimus, or when pregnancy is planned since mycophenolate and sirolimus have significant teratogenic potential and should be avoided. With limited indications, anemia resulting from AZA use is not commonly encountered in clinical practice. In contrast to SRL-associated microcytic anemia, azathioprine (AZA) use is associated with macrocytic anemia and is dose-related. While leukopenia is more common, anemia can still be seen in a significant number of patients.



HGPRT: Hypoxanthine guanine phophoribosyltransferase

XO: Xanthine oxidase

TPMT: Thiopurine methyltransferase

Fig. 11.1 Metabolism of azathioprine

AZA is first metabolized to 6-mercaptopurine (6-MP), which is then activated via the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) pathway. As shown in Fig. 11.1, 6-MP is inactivated by two pathways; thiol methylation via the thiopurine S-methyltransferase (TPMT) pathway, and oxidation via the xanthine oxidase (XO) pathway. Inhibition of either pathway can lead to accumulation of its metabolite, 6-MP, and result in significant bone marrow toxicity. Co-administration of drugs that inhibit XO pathway (Allopurinol) can cause bone marrow toxicity. Similarly, patients who are born with low or absent TPMT activity are also at increased risk of severe bone marrow toxicity.

When severe bone marrow suppression is noted after AZA use, genetic polymorphism of TPMT should be considered. In addition to genotyping tests, phenotyping can be done by measuring red cell TPMT activity. Approximately 10% of Caucasians and African Americans are heterozygous, conferring intermediate TPMT activity.

Mycophenolate and Anemia

Mycophenolate, an inhibitor of inosine-5′-monophosphate dehydrogenase enzyme, is associated with bone marrow suppression and cytopenia [35]. Anemia has been reported in almost a quarter of patients who take mycophenolate. Anemia and leukopenia are more common, when mycophenolate is used in combination with tacrolimus. This is probably related to higher level of mycophenolic acid resulting from drug interaction with tacrolimus. Inhibition of IMP-dehydrogenase in erythroid cells appears to be the cause of anemia [36]. Significant suppression of bone marrow EPO-receptors [37] and genetics [38] may also play a role. In addition to direct bone marrow suppression, mycophenolate is also associated with gastrointestinal bleeding, and resultant anemia from blood loss.

Angiotensin Blockade and Anemia

In kidney transplant patients with stable allograft function, renin–angiotensin system (RAS) blockade is often used for blood pressure control, heart failure management, and for reducing proteinuria. Since angiotensin II (AT II) plays an important role in the pathogenesis of CNI nephrotoxicity and chronic fibrosis, it is often tempting to use RAS blockade to prolong graft half-life. However, the use of Losartan had no significant effect on the composite end point of ESRD, death or doubling of serum creatinine value [39]. Even though these medications are well tolerated, anemia is often an under-recognized complication.

During hematopoiesis, hematopoietic stem cells undergo a complex series of proliferation and differentiation within the bone marrow. This is controlled by numerous cytokines and growth factors, including the renin–angiotensin system (RAS) [40, 41]. There is evidence of the existence of a functional RAS system in bone marrow. AT II interacts with bone marrow AT1 receptors leading to higher numbers of burst-forming unit-erythroid (BFU-E) colonies. This erythroid stimulation is blocked by the addition of an angiotensin receptor blocker, resulting in anemia. In addition to regulating ATII synthesis, angiotensin--converting enzyme (ACE) is primarily responsible for the degradation of a tetrapeptide, acetyl-N-Ser-Asp-Lys-Pro (AcSDKP), an endogenous physiologic inhibitor of hematopoiesis. Hence, inhibition of ACE enzyme will lead to lower generation of hematopoietic stimulant, ATII, and also parallel accumulation of hematopoietic inhibitor, AcSDKP. A better understanding of the role of ATII in hematopoiesis has led to physicians to use RAS blockade drugs for the treatment of post-transplant erythrocytosis [42–45].

Dapsone and Anemia

Dapsone (4,4′-diaminodiphenyl sulfone), a sulfone antibiotic that inhibits folate synthesis, is rarely used in post-transplant patients for prophylaxis against *Pneumocystis jirovecii* infection [46]. In patients with allergy to sulfamethoxazole-trimethoprim, most centers now use atovaquone or aerosolized pentamidine, instead of dapsone. Dapsone is also occasionally used as alternate therapy for transplant patients with severe toxoplasmosis.

Methemoglobinemia can be a life-threatening condition and has been reported with use of oral dapsone. Methemoglobin is an abnormal form of hemoglobin resulting from the oxidation of iron in the heme molecule from ferrous to ferric form. This change shifts the oxygen-hemoglobin dissociation curve to the left, thereby impairing the release of oxygen to the tissues.

Dapsone is metabolized via cytochrome P450 2C and 3A4 pathways to metabolites. Both the parent drug and metabolites cause oxidant stress to the red cell, and are responsible for the oxidation of heme iron to ferric form, thereby causing methemoglobinemia and resultant hemolytic anemia [47]. Symptoms of methemoglobinemia can range from cyanosis, headache, fatigue to serious events such as cardiac arrhythmias, seizures, and death. While direct measurement of methemoglobin concentration will clinch the diagnosis, oxygen saturation differential between pulse oximetry reading and arterial blood gas measurement should alert the transplant

physician. In addition to discontinuation of dapsone, patients may require intravenous methylene blue, or in severe cases, dialysis.

Concurrent G6PD deficiency increases the risk of oxidative stress from glutathione depletion. Since the use of Dapsone is limited to a small number of transplant patients and since it is often co-administered with other medications that cause anemia, it is prudent to check for concurrent G6PD deficiency before starting Dapsone. Dapsone and nitrofurantoin are likely to be unsafe in patients with moderate to severe enzyme deficiency. Other drugs that can cause methemoglobinemia include metoclopramide and sulfonamides.

Isoniazid and Anemia

Isoniazid (INH) is often used in transplant patients to treat latent tuberculosis infection. INH causes sideroblastic anemia by interfering with pyridoxine (vitamin B6) metabolism. INH metabolite can inhibit pyridoxine phosphokinase, an enzyme that converts pyridoxine to active pyridoxal-5-phosphate. Active pyridoxal-5-phosphate plays a pivotal role in heme biosynthesis. During heme biosynthesis, the initial step of glycine and succinyl CoA interaction to form 5′-aminolevulinic acid in the erythroid cell is dependent on a mitochondrial enzyme that requires pyridoxal-5-phosphate as a cofactor. So, the absence of active pyridoxal-5-phophate in INH-treated patients will interfere with heme synthesis and result in ineffective hematopoiesis and anemia. Bone marrow aspirate showing the presence of nucleated erythroblasts with abnormal iron-laden mitochondria in the perinuclear area (ringed sideroblasts) is characteristic of this condition. To avoid this complication, INH is routinely administered with vitamin B6. Since alcohol also causes sideroblastic anemia and can worsen liver injury, patients taking INH should abstain from alcohol use.

Pure Red Cell Aplasia

Pure red cell aplasia (PRCA) can occur in transplant recipients from a wide variety of causes. PRCA is a syndrome characterized by anemia, usually severe, very low reticulocyte count and complete absence of erythroid precursors on bone marrow examination. In PRCA, WBC and platelet counts are normal. PRCA has been rarely reported with the use of mycophenolate and the exact mechanism is unknown. PRCA is sometimes reversible with dose reduction or withdrawal of the drug. Other medications that have been associated with PRCA include azathioprine, sulfonamides, INH, phenytoin, zidovudine, valproic acid, and clopidogrel.

In addition to medications, PRCA can also be associated with parvovirus B-19 infection. By attaching to the blood group P antigen receptor, parvovirus directly destroys proerythroblasts, resulting in PRCA. In this condition, bone marrow examination is characterized by the presence of giant proerythroblasts. The diagnosis is confirmed with the presence of parvovirus B-19 DNA in the serum, and also with anti-B19 IgM antibodies in serum. In some patients, anemia is refractory and may need frequent blood transfusions and multiple doses of intravenous immune globulin (IVIg) infusions. In addition to Parvo-viral infection, several other viral

etiologies including viral hepatitis, cytomegalovirus, Epstein–Barr virus, and HIV infection have been reported to cause PRCA.

Diagnosis

An acute drop in hemoglobin concentration in the immediate post-transplant period is most likely related to a combination of intra-operative blood loss and dilutional component from over-zealous fluid replacement in the post-operative period, especially when there is delayed graft function. If the drop is significant and disproportionate, imaging study is warranted to rule out bleeding complication and hematoma formation.

Anemia noted in the outpatient setting is often related to persistent EPO or nutritional deficiencies, transplant drugs or infections. Anemia should warrant complete work up including checking red blood cell indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), reticulocyte count, iron studies (serum iron, total iron binding capacity, serum ferritin), folate, and vitamin B12 concentrations. In patients with iron deficiency, stool occult blood should be checked and if positive, will need complete gastrointestinal work up including endoscopy. In women, menstrual blood losses should be ruled out as the cause of anemia. Since nutritional deficiencies are easily correctable, it is prudent to rule them out first. If there is no nutritional deficiency, peripheral blood smear is often helpful, along with search for evidence of hemolysis, including serum LDH, indirect bilirubin, and haptoglobin levels. Peripheral smear is often helpful to diagnose microangiopathic hemolysis (schistocytes).

When drugs are suspected, there is usually a temporal relationship between the introduction of a new drug and subsequent anemia. Serial monitoring of lab values will confirm if anemia correlates with the recent addition of sirolimus or RAS blocking drug. Even though these drugs or PRCA can cause isolated anemia, cytopenia caused by most immunosuppressive transplant drugs involve other cell lines, including white cells and platelets.

When etiology of anemia is not evident, bone marrow examination may be required. Since most information required for anemia diagnosis is often obtained with laboratory blood tests, bone marrow biopsy is often not required. But when lab tests are inconclusive, bone marrow examination can show evidence of ineffective erythropoiesis, megaloblastic erythropoiesis, absence of erythroid precursors with presence of giant proerythroblasts suggesting PRCA, or nucleated erythroblasts with abnormal iron-laden mitochondria in the perinuclear area (ringed sideroblasts) suggesting sideroblastic anemia.

Treatment

Since post-transplant anemia impacts graft and patient survival, most physicians agree to treat anemia in this setting. However, the desired goal of hemoglobin correction is not well understood in the absence of randomized studies in this

population. Randomized studies in chronic kidney disease and dialysis patients have shown convincingly that higher hemoglobin does not reduce cardiac and vascular events, but in fact may increase the risk [48–50]. It is still debated if the true culprit is the use of erythropoiesis-stimulating agents or higher target hemoglobin. In the absence of randomized studies in PTA, lessons learnt from these CKD trials have largely influenced transplant physicians' decision-making.

The etiology of anemia often dictates treatment approach. In patients with clearly defined etiologies for PTA, addressing the cause of anemia is the prudent first step. If the initial blood work up indicates nutritional deficiency of iron, folate or vitamin B12, supplementation is recommended. As repletion is ongoing, it is important to identify the cause of nutritional deficiency and address them in parallel. For example, iron deficiency is commonly related to gastrointestinal and gynecologic blood losses. The patient should be referred to appropriate consultant for further work up and treatment. If there is no evidence of ongoing blood loss, patient needs to be evaluated for gastrointestinal malabsorption of iron. Iron malabsorption should also be considered if iron deficiency is refractory to oral supplementation.

Oral iron is often tolerated well. If intolerant to oral iron or ongoing iron and blood loss overwhelms oral iron therapy, parenteral iron is advised. Among kidney transplant patients, there is no difference in infection rates or severe gastrointestinal side effects between oral and intravenous iron preparations [51].

While diagnosing and treating nutritional deficiency anemia is straightforward, PTA caused by immunosuppressive drug is often a diagnosis by exclusion. Treatment is equally complicated. This is especially true in the subset of patients who are at high risk for rejection, in whom dose reduction may not be possible. In sirolimus-related anemia, there are reports of improvement in anemia with iron supplementation, especially if there is laboratory evidence of iron deficiency. Persistent and unexplained anemia in patients taking azathioprine should be investigated with TPMT phenotype and genotype testing. If the patient carries two nonfunctional alleles with low or deficient activity, AZA should be avoided. For individuals carrying one functional and one nonfunctional allele, a 30–50% reduction in AZA dose is recommended. In these patients, it is important to avoid concomitant use of allopurinol and other drugs that can potentially inhibit TPMT pathway, including olsalazine, sulfasalazine, or mesalazine. For drug-induced microangiopathic hemolytic anemia, treatment often includes discontinuation of the offending drug, calcineurin inhibitor or mTOR inhibitor, and infusion of FFP and/or plasmapheresis. After treatment, reintroduction of another CNI is often tolerated without recurrence of TMA, but in some patients disease recurs [52]. When CNI inhibitors are reintroduced, it is very important to follow these patients for recurrence. Pure red cell aplasia related to Parvovirus needs special mention. Usually, these patients are resistant to conventional doses of erythropoietin. In resistant cases, intravenous immunoglobulin can be tried.

Erythropoietin

There are only few studies that have prospectively addressed the utility of anemia correction, specifically in post-transplant patients with erythropoietin. In the open-label, randomized controlled, CAPRIT study (Correction of Anemia and Progression of Renal Insufficiency in Transplant patients), Choukroun et al. have shown that higher hemoglobin target reduces the progression of chronic allograft nephropathy [53]. When patients with estimated creatinine clearance of <50 mL/min/1.73 m^2 were treated with epoetin-beta, complete correction to normalize hemoglobin value was associated with smaller decline in kidney function in 2 years when compared with patients with partial correction of anemia (2.4 vs. 5.9 mL/min per 1.73 m^2). Also, fewer patients in the normalization group progressed to ESRD, and cumulative death-censored graft survival was better in that group. Anemia correction also led to improvement in the quality of life. Unlike anemia studies in CKD patients, there was no increase in cardiovascular events in patients in the higher hemoglobin group. But it is worth mentioning that this observation has not been confirmed with another randomized study, and also the sample size was small compared to CKD studies.

Despite CAPRIT study, use of ESA after kidney transplantation is not widespread. There are anecdotal reports of erythropoietin use in the peri-transplant period to reduce delayed graft function, shorten the duration of anemia, and eventually improve patient and graft survival. While studies have shown that PTA is associated with poor patient and allograft survival, it is unclear if correction of PTA improves survival. Most physicians reserve the use of erythropoietin to patients with anemia and poor allograft function.

Transfusion

Since transplant patients are closely followed, anemia is often addressed early and not severe to warrant blood transfusion, unless there is an acute bleed. Administration of intravenous iron and erythropoiesis-stimulating agents in dialysis population has also significantly reduced the need for blood transfusions in the immediate post-transplant period. Utilization of blood products has also decreased since transfusion thresholds have changed significantly over the years. Currently, most centers do not transfuse until hemoglobin is <8 g/dL. Even in patients with preexisting coronary artery disease, transfusion threshold of 8 g/dL seems reasonable. Since kidney transplant patients undergo extensive cardiac evaluation prior to transplant, it is reasonable to maintain this transfusion threshold. In one study, perioperative blood transfusion was associated with reduced long-term graft survival [54]. It is unclear if the link is related to anemia, cause of anemia, transfusion, or the higher rate of delayed graft function noted in the transfusion group. When blood transfusions are needed, most programs give leukocyte-poor filtered blood or CMV sero-negative blood products to minimize transmission of viruses.

Conclusion

PTA is common after kidney transplantation and is associated with poor allograft and patient survival. Blood loss, poor allograft function and erythropoietin deficiency, opportunistic infections, and medications commonly used to prevent and treat rejection and infections in the post-transplant period are common causes of PTA. Similar to clinical trials in CKD patients, we need randomized studies to address the impact of correction of anemia in the post-transplant period.

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Special Populations with Anemia: Anemia in the Pediatric Patient

12

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Introduction

Anemia is probably the most common hematological abnormality seen in the pediatric population. It is estimated that approximately 20% of children in the United States and 80% of children in developing countries develop anemia at some point during their childhood and adolescence [1]. The most recent Pediatric Nutritional Surveillance system report showed a slight increase in the overall prevalence of anemia in children in the US from 13.4% in 2001 to 14.6% in 2010 [2]. Certain ethnic groups do have a higher incidence such as the African-American (24.6%) and Hispanic (18.4%) populations [3]. Anemia is a sign, not a diagnosis by itself, and it is often a symptom of an underlying disease. Though it is not a primary disease process, anemia can lead to severe morbidity and even cause death on rare occasions. In a recent systematic analysis of the global anemia burden, Kassebaum et al. reported that anemia accounted for 8.8% of the total disability from all conditions, and women and children <5 years of age had the highest burden [4]. Therefore, it is important to recognize anemia, evaluate the underlying disease process and ideally, intervene therapeutically to decrease the burden of associated symptoms.

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Table 12.1 Normal reference values for hemoglobin and mean corpuscular volume

	Hemoglobin (g/dL		Mean corpuscular volume (fL)	
Age	Mean	Lower limit[a]	Mean	Lower limit[a]
Birth (cord blood)	16.5	13.5	108	98
1–3 days (capillary)	18.5	14.5	108	95
1 week	17.5	13.5	107	88
2 weeks	16.5	12.5	105	86
1 month	14.0	10.0	104	85
2 months	11.5	9.0	96	77
3–6 months	11.5	9.5	91	74
0.5–2 years	12.0	11.0	78	70
2–6 years	12.5	11.5	81	75
6–12 years	13.5	11.5	86	77
12–18 years female	14.0	12.0	90	78
12–18 years male	14.5	13.0	88	78
18–49 years female	14.0	12.0	90	80
18–49 years male	15.5	13.5	90	80

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[a]Two standard deviations below mean

Definition of Anemia

Anemia is defined as a reduction in hemoglobin concentration, hematocrit, or red cell mass below the lower limit of the normal range. The lower limit for the pediatric patient is usually set at two standard deviations below the mean for age and sex for the general population. The age and gender-related reference values for hemoglobin are provided in Table 12.1 [5]. Caution should be executed when interpreting these values for a particular patient. For example, a hemoglobin value of 13 g/dL is perfectly normal for an 8-year-old child, but it is below the lower limit of the normal range for a newborn or a postpubertal adolescent. Also, children and adolescents with cyanotic congenital heart disease, chronic pulmonary disease, or hemoglobinopathies with abnormal oxygen affinities can have normal or higher than normal hemoglobin values, but can be functionally anemic (e.g., poor tissue oxygenation). Therefore, it is important to characterize anemia based not only on hemoglobin levels, but also based on the patient's age, gender, cardiopulmonary status, living conditions (high altitude), etc.

Etiology

The causes of anemia in children can be classified by the physiological process of red blood cell (RBC) production and loss or by morphology based on the size of the RBCs. The most common causes based on this classification are listed in Table 12.2.

Table 12.2 Classification of anemia

Physiologic
I. Impaired red blood cell production
A. Nutritional deficiencies
a. Iron deficiency
b. Folic acid deficiency
c. Vitamin B12 deficiency
d. Protein calorie malnutrition
i. Marasmus,
ii. Kwarshiorkar
e. Vitamin B6 deficiency
f. Scurvy
B. Bone marrow failure
a. Pure red cell aplasia
i. Diamond Blackfan anemia
ii. Transient erythroblastopenia of childhood
b. Aplastic anemia
c. Inherited bone marrow failure syndromes
d. Marrow infiltration
i. Malignancies
ii. Osteopetrosis
iii. Storage disorders
C. Dyserythropoiesis
a. Thalassemia syndromes
b. Lead poisoning
c. Sideroblastic anemia
d. Congenital dyserythropoietic anemia
e. Erythropoietic protoporphyria
D. Miscellaneous
a. Anemia of chronic disease
b. Hypothyroidism
c. Anemia due to chronic kidney disease
d. Connective tissue disorders
e. Hemoglobin mutants with decreased affinity for oxygen
II. Hemolytic anemia (increased red blood cell destruction)
A. Defects of hemoglobin
a. Quantitative disorder (Thalassemias)
b. Qualitative disorder (sickle cell disease)
B. RBC membrane defects
a. Hereditary spherocytosis
b. Hereditary elliptocytosis
c. Hereditary stomatocytosis
C. Enzyme deficiencies
a. Glucose 6-phosphate dehydrogenase deficiency
b. Pyruvate kinase deficiency

(continued)

Table 12.2 (continued)

D. Immune
a. Isoimmune
i. Rh incompatibility
ii. ABO incompatibility
b. Autoimmune
i. Primary or idiopathic
ii. Secondary
1. Lupus
2. Other connective disorders
E. Miscellaneous
a. Microangiopathic hemolytic anemia
i. Thrombotic thrombocytopenic purpura
ii. Hemolytic uremic syndrome
b. Paroxysmal nocturnal hemoglobinuria
c. Burns
d. Hypersplenism
III. Blood loss
a. Acute
i. Fetomaternal hemorrhage
ii. Trauma
iii. Splenic sequestration
b. Chronic
i. Gastrointestinal blood loss
Morphologic
I. Microcytic
a. Iron deficiency anemia
b. Thalassemia: Alpha thalassemia, Beta thalassemia
c. Hemoglobinopathies
d. Anemia of chronic disease (later stages)
e. Chronic lead poisoning
f. Sideroblastic anemia
II. Macrocytic
a. Folic acid deficiency
b. Vitamin B12 deficiency
c. Aplastic anemia
d. Hypothyroidism
e. Liver disease
f. Diamond Blackfan anemia
g. Inborn error of metabolism: Hereditary orotic aciduria
III. Normocytic
a. Acute blood loss
b. Hemolytic anemia
c. Chronic renal disease
d. Anemia of chronic disease (early stages)
e. Splenic sequestration

The physiological causes of anemia consist of decreased RBC production, bone marrow failure, dyserythropoiesis, hemolytic anemias, and blood loss. Morphological classification of anemia is divided into three major groups based on the mean corpuscular volume (MCV) of the RBC as microcytic (MCV below the lower limit for age), macrocytic (>100 fL), and normocytic (78–100 fL). However, these categories are not mutually exclusive as may occur when there is more than one mechanism causing anemia. For example, anemia associated with chronic kidney disease could be related to decreased erythropoietin production, iron deficiency, effect of uremia on red blood cell membranes, and/or chronic inflammation. Macrocytic RBCs are frequently seen in hemolytic anemias due to either an increased number of macrocytic reticulocytes or a relative deficiency of folic acid due to increased RBC turnover. Likewise, a microcytic, hypochromic (decrease in mean corpuscular hemoglobin) anemia can occur due to the coexistence of iron deficiency and lead poisoning. Finally, the classification of anemia can change during the course of the disease from one category to another. For example, RBCs are typically normocytic during the initial stages of chronic disease but can become microcytic in the later stage due to associated iron deficiency.

Diagnostic Approach

Clinical

Anemia in children can be identified through universal screening or based on signs and symptoms. The American Academy of Pediatrics recommends universal screening with a hemoglobin or hematocrit measurement for all infants at 12 months of age [6]. In addition, newborn screening is mandated and can identify patients with hemoglobinopathies (sickle cell disease, beta thalassemia). The signs and symptoms of anemia that may be present largely depend upon how abrupt the onset of anemia is. The onset can be very rapid (e.g., acute blood loss, hemolysis) or insidious (iron deficiency, anemia of chronic disease, lead poisoning). Family and medical history often provide valuable information in the diagnostic evaluation of an anemic patient. The majority of children with mild anemia of insidious onset are clinically asymptomatic. Infants and young children often present with non-specific symptoms such as poor feeding, irritability, excessive sleepiness, or tiredness. Older children and adolescents may have shortness of breath, palpitations, syncope, or exercise intolerance. Parents also often report pallor as a presenting symptom, which has poor sensitivity for predicting mild anemia, but correlates well with severe anemia [7, 8]. Physical examination may show pallor, jaundice, lymphadenopathy, hepatosplenomegaly, frontal bossing and congenital anomalies, and reflect a variety of causes of anemia. Vital signs (heart rate, respiratory rate, blood pressure, and oxygen saturation) should be evaluated in all anemic patients to ensure hemodynamic stability. In addition, patients may have signs and symptoms associated with a chronic condition such as systemic lupus erythematosus, rheumatoid arthritis, chronic kidney disease, or hepatic disease. Clinical clues for some causes of anemia are enumerated in Table 12.3.

Table 12.3 Historical clues in the evaluation of anemia in children

Clinical clue	Suspected cause of anemia
Excess intake of cow's milk	Iron deficiency
Chronic blood loss	Iron deficiency
Exclusive intake of goat's milk	Folic acid deficiency
Strict veganism	Vitamin B12 deficiency
Pica	Iron deficiency, lead poisoning
Family history of splenectomy, cholecystectomy	Hereditary spherocytosis
Family origin of Mediterranean and Middle Eastern ancestry	Beta thalassemia, glucose 6-phosphate dehydrogenase (G6PD) deficiency
Family origin of South East Asian ancestry	Alpha thalassemia, HbE disease (Cambodia, Laos)
Exposure to drugs, toxins	G6PD deficiency, folic acid deficiency, lead poisoning
Ingestion of certain foods, e.g., Fava beans	G6PD deficiency

Laboratory

The initial laboratory evaluation of anemia includes a complete blood count, reticulocyte count, and a peripheral smear. Most physicians use the morphological classification of anemia based on MCV (microcytic, macrocytic, or normocytic) to help guide the diagnostic process (Table 12.2). An elevated reticulocyte count (or percentage) suggests hemolysis or chronic blood loss as the cause of anemia, whereas a decreased count (or percentage) suggests decreased RBC production. A reticulocyte index (reticulocyte count x patient's hematocrit/normal hematocrit) is a more accurate marker of erythropoietic activity. In general, a reticulocyte index ≥3 indicates chronic blood loss or a hemolytic process, while an index of <3 (often <1.5) is seen in patients with impaired RBC production [9]. Evaluation of a peripheral blood smear of a child may provide particularly valuable clues toward a particular etiology of anemia. For example, the presence of a large number of spherocytes strongly suggests the diagnosis of hereditary spherocytosis. Table 12.4 lists some of the important blood smear findings and their associated clinical significance. The use of special RBC stains is necessary to identify inclusion bodies such as Heinz bodies (RBC enzymopathies, chronic liver disease, asplenia), siderocytes (aplastic anemia, chronic infection), and intraerythrocytic parasites (malaria, babesiosis).

After the initial laboratory evaluation has been conducted, additional studies may be helpful to identify the physiological process resulting in anemia (Table 12.2). Reduced serum ferritin (<12 ng/mL) levels with an elevated total iron binding capacity (TIBC > 450 μg/mL) suggests iron deficiency anemia. An elevated lactate dehydrogenase level, a low haptoglobin level, and indirect hyperbilirubinemia point toward a hemolytic process. Other tests required for diagnostic confirmation include the osmotic fragility test (hereditary spherocytosis), hemoglobin electrophoresis (sickle cell disease, thalassemia, and other hemoglobin variants), RBC enzyme panel (G6PD and pyruvate kinase deficiency), direct and indirect antiglobulin test (autoimmune hemolytic anemia), and CD55/59 flow cytometric assay (Paroxysmal nocturnal hemoglobinuria).

Table 12.4 Diagnostic significance of peripheral blood smear

Finding	Conditions seen
Target cells	Hemoglobin SC disease, HbE disease, thalassemias, severe iron deficiency
Spherocytes	Hereditary spherocytosis, immune hemolytic anemia
Elliptocytes or Ovalocytes	Hereditary elliptocytosis
Stomatocytes	Hereditary stomatocytosis, Rh null blood group, cold hemolysis
Irreversibly sickled RBCs	Sickle cell syndromes
Howell-Jolly bodies (nuclear remnants)	Hyposplenism, asplenia, dyserythropoietic anemias, severe iron deficiency
Basophilic stippling (ribosomal aggregation)	Lead poisoning, thalassemias, severe iron deficiency
Cabot's rings	Lead poisoning, pernicious anemia
Fragmented RBCs, Schistocytes	Microangiopathic hemolytic anemia, disseminated intravascular coagulation
Crenated RBCs	Uremia, acute hepatic necrosis

Specific Causes of Anemia in Children

Anemia in the Newborn

Anemia in the neonate can be classified into three major categories: anemia due to blood loss, hemolysis, or decreased RBC production. Anemia as a result of blood loss can occur due to twin-to-twin transfusion, internal hemorrhage that occurs with a cephalohematoma or splenic rupture from a traumatic birth, an intraventricular hemorrhage that occurs in premature, low birth weight babies, or fetomaternal hemorrhage. Fetomaternal hemorrhage is diagnosed by the Kleihauer-Betke acid elution test which is based on the resistance of fetal hemoglobin to elution from the intact cell in an acid medium [10]. Fetal–maternal transfusion occurs in approximately 50% of pregnancies, but does not usually result in significant blood loss [11]. Additional risk factors for substantial blood loss in the neonate include rupture of the umbilical cord, placental abruption, placenta previa, placental tumors, and amniocentesis during the third trimester of pregnancy.

Immune hemolytic anemia occurs as a result of blood group incompatibility [Rh (D antigen), ABO, and minor blood group incompatibilities] between the mother and the fetus. Newborns with immune hemolytic anemia usually present with anemia, jaundice, and a positive Coomb's test within the first 24 h of life. These infants need to be monitored closely with appropriate interventions (phototherapy, exchange transfusion) as indirect bilirubin levels can rise rapidly and lead to kernicterus. Although hemolytic anemia due to Rh incompatibility is usually severe, its incidence has decreased dramatically thanks to antenatal screening and the use of Rh immunoglobulin (RhoGam) in Rh-negative mothers. In contrast, hemolytic anemia secondary to ABO incompatibility is usually milder and occurs in the setting of a mother with blood Group O and an infant who has either Group A or B blood type.

Other conditions that may manifest as hemolytic anemia in the newborn period include G6PD deficiency, hereditary spherocytosis, and thalassemia syndromes.

Anemia as a result of decreased RBC production in the neonate can occur due to physiological anemia of infancy or anemia of prematurity (AOP), Diamond-Blackfan anemia (discussed later in the chapter), and congenital infections. After birth, there is initially a decrease in RBC mass due to transition from a hypoxic state in utero to a hyperoxic state with increased tissue oxygenation. This process continues until oxygen delivery is inadequate which in turn stimulates erythropoiesis again. Due to this physiological adaptation to postnatal life, all term infants develop a normocytic and asymptomatic anemia (hemoglobin 9–11 g/dL) at 8–12 weeks of age. AOP, on the other hand, is an exaggerated physiological response, characterized by a normocytic, normochromic anemia with a decreased reticulocyte count and decreased serum erythropoietin (EPO) levels. The low hemoglobin concentration in these premature infants is due to a lack of third-trimester hematopoiesis and iron transport, suboptimal EPO level and response, shorter RBC life span and iatrogenic blood loss in relation to their body weight. The nadir of hemoglobin (7–9 g/dL) typically occurs by 4–8 weeks after birth and usually improves by 3–6 months of age [12]. The management of AOP includes optimal nutrition with iron supplementation and judicious use of blood sampling and blood transfusion. Routine use of recombinant EPO for the treatment of AOP is not recommended due to its limited benefit in reducing blood transfusions and exposure to blood donors, and because its early use (first 8 days after birth) can increase the risk of retinopathy of prematurity [13].

Pure Red Cell Aplasia

Diamond-Blackfan anemia (DBA) is a rare congenital pure red cell aplasia that results from defective ribosomal biosynthesis in bone marrow erythrocytic progenitors that leads to early apoptosis of RBCs [14]. Mutation in the RPS19 gene is the most common genetic defect and is present in 25% of patients with DBA. Most patients present during the first year of life with progressive severe macrocytic anemia, reticulocytopenia, elevated fetal hemoglobin (HbF) and adenosine deaminase (ADA) levels and a normocellular marrow with a paucity of erythroid precursors. About 30% of patients have associated anomalies such as short stature, dysmorphic facies, skeletal abnormalities (e.g., triphalangeal or bifid thumb), or congenital heart disease. Patients with DBA are at risk to develop secondary malignancies such as acute myeloid leukemia and osteosarcoma. Treatment options for DBA include glucocorticoids, chronic blood transfusions, and bone marrow transplantation for patients with severe anemia who are transfusion-dependent.

Transient erythroblastopenia of childhood (TEC) is an acquired form of pure red cell aplasia characterized by anemia with reticulocytopenia [15]. In contrast to DBA, patients with TEC are usually older (mean age 2–3 years), have normocytic anemia, normal HbF and ADA levels and do not have associated physical anomalies. Parvovirus B19 has been implicated in the pathogenesis of TEC, especially in the setting of underlying chronic hemolytic anemia such as hereditary spherocytosis

or sickle cell disease. Most cases resolve spontaneously without any specific interventions and almost never recur. Blood transfusion may be required in patients who present with severe symptomatic anemia.

Iron Deficiency Anemia

Iron deficiency is the most common cause of anemia in children. Despite the increased efforts promoting breastfeeding and the use of iron-fortified formula, iron deficiency anemia (IDA) still occurs in approximately 3–7% of young children and 9% of adolescent females [16, 17]. Increased demand due to rapid growth coupled with excess intake of cow's milk (low iron content) and heavy menstrual bleeding with inadequate iron intake are the major reasons for IDA in these populations, respectively. Since dietary deficiency of iron is uncommon in school age children and adolescent males, the presence of IDA in these patient groups warrants additional investigations to rule out sources of chronic blood loss (e.g., Meckel's diverticulum). IDA is also the most common cause of microcytic, hypochromic anemia. Other important causes of microcytic anemia include alpha or beta thalassemia trait and lead poisoning.

IDA in children is associated with a variety of clinical manifestations including pica (irresistible craving for ice, paper, clay), restless leg syndrome, breath-holding spells, and poor neurodevelopmental outcome [18]. In addition to the microcytic, hypochromic appearance of the RBCs, IDA is characterized by a low reticulocyte count, an increased RBC distribution width (RDW) and a Mentzer index >13 [Mentzer index = MCV (fL)/RBC count ($\times 10^6$ per mm^3)] [19]. In contrast, microcytic anemia in beta thalassemia trait is associated with a normal or slightly increased reticulocyte count, normal RDW, and a Mentzer index <13. An elevation of the reticulocyte count (after 1 week) and hemoglobin value (after 1 month) subsequent to the initiation of oral iron therapy confirms the diagnosis of IDA in most cases. Additional laboratory results such as a low serum ferritin level, increased total iron binding capacity, and low transferrin saturation percentage confirms iron deficiency and may be needed in certain situations. Standard oral iron therapy consists of the provision of 3–6 mg/kg/day of elemental iron in 2–3 divided doses for 1–2 months to correct the anemia, followed by an additional 2–3 months of therapy to increase hepatic iron stores. Ferrous sulfate is the most commonly used iron formulation used to treat IDA, but may cause gastrointestinal side effects. Newer iron preparations such as NovaFerrum™ are associated with fewer side effects and improved palatability, but may be expensive [18]. Intravenous iron therapy can be useful for patients with poor adherence or those who are intolerant to oral iron preparations.

Macrocytic Anemia

Macrocytic anemia (MCV > 100 fL) is uncommon in children and can be classified into two categories: megaloblastic and non-megaloblastic. Megaloblastic anemia is usually due to folic acid or Vitamin B12 deficiency. Inadequate diet, rapid growth,

increased demand (e.g., chronic hemolytic anemia), malabsorption syndromes, and drug therapy (trimethroprim, phenytoin) can all cause folic acid deficiency [20]. Exclusive intake of unfortified goat's milk is an important cause of megaloblastic anemia as it is a poor source of folic acid [21]. Nutritional deficiency of Vitamin B12 is very rare in children but can occur due to strict veganism, ileal disease, and pernicious anemia. Pernicious anemia is a rare autoimmune gastritis resulting from anti-parietal cell antibodies that leads to decreased intrinsic factor (IF) production which can compromise Vitamin B12 absorption [22]. Though rare, Vitamin B12 deficiency can be associated with neuropsychiatric manifestations such as subacute combined degeneration of the spinal cord, seizures, and dementia. The usual laboratory findings in megaloblastic anemia are macrocytic anemia, granulocytopenia, thrombocytopenia, reticulocytopenia, hypersegmented neutrophils on the peripheral blood smear and either decreased RBC folate (<160 ng/mL) and/or serum Vitamin B12 levels (<100 pg/mL) [20]. Treatment of megaloblastic anemia includes oral replacement of folic acid or parenteral administration of Vitamin B12 and correction of underlying cause. The causes of non-megaloblastic anemia include aplastic anemia, hypothyroidism, dyserythropoietic anemias and liver disease.

RBC Enzymopathies

There are two major enzymatic pathways in RBCs, namely the Hexose-Monophosphate (HMP) shunt and glycolytic pathways. Enzyme deficiencies in either pathway can lead to hemolytic anemia. Glucose 6-phosphate dehydrogenase (G6PD), a major enzyme in the HMP shunt pathway is responsible for producing reduced glutathione in RBCs which protects the cells from oxidative damage. In turn, deficiency of this enzyme leads to hemolysis due to decreased oxidative protective mechanisms for the sulfhydryl groups of the RBC membrane and hemoglobin. G6PD deficiency is an X-linked recessive disorder and is the most common human enzyme defect. There are two major types of G6PD deficiency: A-minus and the Mediterranean type [23]. The A-minus variety is more common in African Americans and is associated with mild to moderate enzyme deficiency (10–60% of normal). Patients with the A-minus type have normal levels of G6PD in young erythrocytes (reticulocytes), but have very low levels in mature RBCs. In contrast, the Mediterranean type is associated with severe enzyme deficiency (<10% of normal) due to uniformly low levels of G6PD across all stages of the erythrocyte, and is most commonly present in Mediterranean and Asian populations. Clinically, patients with G6PD deficiency present with acute hemolysis characterized by anemia, reticulocytosis, indirect hyperbilirubinemia and hemoglobinuria (dark colored urine) upon exposure to certain drugs (antimalarials, dapsone, etc.), ingestion of Fava beans (Favism), and viral infections. Whereas a reduced G6PD level will confirm the diagnosis, a normal level does not rule out the diagnosis during an acute hemolytic episode with associated reticulocytosis. In this situation, repeat testing is recommended several months after the resolution of active hemolysis. Treatment for G6PD deficiency consists of supportive care and avoidance of agents that cause

oxidative stress. Patients and families should be educated about avoidance of these stressors, the importance of heightened vigilance during infection and recognition of the signs and symptoms of hemolytic anemia. Additional information about medications and foods that should be avoided can be found at: www.g6pddeficiency.org.

Pyruvate kinase is an enzyme in the glycolytic pathway, the deficiency of which leads to hemolysis due to decreased ATP production. Pyruvate kinase deficiency is an autosomal recessive disorder characterized by variable degree of anemia, jaundice, splenomegaly, and reduced erythrocyte PK activity [24]. Treatment includes folic acid supplementation and blood transfusions on an as-needed basis. Splenectomy can be considered in patients with persistently increased requirements for blood transfusions.

RBC Membrane Disorders

Deficiency in one of the structural proteins of the RBC cytoskeleton can lead to RBC membrane disorders which result in anemia in children. Hereditary spherocytosis (HS), the most common RBC membrane disorder, results from a deficiency of the major red cell membrane protein, spectrin [25]. Deficiency of this protein causes decreased erythrocyte deformability as well as the loss of membrane lipids and surface area due to repetitive sequestration of RBCs in the spleen, all of which ultimately leads to the formation of spherocytes. These spherocytes are subsequently destroyed in the spleen which leads to a chronic hemolytic anemia. HS is usually inherited in an autosomal dominant fashion, with a positive family history found in about 75% of cases [26]. The severity of anemia in HS is variably ranging from mild to severe depending on the rate of hemolysis. Approximately 50% of cases present during the newborn period in an infant with neonatal jaundice. Clinical and laboratory findings of HS include pallor, jaundice, splenomegaly, normocytic anemia with an elevated mean corpuscular hemoglobin concentration (>36 g/dL), spherocytes on the peripheral blood smear (usually >10%), increased reticulocyte count, and increased osmotic fragility of RBCs. Since spherocytes and abnormal osmotic fragility can also be seen with autoimmune hemolytic anemia, it is important to perform a Coomb's test which is usually negative in HS but positive in autoimmune hemolytic anemia. Patients with HS can develop aplastic crisis (due to parvovirus B19), splenic sequestration crisis, bilirubin gall stones, and iron overload.

Treatment of HS is usually supportive and includes folic acid supplementation (1 mg/day) and judicious use of blood transfusion for symptomatic anemia. Splenectomy is a curative option, but splenic loss is accompanied by a life-long increased risk for serious bacterial infections [27]. Recent data suggest that patients with HS who undergo splenectomy are also at increased risk for developing delayed vascular complications such as myocardial infarction, pulmonary hypertension, and stroke later in life [28–30]. Thus, the decision to perform a splenectomy should be carefully considered after a detailed discussion of its risks and benefits with the patient/family. Splenectomy is typically reserved for patients with severe anemia, growth and developmental delay requiring frequent blood transfusions, persistent

severe left upper quadrant pain or those with recurrent gallstones. Splenectomy is usually delayed until 5–6 years of age to minimize the risk of post-splenectomy sepsis from encapsulated bacteria. Special immunizations (23 valent pneumococcal vaccine and meningococcal vaccines) and postsplenectomy antibiotic prophylaxis should be given to all patients. Hereditary elliptocytosis and hereditary stomatocytosis are other rare membrane disorders with variable degrees of hemolytic anemia.

Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) results from the extravascular or intravascular destruction of RBCs due to autoantibodies with or without complement fixation. It can be primary (idiopathic) or secondary that occurs in association with a systemic disease [31, 32]. The onset of anemia is sudden and is usually preceded by a viral-like illness. Patients with AIHA present with pallor, jaundice, mild organomegaly, and sometimes with dark colored urine. Laboratory investigations reveal anemia (often with hemoglobin 4–7 g/dL), indirect hyperbilirubinemia, reticulocytosis, microspherocytes on the peripheral blood smear and most importantly, a positive direct antiglobulin test (DAT or Coomb's test). The DAT involves the Coomb's reagent which is an antihuman γ-globulin (or complement) that agglutinates RBCs by reacting with the autoantibodies or complement on the cell surface [33]. Based on this assessment, AIHA can be classified into three subtypes: warm-reactive AIHA, cold agglutinin disease, and paroxysmal cold hemoglobinuria.

Warm-reactive AIHA can be primary or seen in patients with systemic lupus erythomatosus, lymphoma (Hodgkin's and non-Hodgkin's) and common variable immunodeficiency. In warm-reactive AIHA, the autoantibodies are usually IgG and have their maximum activity at 37 °C with or without complement fixation; the hemolysis primary occurs in the spleen. Corticosteroids are the initial treatment of choice in patients with warm-reactive AIHA. Prednisone at 2 mg/kg/day for 1–2 weeks with a slow taper is the standard regimen used. Although blood transfusion should be avoided whenever possible, the least incompatible RBC unit may be used in patients with severe symptomatic anemia. The other treatment options include IVIG, rituximab, and splenectomy for refractory and relapsing cases.

Cold agglutinin disease in children commonly follows Mycoplasma infections. Hemolysis is primarily intravascular as the autoantibodies are usually IgM and have maximum reactivity at 4 °C complement fixation. Cold agglutinin disease is usually self-limited and avoidance of cold temperatures and warming the patient's room is the mainstay of treatment. As in warm AIHA, blood transfusion should be avoided if possible. If transfusion is indicated, warming the blood to 37 °C will help minimize hemolysis. Steroids are not effective in patients with cold agglutinin disease. Patients with severe hemolysis can also benefit from plasmapheresis as IgM is largely intravascular.

Paroxysmal cold hemoglobinuria is a rare condition that typically occurs after a viral-like illness in children. It is characterized by an IgG autoantibody

(Donath-Landsteiner) that fixes compliment at cold temperatures with resultant hemolysis and hemoglobinuria. Keeping the patient warm and warming the blood product prior to transfusion if needed is the mainstay of therapy. Steroids and plasmapheresis can also be used in severe cases.

Sickle Cell Disease

Sickle hemoglobin (HbS) is the result of a point mutation that causes substitution of valine for glutamic acid at the sixth carbon position of the β globin gene. The term sickle cell disease (SCD) generally refers to any sickling hemoglobinopathy that contains HbS such as HbSS disease, HbSC disease, and HbS beta thalassemia, but excludes sickle cell trait. Sickle cell disease is the most common inherited blood disorder and affects approximately 100,000 people in the US [34, 35]. It is estimated that SCD affects 1 in 500 African Americans, whereas sickle cell trait occurs in 1 in 8–10 African Americans in the US [34, 35]. In the US, most SCD patients are diagnosed through the newborn hemoglobinopathy screening. There are four major phenotypes of SCD: HbSS disease (65–70%), HbSC disease (20–25%), Hb $S\beta^0$, or $HbS\beta^+$ thalassemia (2–5%). HbSS disease and Hb $S\beta^0$ thalassemia are the most severe phenotypes (baseline Hb 7–9 g/dL) and are often referred to as sickle cell anemia, whereas HbSC disease and Hb $S\beta^+$ thalassemia are usually milder (baseline Hb 9–11 g/dL).

Microvascular occlusion due to sickled erythrocytes resulting from polymerization of deoxygenated HbS is the fundamental pathophysiological hallmark of SCD. Repeated microvascular occlusions in combination with accelerated hemolysis (the half-life of sickled RBCs is 15–45 days) often leads to chronic end-organ damage in SCD patients. The most common acute complications of SCD are vaso-occlusive painful episodes, dactylitis, infections, acute chest syndrome, stroke, and priapism. Increased susceptibility to infection with encapsulated bacteria results from poor splenic function due to recurrent microvascular occlusions in the spleen. Chronic end-organ damage in these patients leads to the development of pulmonary hypertension, silent cerebral infarcts, gallstones, avascular necrosis of bone and retinopathy.

Treatment of SCD is usually supportive and complemented by a periodic comprehensive follow-up. Comprehensive management of SCD includes early initiation of penicillin prophylaxis (2–3 months of age), provision of routine and special immunizations (e.g., 23 polyvalent vaccine at 2 years of age), appropriate screening procedures (e.g., transcranial Doppler for stroke screening), and education and counseling of patient/parents about the disease and its complications. Hydroxyurea (HbF inducer) is frequently prescribed to patients with recurrent painful episodes and/or acute chest syndrome. Bone marrow transplantation is the only curative option and is generally reserved for patients with severe sickle cell disease with an HLA identical sibling.

Beta Thalassemia

Beta thalassemia is due to point mutations in one or both of the globin genes with resultant decreased globin synthesis that leads to ineffective erythropoiesis and hemolysis [36]. It mostly affects children of Mediterranean and Southeast Asian ancestry. It can be classified into three clinical syndromes based on the amount of β-globin synthesized. Beta thalassemia trait is a mild disorder characterized by mild anemia (Hb 10–13 g/dL), microcytosis, normal or slightly elevated RBC count and a Mentzer index <13. Finding of an increased HBA_2 ($\alpha_2\beta_2$) > 3.5% on hemoglobin electrophoresis usually confirms the diagnosis. Most patients are asymptomatic and rarely require a blood transfusion. In contrast, patients with beta thalassemia major (severe reduction or complete absence of β-globin chains) manifest with severe anemia in infancy and are transfusion-dependent. They are at risk for developing iron overload, alloantibodies, and infections from frequent blood transfusions. Bone marrow transplantation from an HLA-matched sibling is curative in most patients. Patients with beta thalassemia intermedia have moderate to severe anemia and require frequent but intermittent blood transfusions.

Alpha Thalassemia

Deletion of one or more of the 4 α-globin genes with decreased synthesis of α-globin chains leads to alpha thalassemia [37]. It is more common in persons of Southeast Asian and African ancestry. Deletion of one α-globin gene results in a silent carrier state with no hematological abnormality. Alpha thalassemia trait is a mild hypochromic, microcytic anemia that results from deletion of two α-globin genes. Patients are usually asymptomatic and rarely need transfusion. In contrast to beta thalassemia trait, hemoglobin electrophoresis will be normal (without elevation of HbA_2) in patients with alpha thalassemia trait. In alpha thalassemia trait, deletion of these two genes can occur on the same chromosome (cis) as is often the case in the Southeast Asian population, or on opposite chromosomes (trans) as is common in the black population [38]. Thus, genetic counseling is very important for patients of Southeast Asian descent as they have a higher risk of having an offspring with hydrops fetalis.

HbH disease is due to deletion of three α-globin genes and often associated with moderate to severe hemolytic anemia, splenomegaly, and increased transfusion need. Deletion of all 4 α-globin genes leads to hydrops and fetal death.

Lead Poisoning

Lead poisoning is an important cause of microcytic, hypochromic anemia in children. Lead intoxication occurs primarily via ingestion of contaminated paint, plaster or breathing contaminated dust in older buildings in the inner city areas. Through the inhibition of aminolevulinic acid dehydratase and ferrochetalase, key enzymes

in hemoglobin synthesis, and as a result of interfering with intestinal iron absorption, lead poisoning leads to microcytic, hypochromic anemia with an elevated erythrocyte protoporphyrin level [39]. Basophilic stippling of RBCs is frequently seen on the peripheral blood smear. The majority of patients have concomitant iron deficiency. Management of lead poisoning includes environmental investigation to identify and limit ongoing exposure, serial blood lead level monitoring, chelation therapy with dimercaprol or EDTA and iron supplementation.

Anemia of Chronic Disease

The anemia of chronic disease (ACD), also known as the anemia of inflammation, is an acquired form of anemia that is observed in a variety of clinical conditions in children including infection, autoimmune disease, organ failure, and malignancy. Hepcidin, a 25-amino acid antimicrobial peptide encoded by the *HAMP* gene and produced in the liver, has emerged as the key mediator of ACD [40]. Hepcidin regulates both intestinal iron absorption and body iron distribution through its post-translational suppression of cell-membrane expression of ferroportin, which is the sole cellular iron exporter. Hepcidin binding to ferroportin causes internalization and degradation of ferroportin, which results in downregulation of dietary iron absorption via intestinal enterocytes, and inhibits the release of stored iron from reticuloendothelial cells [41]. In this way hepcidin mediates iron-restricted erythropoiesis by preventing the utilization of absorbed or stored iron for erythropoiesis by the bone marrow, a process which in the short term may serve as a host-defense mechanism intended to sequester iron from invading pathogens or malignant cells [42]. Unfortunately in the longer term, hepcidin mediates chronic anemia and iron restriction which may be associated with poor response to iron supplementation along with adverse clinical effects on growth and cognitive development in children [43, 44]. Hepcidin expression is induced by inflammation in general and in particular by the inflammatory cytokine IL-6. It is cleared from the circulation by glomerular filtration, leading to increased levels also occurring in the setting of decreased renal function [45]. Distinguishing ACD from iron deficiency anemia can present a clinical challenge, as both disorders can be characterized by anemia which may be microcytic, with low reticulocyte counts, decreased serum iron concentration, and low transferrin saturation. Serum ferritin levels can be helpful in distinguishing the disorders; iron deficiency is associated with a low ferritin concentration, while ACD is often characterized by normal or elevated serum ferritin, reflecting iron sequestration in the reticuloendothelial system [43].

Anemia Due To Chronic Kidney Disease

Anemia is one of the most common and clinically significant complications of chronic kidney disease (CKD) in children, and is associated with a variety of adverse clinical consequences in this population including an increased risk for

hospitalization and mortality [46–48]. Although a complex interaction of factors is responsible for the decrease in hemoglobin seen with progressive CKD, impaired erythropoietin (EPO) production by failing kidneys and iron restriction are central causes. Human EPO is produced prenatally by hepatocytes, but primary production shifts to the peritubular fibroblasts in the renal cortex at birth [49]. Circulating EPO regulates erythroid proliferation and survival via an anti-apoptotic mechanism, and also acts as a growth factor to enhance red blood cell maturation [50, 51]. EPO production in CKD can be downregulated both due to destruction of peri-tubular fibroblasts, and as a result of dysregulation of the renal oxygen-sensing mechanism which includes decreased stability and transcriptional activity of hypoxia-inducible factors (HIFs), independent of damage to EPO-producing cells [52]. EPO levels in CKD patients are normal to slightly increased, but are inappropriately low for the degree of anemia and are 10–100 times higher in anemic patients with normal renal function [50, 53]. The development of recombinant human erythropoietin (rHuEPO) in the 1980s revolutionized the treatment of the anemia of CKD in children, eliminating the dependence on red blood cell transfusions which could be complicated by transfusion-associated viral infections, iron overload, and allosensitization, the latter issue being particularly significant to those patients requiring a kidney transplant [54–56]. However, treatment with rHuEPO or another erythropoiesis-stimulating agent (ESA) alone is usually not sufficient to correct the anemia of CKD; up to 40% of children with advanced CKD demonstrate persistently low hemoglobin levels while being treated with an ESA [57]. An additional contributor to anemia is iron-restricted erythropoiesis, which may be secondary to absolute iron deficiency, functional iron deficiency in which accessible iron stores are depleted by an ESA-stimulated bone marrow, or to impaired iron trafficking in the setting of inflammation [58]. Thus, iron supplementation also forms a cornerstone of anemia treatment. While oral iron supplementation is safe and efficacious in children with CKD, those with more advanced disease or especially those on hemodialysis often benefit from IV iron preparations due to poor enteral absorption. An ongoing challenge for the clinician is recognizing when inflammation and upregulation of hepcidin production may be contributing to iron restriction and poor utilization of iron supplements, and deciding when an intravenous route of iron administration or even transfusion may be more effective than escalation of ESA dose. CKD-associated hyperparathyroidism can also contribute to anemia through decreased bone marrow production of red blood cells in the setting of myelofibrosis [50, 59]. Finally, accumulated uremic toxins and associated oxidative stress induce changes in erythrocyte cell membranes which promote hemolysis and shorten cell lifespan, with cell survival time decreased by as much as 50% compared to healthy subjects [60]. Given the limitations of ESAs and conventional iron supplementation in the treatment of anemia of CKD, there are a number of novel therapies currently in varying stages of development including HIF stabilizers to promote endogenous EPO production, anti-hepcidin antibodies or binders, and more bioavailable iron preparations delivered via the intravenous and dialysate routes [52, 61–63]. However, the safety and efficacy of these therapies has yet to be systematically assessed in children with CKD.

Anemia in Gastrointestinal and Hepatic Disorders

Anemia is a common manifestation of several gastrointestinal and liver diseases in children. Malabsorption syndromes such as celiac disease are often associated with deficiency anemias due to defective absorption of iron, folate, and Vitamin B12. Iron deficiency occurs from chronic bleeding from peptic ulcer, Meckel's diverticulum, and colonic polyps. Anemia in chronic liver disease usually results from hemorrhage, hypersplenism, and aplasia [64]. Coomb's negative hemolytic anemia can be the presenting manifestation of Wilson's disease (hepatocellular degeneration), a rare disorder of hepatic copper accumulation [65]. Recurrent anemia is also seen in approximately one-third of patients with inflammatory bowel disease (IBD) [66]. The etiology of anemia in IBD is multifactorial, frequently a combination of iron deficiency, anemia of chronic disease, deficiencies of folate and Vitamin B12 and rarely hemolysis from the medications used for the treatment of IBD. Management of anemia in IBD involves control of the disease, iron supplementation, and recombinant EPO. Erythropoietin therapy not only improves the hemoglobin level, but also helps decrease inflammation and disease activity [67].

Conclusions

In summary, anemia remains an important health problem in infants, children, and adolescents. Proper implementation of screening programs are crucial to the detection of anemia in the newborn and appropriate education about nutritional habits can help prevent iron deficiency, the most common cause of anemia in the pediatric patient. Health care providers should also be aware of the vast array of other etiologies of anemia, along with their presenting features and associated treatment recommendations, so as to decrease the health burden due to anemia and optimize the outcome of affected patients.

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Management of Sickle Cell Anemia

13

Jason Mouabbi and Zyad Kafri

Introduction

Sickle cell disease (SCD) is a multi-system disease, associated with episodes of acute illness and progressive organ damage. Sickle cell anemia (SCA) is an autosomal recessive disease where a single point mutation in the gene encoding the β-globin chain produces a mutant hemoglobin molecule called hemoglobin S (HbS). SCA Hg SS disease is the most common genotype occurring in 60–75% of patients and reduces the solubility of HbS promoting the formation of large insoluble aggregates under deoxygenation stress (Fig. 13.1). This crystallization disrupts erythrocytes and its architecture and flexibility leading to cellular dehydration, with physical and oxidative cellular stress. The rate and extent of HbS polymerization is proportional to the extent and duration of hemoglobin deoxygenation [1].

Twenty-five percent of sickle cell patients have compound heterozygosity of HbS with another β-globin chain variant, such as hemoglobin C, D-Punjab, O-Arab, and E. The third form of SCD occurs when HbS is inherited with a β-thalassemia allele, causing HbS/β-thalassemia.

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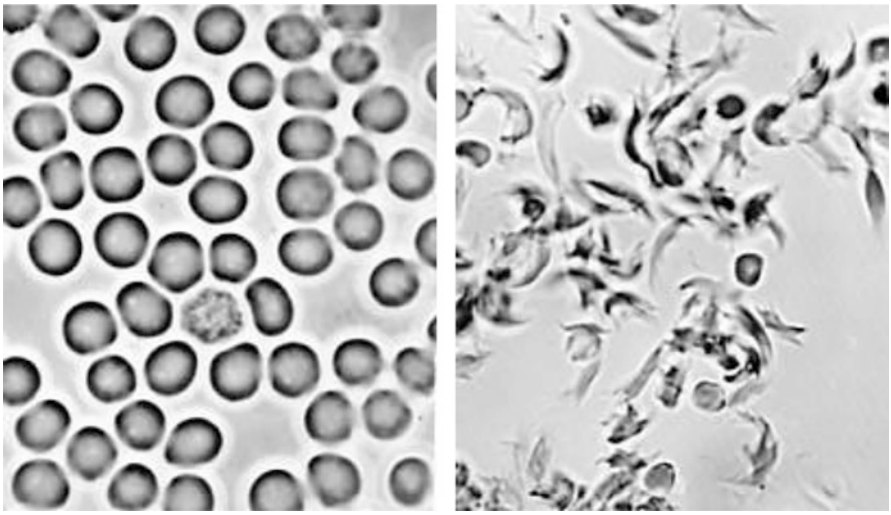

Fig. 13.1 Red blood cells of a patient with sickle-cell disease. *Left*: Oxygenated. *Right*: Deoxygenated. The shape of the cells when deoxygenated causes them to break easily. [Courtesy of Dr. Anthony C. Allison.]

Epidemiology

Historically, SCA have been shown to prevail in malarious areas, since HbS carries a selective advantage against malaria. Subsequent migration from malaria-endemic regions lead to the widespread SCA [2].

SCD is the most common inherited blood disorder in the United States. It is responsible for approximately 113,000 hospitalizations and $488 million dollars in hospitalization costs annually. It is estimated that 90,000–100,000 Americans of all races suffer from SCD. SCD predominantly affect African-Americans where 1 in 13 have sickle trait (heterozygous) and 1 in 365 have SCA [3].

Pathophysiology

SCD manifestations are driven by two major pathophysiological processes: vaso-occlusion with ischemia–reperfusion injury and hemolytic anemia.

Acute vaso-occlusive pain is thought to be caused by entrapment of erythrocytes and leucocytes in the microcirculation, causing vascular obstruction and tissue ischemia. Although this process requires HbS polymerization, the event that triggers the vascular obstruction by sickle erythrocytes is often inflammatory where hypoxia induces endothelial–leucocyte–erythrocyte adhesive interactions in the post-capillary venules. Vascular occlusion is the result of a dynamic interaction between erythrocytes and the vascular endothelium, resulting in episodic microvascular occlusion and ischemia, followed by restoration of blood flow, which further

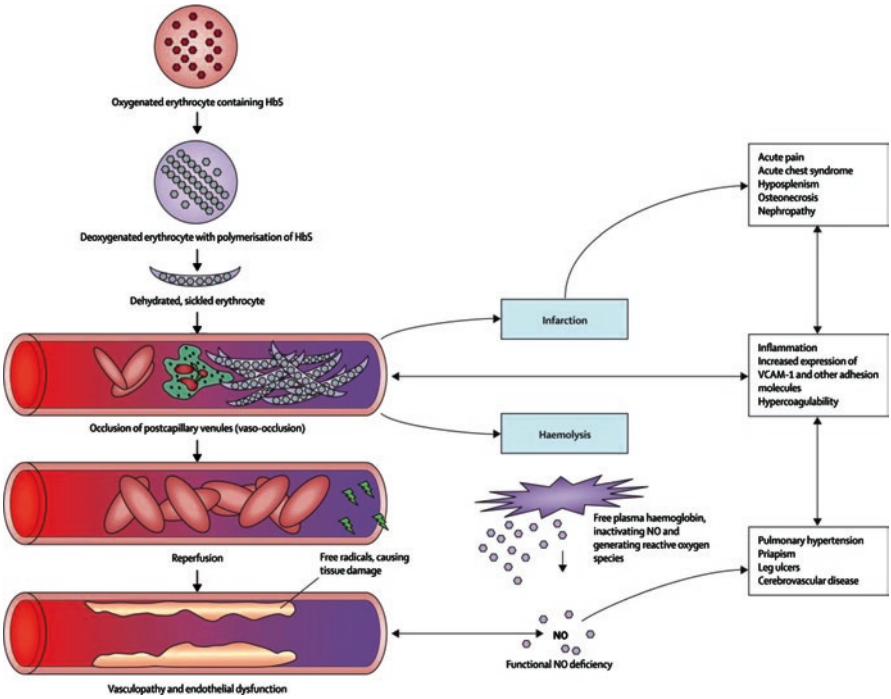

Fig. 13.2 Pathophysiology of sickle cell disease

promotes tissue injury mediated by reperfusion. Necrotic marrow fat and cellular elements (Fat emboli) contribute to vascular occlusion, particularly in the lungs, where it causes acute chest syndrome.

The second pathophysiological process in sickle-cell disease is hemolytic anemia, which is also driven by HbS polymerization. Hemolysis cause anemia, fatigue, cholelithiasis, and more importantly contributes to the development of progressive vasculopathy. Therefore, patients develop pulmonary hypertension, cutaneous leg ulceration, priapism, endothelial dysfunction, cerebrovascular events, and proliferative changes in the intima and smooth muscle of blood vessels (Fig. 13.2) [4].

Diagnosis and Screening

Diagnosis of SCD is based on analysis of hemoglobin. Typically, this analysis involves protein electrophoresis or chromatography, which are inexpensive techniques and widely available worldwide. Antenatal screening is available to women in some countries to help identify couples who are at risk of having a baby with SCD [5].

Management by Complications

Anemia

SCD produces a chronic, compensated hemolytic anemia with episodic acute drops in hemoglobin leading to symptomatic anemia. Other contributing factors to chronic anemia include inappropriately low serum erythropoietin (EPO) concentration (due to renal disease or increased plasma viscosity) and/or folate or iron deficiency.

Chronic Hemolytic Anemia

Sickled cells undergo hemolysis, with a typical red blood cell (RBC) lifespan of approximately 17 days (one-seventh that of normal RBCs), leading to chronic hemolytic anemia. Blood counts will show a hemoglobin baseline level between 8 and 10 g/dL (hematocrit 20–30%). High MCV can be seen if the patient has significant reticulocytosis or is taking hydroxyurea. White blood cells (WBC) are mildly increased and peripheral smears may show abundant reticulocytosis (3–15%), sickled cells (drepanocytes), and Howell Jolly bodies (nuclear remnants that have not been phagocytosed due to reduced splenic function). Other findings related to hemolysis include elevated serum lactate dehydrogenase (LDH) and low serum haptoglobin [6].

Aplastic Crisis

Aplastic crisis is characterized by an acute drop in hemoglobin level caused by a transient arrest of erythropoiesis, leading to abrupt reductions in red cell precursors in the bone marrow and a markedly reduced number of reticulocytes in the peripheral blood (<1.0%). Although TAC usually manifests as a pure red cell aplasia, white cell and platelet counts may also decline resulting in pancytopenia. Management is with transfusion for hemoglobin close to the known patient baseline. Eighty percent of SCD patients who develop TAC were found to be secondary to acute parvovirus [discussed in more detail under section “Infections”] [7].

Splenic Sequestration Crisis

Splenic sequestration crisis is characterized by an acute drop in hemoglobin level caused by vasoocclusion within the spleen and splenic pooling of RBCs. A large percentage of blood can become sequestered in the spleen, leading to hypovolemic shock and death. Patients with splenic sequestration crisis present with a rapidly enlarging spleen and a marked decrease in hemoglobin level despite persistent reticulocytosis. The mortality rate is as high as 10–15% with up to 50% of the patients having recurrent episodes. Treatment requires, immediate diagnosis with swift volume resuscitation using intravenous normal saline and blood transfusions. Splenectomy is often used after the first acute event to prevent recurrence [8].

Hyper-hemolytic Crisis

Hyper-hemolytic crisis refers to the sudden exacerbation of hemolysis with worsening anemia despite ongoing reticulocyte production. This complication is rare and

the causes are unknown. Some believe it is a form of a delayed hemolytic transfusion reaction. Infections and/or drug exposure may be responsible for increased hemolysis in some cases. In a study, 7/8 children with hyper-hemolytic crisis had associated glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Some reports have noted improvement with immunosuppressive therapies including intravenous immune globulin (IVIG) plus glucocorticoids. Rituximab also has been used with some success. The successful use of immunosuppressive agents hint at an underlying antibody-mediated etiology for the hemolysis [9].

Acute VASO-Occlusive Pain Crisis

Vaso-occlusive crises are recurrent episodes of severe pain in sickle cell disease. The first episode may occur as early as 6 months of age, often presenting as dactylitis (vasoocclusive pain in the small bones of the hands and feet), and thereafter may occur with variable frequency, usually in the extremities, chest, and back.

Epidemiological studies of the frequency and severity of vaso-occlusive crises indicate an association with high concentrations of HbS, low concentrations of HbF, and high steady-state leukocyte counts and hemoglobin levels.

Many patients have specific triggers for pain such as cold, wind, low humidity, dehydration, stress, alcohol, and menses, which they then develop strategies to minimize or avoid. However, most painful episodes have no identifiable cause.

Although acute vaso-occlusive pain is typically self-limiting and does not result in permanent organ damage, it is the most important complication from the patient's perspective, and increased frequency of pain is associated with early death and the risk for other complications such as acute chest syndrome (ACS), requiring rapid triage, evaluation, and administration of analgesics. Patients with SCD experiencing acute pain crises may be incorrectly identified as those with drug-seeking behavior or addiction.

Appropriate opioid agents include morphine hydromorphone, or fentanyl. Medication can be provided by intermittent dosing or continuous infusion. Of these, continuous infusion by patient-controlled analgesia (PCA) is preferred because it provides the most even therapy with the patient in the therapeutic window of pain control without side effects, along with the option for self-administration of additional doses for breakthrough pain. Importantly, as-needed (PRN) pain medication as the sole source of pain relief should be avoided based on the pharmacological principle that the patient will have sub-therapeutic pain medication levels resulting in unnecessary pain [10].

For individuals receiving chronic hydroxyurea therapy, hydroxyurea should be continued at the patient's regular dose during acute events including hospitalizations, unless there is a hydroxyurea-induced cytopenia or other laboratory abnormalities. For individuals not receiving hydroxyurea at the time of hospitalization, the possibility of starting hydroxyurea to reduce future pain episodes generally is discussed with the primary hematologist on an outpatient basis when the patient is

feeling well; the benefits of hydroxyurea for preventing pain typically do not occur until at least 3 months after starting therapy.

During painful episodes, individuals with SCD are frequently hypovolemic. An initial intravenous (IV) bolus of 0.5–1 L of normal saline should be administered thereafter maintenance IV fluids along with PO fluid intake should be established. A non-RCT study in 1971 suggested that treatment of sickle cell crisis with rapid infusion of hypotonic saline can lower serum osmolality, decreases pain and is well tolerated. However, high-quality evidence to support a specific route, type, or quantity of hydration is lacking [11].

Infections

Bacterial infections are a major cause of morbidity and mortality in patients with SCD. Mechanisms include functional hyposplenism or asplenism, reduced tissue perfusion, presence of an indwelling catheter (e.g., for chronic transfusion), splinting, and hypoventilation.

Functional hyposplenism develops in early childhood (often starting as early as 4 months of age), and infants and young children are at greatest risk of certain infections. Splenic auto-infarction typically renders patients functionally asplenic by 2–4 years of age, which greatly increases the risk of serious infection with encapsulated organisms.

Most reported sites of infection include bacteremia, meningitis, osteomyelitis, bone marrow (especially by Parvovirus B19), and pulmonary infections (pneumonia and acute chest syndrome [ACS]).

Meningitis has been associated with SCD given the prevalence of encapsulated organism infections [12].

Parvovirus B19 infection which invades proliferating erythroid progenitors leading to transient arrest of erythropoiesis (Transient aplastic crisis) is a relatively common event for patients with SCD. Parvovirus B19 infection has an incidence of 11.3 per 100 patient-years with transient aplastic crisis occurring in 65–80% of them [12].

The incidence of osteomyelitis is also increased in individuals with SCD. Long bones are usually affected, often at multiple sites, resulting from infection of infarcted bone. The most common organisms are Salmonella species. Diagnosis is stablished by biopsy or aspiration of a suspected locus of infection by imaging. Plain films, bone scintigram, and MRI can all be used to find a locus of infection.

Prevention of infections is crucial in decreasing morbidity and mortality in SCD patients. Two major measures for preventing infection in individuals with SCD are immunization for all patients, and prophylactic penicillin (prophylaxis is done up to age 5). All patients with SCD should be immunized with both pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). The United States Advisory Committee on Immunization Practices (ACIP) recommends revaccination with PPSV23 every 5 years. All patients should also receive H. Flu

type b (Hib) conjugate vaccine, meningococcal vaccine and, yearly influenza vaccine [12].

Fever or rigors is usually the first sign of bacteremia which might lead to fulminant septicemia. Asplenic individuals, especially those who no longer take daily prophylactic antibiotics, should self-medicate with high-dose oral antibiotics. These patients should have prescribed antibiotics on hand and take them immediately. Amoxicillin-clavulanate is the drug of choice or extended-spectrum fluoroquinolones for penicillin-allergic patients. This an acceptable approach up until the age of 18 then antibiotics should be used cautiously and only after proving the patient has an active infection as the risk of pneumococcal infection reduces significantly with age: 9.8 per 100 patients before age 5 to 0.67 per 100 patients after age 5. Furthermore, long-term penicillin prophylaxis is not without its problems. Prolonged or intermittent antibiotic use can promote the development of resistance, so the recommendation to continue must represent a balance between the risk to the individual of pneumococcal infection versus the danger resistant organisms pose to the whole population [12].

Neurological Complications

Stroke is a common and potentially devastating manifestation of sickle cell disease that can affect both children and adults. It is usually seen with SCA (HbSS). Many cases are associated with vasculopathy affecting the distal internal carotid and middle cerebral arteries. Although the mechanisms for stroke remain uncertain, contributory factors to this vasculopathy include anemia, leukocytosis, hypoxemia, functional nitric oxide deficiency associated with hemolysis, and impaired regulation of blood flow causing hyperemia. The vasculopathy seems to start in infancy, with 11% of patients with SCD have had a stroke by the age of 20 years and 25% by age 45. Once a stroke has occurred, the risk of recurrence is more than 60% [13].

Vasculopathy can be detected at an early stage by use of transcranial doppler (TCD) ultrasonography. TCD is done on the distal internal carotid artery or proximal middle cerebral artery. High-risk patients were identified as patient that exhibit high TCD flow velocities ($\geq$200 cm/s) since their stroke risk was up to 40%. The Stroke Prevention in Sickle Cell Anemia (STOP) study, done in pediatric population 2–16 years of age, showed that regular blood transfusion (both simple and exchange transfusions were used) to keep HbS below 30% reduced the risk of stroke by 90% in high-risk patients. The study also showed that patients on transfusion therapy were more likely to have normal TCD results. Transfusions did however result in iron overload and alloimmunization, but not more frequent infections. A follow-up study (STOP II) was done based on the hypothesis that the former patients were potential candidates for stopping transfusion; unfortunately, this did not prove to be the case. Discontinuation of transfusion after at least 30 months in patients who have converted to normal TCD velocities was associated with high risk of reversion to abnormal and chance of stroke [13].

The role of hydroxyurea and phlebotomy was studied in the Stroke with Transfusions Changing to Hydroxyurea (SWiTCH) study. SWiTCH compared the efficacy of regular blood transfusions and iron chelation with hydroxyurea and phlebotomy in children with SCD and stroke. However, the study was stopped early due to the high number of strokes in the hydroxyurea group; no strokes occurred in patients receiving blood transfusions [14].

Primary prevention to reduce the risk of a first stroke is based on the use of regular transcranial Doppler measurements for risk stratification. Screening beyond the age of 16 years is not recommended given the changing patterns of cerebral blood flow velocities above this age.

High-risk patients are treated with chronic prophylactic transfusion with a goal to lower and maintain HbS level at ≤30% of total hemoglobin, while maintaining a pre-transfusion hemoglobin concentration of approximately 9 g/dL or 27% hematocrit and up to 10 g/dL in chronically transfused patients.

New studies have showed that allogenic stem cell transplant allowed the safe stop of all transfusions in all patients, even in those with abnormal TCD velocities prior to transplant [15].

Intracranial bleeds occur in patients of all ages, but are most common between the ages of 20 years and 30 years; they are typically associated with either a moyamoya-like syndrome or cerebral aneurysms. Treatment is neurosurgical and outcomes are generally poor.

In patients with radiologically confirmed acute ischemic stroke, transfusion therapy should be initiated promptly. The goal is to lower the percentage of HbS to ≤30% of total hemoglobin and to aim for a total hemoglobin level of ≤10 g/dL. Both simple and exchange transfusion can be used. Thrombolytic therapy is not generally used, although there are no good quality data to guide practice. Antiplatelet therapy with aspirin is a mainstay of treatment in patients with SCD who have an acute ischemic stroke [16].

Acute Chest Syndrome

Acute chest syndrome (ACS) is defined as new infiltrate involving at least one complete lung segment (usually predominate the lower lobes), that is accompanied by fever, chest pain, tachypnea, wheezing, and/or cough in a patient with SCD. Pain crisis is a prodrome of the ACS; ACS usually occurs 24–72 h after the onset of severe pain in the arms, legs, or chest.

ACS is a leading cause of morbidity and mortality in patients with SCD. The Cooperative Study of Sickle Cell Disease (CSSCD), the largest natural history study of SCD (939 patients) found that approximately 50% of patients with SCD will have an ACS. While the etiology of respiratory dysfunction in SCD is multifactorial, pulmonary embolism (PE) and infection contribute to ACS in a significant proportion of cases. The National Acute Chest Syndrome Study Group (the largest study on ACS with 538 patients) noted that pulmonary infections by a community-acquired pathogen was identified in 54% of patients with ACS. Chlamydia was the

most commonly isolated infective organism associated with ACS, although the rate of infection with this organism was similar to that in the general population. PE (both venous thromboembolism (VTE) and fat embolism) were the second most common cause of ACS in 17% of episodes.

To minimize the risk of ACS in SCD patients, influenza and pneumococcal vaccines should be administered (as discussed earlier). Furthermore, those admitted for painful crises should be considered to be in the prodromal phase of ASC; they require incentive spirometry and daily monitoring for pulmonary disease.

Once ACS has been diagnosed, broad-spectrum antibiotics (including a macrolide) are required. Given that bronchodilator therapy has shown to be effective in improving pulmonary function in ACS patients, they should be initiated even if airway hyper-reactivity is not evident. Routine, early exchange or simple transfusions have shown to improve oxygenation in ACS and are indicated for patients at high risk for complications, including adults and those who have a history of cardiac disease and severe pain in the arms and legs at presentation. Those who present with severe anemia and thrombocytopenia should receive exchange transfusion before respiratory distress (ARDS) develops. New data suggest that corticosteroids and nitric oxide are beneficial in severe cases that have not responded to other treatments [17, 18].

Pulmonary Hypertension

Pulmonary hypertension (PH) has been increasingly recognized as a prevalent complication in adults with SCD, affecting approximately 10% of patients and resulting in an increased risk of death.

Screening for PH is done by measuring tricuspid regurgitation velocity (TRV) using transthoracic echocardiography (TTE) with doppler and plasma N-terminal of the prohormone brain natriuretic peptide (NT-proBNP). Studies showed that SCD patients association of a TRV of ≥2.5 m/s with increased mortality risk. Similarly, studies on NT-pro-BNP showed that the degree of elevation correlated with mortality in SCD patients, with the highest risk observed at NT-proBNP ≥160 pg/mL (mortality risk of 6.24 compared with those with lower levels) [19].

Because of the known association of obstructive sleep apnea (OSA) with PH in non-SCD populations, the clinical guidelines for diagnosis of PH in SCD recommend a formal sleep study for all SCD patients with an elevated TRV or PH.

Since SCD patients with PH have higher mortality rates, it has been recommended that the underlying SCD be aggressively controlled. Patient should start on hydroxyurea, and a regular simple or exchange transfusion program should be initiated with target HbS level of 20% after transfusion.

A large multicenter trial (Walk-PHaSST trial) evaluating sildenafil in SCD patients with PH was stopped early due to significant increase in severe sickle cell pain crisis hospitalizations in patients taking sildenafil versus placebo [19].

The American Thoracic Society Clinical Practice Guideline suggests indefinite anticoagulant therapy for patients with Right heart catheterization-confirmed PH

plus VTE, and no additional risk factors for bleeding regardless of candidacy for surgical endarterectomy. More recently, pulmonary thromboendarterectomy surgery has become the preferred treatment for selected patients with chronic thromboembolic pulmonary hypertension (CTEPH). This intervention may result in a substantial improvement in cardiopulmonary hemodynamics [19].

Heart Diseases

Cardiac complications are a common feature of SCD and are felt to be an important cause of the morbidity and mortality associated with this disease.

Myocardial ischemia and infarction have been reported to occur in SCD, but in almost all cases evaluated coronary angiography reveals normal coronary arteries. Myocardial ischemia is likely related to acute and chronic microvascular occlusion in vaso-occlusive sickling events and systemic fat emboli syndrome. Reversal of the cardiac abnormalities has been seen after exchange transfusion. Evidence-based guidelines for treatment with antiplatelet agents, anticoagulation, and thrombolytics are lacking.

Large autopsy series have shown that cardiopulmonary causes account for the majority of SCD deaths.

An interesting fact about SCD patients is that the incidence of hypertension is markedly lower than in the general African-American population (2% vs. 28%) [20].

Renal Complications

Renal damage is almost inevitable in sickle-cell disease. There is a strong tendency for HbS to polymerize in the renal medulla, because of the low partial pressure of oxygen, the low pH, and the high osmolality causing erythrocyte dehydration. The consequent vaso-occlusion causes renal infarction with hematuria, papillary necrosis, and medullary fibrosis with focal segmental glomerulosclerosis (FSGS). Glomerular hyperfiltration and tubular dysfunction also occur, and are possibly associated with anemia and increased sensitivity to prostaglandins.

Gross and painless hematuria due to papillary infarcts is often the most dramatic event in SCD. It occurs at any age and is reported most often in patients with sickle cell trait. It is usually unilateral and occurs most commonly in the left kidney due to increased venous pressure in the longer left renal vein by normal anatomic "kinking" and compression from adjacent vessels (the L. renal artery runs between the aorta and superior mesenteric artery).

Renal papillary necrosis (RPN) is usually accompanied by hematuria (but not always). It has been shown that 40% of patients with SCA have RPN. Treatment options for hematuria and RPN is usually conservative with hypotonic IVF along with diuretics (thiazide or loop) to maintain high rate of urine flow. Refractory hematuria is treated with aminocaproic acid (EACA). EACA inhibits fibrinolysis,

enabling the formation of clots. Given the high risk of thrombosis in SCD patients, EACA must not be used unless other conservative measures have failed.

Renal dysfunction leading to glomerular hyperfiltration is apparent from an early age in patients with SCD. Micro-albuminuria is common in childhood and up to 20% of adults develop nephrotic-range proteinuria (>3.5 g proteinuria in 24 h). Chronic renal failure can be seen in up to 30% of patients. Treatment should focus on the early use of hydroxyurea and angiotensin-converting enzyme inhibitors in patients with clinically significant albuminuria (persistent urine protein excretion >300 mg per day). If end-stage renal disease develops, transplantation is the treatment of choice. The 10-year survival was 56% for patients after renal transplantation vs.14% for patients receiving dialysis.

Renal medullary carcinoma is a rare highly aggressive malignancy found almost exclusively in young patients with SCD or sickle cell trait. It is believed to arise due to medullary hypoxia leading to oxidative stress and formation of free radicals which lead to tumorigenesis. Patients typically present with painful hematuria (flank pain associated with hematuria) and weight loss. Most patients have metastatic disease at the time of diagnosis and survival after diagnosis is usually less than 6–12 months. Prompt evaluation should be performed to young patients with sickle cell trait or SCD presenting with painful hematuria using computed tomography (CT) scan with intravenous pyelography (IVP) [21].

Venous Thromboembolism

Venous thromboembolism (VTE) is common in patients with SCD. It affects nearly one in four patients and appears to be a risk factor for death in SCD. Traditional risk factors such as high use of central venous catheters, frequent hospitalization, high-risk surgeries such as orthopedic surgery for avascular necrosis (AVN), and pregnancy significantly influence VTE risk in SCD patients. Furthermore, SCD-related factors such as an increased prevalence of certain thrombophilic defects, genotype, and history of splenectomy can also alter VTE risk [22].

In patients with SCD, splenic auto-infarction affects the majority of children with hemoglobin SS disease by age 1 year. While surgical splenectomy is a significant risk factor for VTE, functional asplenia has been hypothesized to contribute to underlying hypercoagulability in SCD. However, no formal studies have evaluated the role of functional asplenia on thrombotic risk in SCD patients.

The largest study performed using the National Hospital Discharge Survey evaluated 1,804,000 SCD admissions from 1979 to 2003 and found that the prevalence of pulmonary embolism (PE) in hospitalized SCD patients was approximately 3.5 times higher than non-SCD African–American. In fact one study of 144 ACS events found that 17% of cases had pulmonary thrombi on CT with concurrent lower extremity (LE) compression ultrasonography showing no evidence of DVT in any of the cases. This may suggest that pulmonary thrombosis during ACS is an in situ, rather than embolic phenomenon [23].

Due to high VTE risk and high VTE recurrence rate in SCD patients, appropriate studies and specific guidelines should be followed [22].

Avascular Necrosis of Bones

Avascular necrosis of bone or osteonecrosis results from infarction of bone trabeculae. The femoral and humeral heads may be affected and is frequently bilateral.

Core decompression and bone marrow transplantations should be recommended at early stages of osteonecrosis to postpone the need for hip arthroplasty [24].

Proliferative Retinopathy

The frequency of retinopathy is greatest in adulthood, but retinopathy has also been described in children. The risk of developing proliferative sickle-cell retinopathy (PSR) is higher between the ages of 25–39 for both sexes. Regression of neovascularization and improvement of visual acuity was seen after 4 weeks of treatment [25].

Sickle Hepatopathy

SCD commonly present with one of several acute hepatic syndromes that manifest clinically as fever, right upper quadrant pain, and jaundice. The differential diagnosis includes acute sickle hepatic crisis (ASHC), sickle cell intra-hepatic cholestasis (SCIC), cholecystitis, choledocholithiasis, and acute viral hepatitis.

Patients with ASHC typically present with increased serum AST and ALT levels up to 300 U/L and an increased serum bilirubin level up to 15 mg/dL. Treatment is largely supportive, and vigorous exchange transfusion has been reported to be beneficial.

Pigmented gallstones develop in up to 58% of patients with SCD. Because the prevalence of cholelithiasis in this patient population is so high, abdominal ultrasonography has limited specificity for diagnosing acute cholecystitis. Thus, cholecystectomy should be considered in patients with SCD, gallstones, and symptoms suggestive of biliary pain or acute cholecystitis.

The collective experience includes seven adults and five children with the spectrum of liver diseases that occur in patients with SCD (ASHC, sickle cell intrahepatic cholestasis (SCIC), chronic hepatitis, cirrhosis, and iron overload). The experience to date suggests that liver transplantation is a feasible option for patients with SCD and liver failure but is fraught with challenges since it was associated with high rate of vascular thrombosis (including graft thrombosis, stroke, and pulmonary embolism) and immunosuppression-related infections [26].

Priapism

The prevalence of priapism in patients with sickle cell disease has been reported to be 5–45%. Approximately 30–45% of patients with sickle cell anemia will present at least one episode of priapism during their lifetime. If priapism persists for 4 h or more without detumescence, the patient is at risk for irreversible ischemic penile injury, which may result in fibrosis and erectile dysfunction. Adrenergic agonists (injectable epinephrine) form mainstay of treatment, with emerging use of phosphodiesterase-5 inhibitors and finasteride [27].

Leg Ulcers

Leg ulceration in sickle cell patients have been historically associated with compromised blood supply secondary to vaso-occlusion. Most recently, endothelial dysfunction and thrombosis have been hypothesized to be contributing factors for this as well.

Severe pain at the wound site is disproportionately greater in SCD than in other populations. The best approach to leg ulcers is prevention, which includes attention to properly fitting shoes, compression bandages to control LE swelling and immediate treatment for early signs of skin injury.

Chronic transfusion therapy accelerates wound healing and is considered the core therapy. However, in patients who cannot receive chronic transfusions due to iron overload or other causes, hydroxyurea may be beneficial. In some reports, initial chronic transfusion with transition to hydroxyurea has shown to effective.

Hyperbaric chamber therapies aimed at increasing local perfusion and oxygen delivery have been studied, however it is not a recommended therapy due to association with severe pain crisis exacerbations.

Data suggest that targeted analgesic interventions at the leg ulcer site are recommended and should be used to decrease patient exposure to systemic opioids [28].

Basic Management

Blood Transfusion and Iron Chelation

Erythrocyte transfusion has an established role in the management of both acute and chronic complications in SCD.

Erythrocytes can be given as a simple additive transfusion or by exchange transfusion. Simple blood transfusions involve adding blood or blood products without removing any. In contrast, an exchange transfusion is when apheresis is used to remove the patient's red blood cells and replace them with cells from blood donors. Exchange transfusion provides greater benefit compared to simple transfusion because only exchange transfusion can significantly lower HbS levels (to <30%). Furthermore, exchange transfusion are associated with lessened effects on viscosity

for a given Hgb level when compared to simple transfusion which is critical in potentially reversing vaso-occlusion.

Chronic blood transfusion is inevitably associated with iron overload. If/when this occurs, treatment with iron chelation agents is indicated with a goal to avoid liver damage(cirrhosis) from iron deposition and to decrease ferritin levels to less than 500 ng/mL. In SCD, serum ferritin of 1000 ng/mL and above is an indication for starting chelating agents. Serum ferritin roughly correlate with iron loading, but the relationship is too inaccurate to use as a reliable method for evaluating iron status. Rather, serum ferritin is used as a biomarker to track qualitative trends of iron loading and chelation efficacy over time.

In recent years, MRI measurement of liver iron concentration (LIC) also known as FerriScan have been used to evaluate iron status. LIC of at least 2 mg iron/g dry weight of liver tissue in patients receiving simple transfusions, and 5 mg iron/g dry weight of liver tissue in patients receiving exchange transfusions are considered indications to initiate chelating agents. FerriScans should be done at the initiation of chelation therapy, and annually in chronically transfused patients.

The current drug of choice is deferasirox which is an oral iron chelator that is taken once daily. Deferiprone is another oral iron chelator that was approved for thalassemia in 2011, however it is not yet approved for SCD.

Therapy should be discontinued immediately if the serum creatinine is greater than two times the upper limit of normal. Chelation therapy should be held if the serum alanine aminotransferase (ALT, formerly called SGPT) is greater than five times the upper limit of normal [29].

Role of Hydroxyurea

Its been observed and well documented that high fetal hemoglobin (HbF) levels have a preventative effect on intra-erythrocytic HbS polymerization and vaso-occlusion. To this end, hydroxyurea (HU), a ribonucleotide reductase inhibitor was studied and found to increase HbF and thus decrease pain crisis episodes in SCD patients. Hydroxyurea has been shown to lower the number of circulating leukocytes and reticulocytes, alters the expression of adhesion molecules, raises RBC volume (higher MCV) which help prevent RBC deformity, and increase nitric oxide formation directly from HU metabolism.

The Multicenter Study of Hydroxyurea in Sickle Cell Anemia [MSH] trial is the largest randomized, double-blind, placebo-controlled trial that studied the efficacy of hydroxyurea. Trial that studied randomly assigned 299 adults with SCD (HbSS genotype) and three or more VOC per year to receive HU or placebo. Patients who received HU were less likely to have an episode of ACS, had a lower annual rate of VOC, had a longer duration until their first and second crises, and required fewer transfusions and hospitalizations.

With respect to mortality, during a subsequent extension study, there was a significant reduction in mortality at 9 years among patients who received HU for at

least 1 year compared with those who either did not receive HU or received HU for less than 1 year (21% vs. 36.3%).

In adults, a multicenter, two-year, double-blind, randomized, placebo-controlled trial of HU showed that it reduced the rate of VOC by 50% and had a 40% reduction in mortality.

The major short-term toxicity of HU is global myelosuppression. Other potential adverse effects of HU such as infertility, susceptibility to infections, or teratogenic effect have also been the subject of concerns and has contributed to the low compliance of SCD patients [30].

Stem Cell Transplant

The only known curative therapy for patients with severe SCD is myeloablative conditioning and allogeneic hematopoietic stem cell transplant (allo-HSCT) from HLA-matched sibling donors. All allo-HSCT to date are limited to SCD patients who have HLA-matched siblings, thus limiting its widespread use.

The biggest study was conducted in France on 87 patients and showed that allo-HSCT SCD patients had 5-year overall survival of 97% and disease-free survival of 85%. Not only allo-HSCT alleviated SCD symptoms but it has also shown to stabilize or reverse organ damage due to SCD. Graft rejections were seen in 7% of patients. Acute graft versus host disease was seen in 13% of patients whereas chronic graft versus host disease was seen in 20% of them [31].

Nutrition

Growing evidence suggests that SCD patients have vitamin and micronutrient deficiencies that may influence the course of their disease.

Folate deficiency has been found to be present in 28–38% of patients with SCD. Increased folate consumption from ongoing hemolytic anemia is often proposed as a rationale for the use of folic acid in these patients.

Studies have showed that 80% of patients with SCD have osteopenia and osteoporosis attributed to vitamin D deficiency and inadequate calcium intake in this population. Current recommendations suggest regular check of patient's vitamin D level with oral vitamin D and calcium supplements initiated if deficient.

Daily multivitamin without iron is recommended for all SCD patients to replace some of the vitamins and micronutrients commonly reported to be deficient in these individuals (40–75% were found to be deficient in zinc, vitamin D, vitamin E, vitamin C, vitamin A, magnesium and selenium) [32].

Future Developments

Novel Therapies to Induce Fetal Hemoglobin Production

Current in vitro studies on adult erythroid cells from SCD subjects showed that gene manipulation by activating factors such as Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) lead to the induction of HbF production. Bardoxolone methyl and Tecfidera (dimethyl fumarate) have shown in mice models to induce Nrf2 and induce the production of HbF in levels higher than HU [33, 34].

Phosphodiesterase-9 inhibitors (PDE9i) inhibit the degradation of cGMP and increase cellular cGMP levels. IMR-687 is a potent PDE9i, has proven in animal models to increase HbF levels, and reduce red cell sickling, leukocytosis and microvascular stasis, without the observed toxicities of HU. IMR-687 may offer a once a day, oral, safe replacement for HU in the treatment of SCD [35].

Drugs Targeting Cell Adhesions

Adhesive interactions (especially P-selectin-mediated and E-selectin-mediated adhesion) have been shown to contribute to vaso-occlusive crisis (VOC) in sickle animal models. Targeted inhibition of both led to reduction of vaso-occlusion in in vitro and in murine models.

Rivipansel (GMI-1070) is an E-selectin inhibitor that is currently in phase 3 clinical trial for treatment of acute VOC. Similarly, SelG1 or crizanlizumab is a humanized monoclonal antibody against P-selectin showed promising results in clinical trials. Results are in favor of reducing the frequency of vaso-occlusive crisis and frequency of hospitalizations [36].

Sevuparin, a derivative of low-molecular-weight heparin (LMWH), is being developed as a P-selectin blocker. Sevuparin retains the P-selectin-binding domain of heparin but largely lacks anticoagulant properties. Studies have shown to inhibit sickle RBC adhesion to endothelial cells in vitro. Sevuparin is now in a phase 2 clinical trial for the management of acute VOC [36].

Drugs Targeting Inflammatory Pathways

Invariant natural killer T cells (iNKT) are implicated in the pathogenesis of ischemia/reperfusion injury. Blockade of iNKT cell activation in mice with SCD reduces pulmonary inflammation and injury. iNKT cell activation can be downregulated by activation of the adenosine A2A receptor (A2AR). Regadenoson & ATL146e are selective adenosine A2A receptor agonists currently undergoing clinical trials in SCD patients. Similarly, NKTT120, a humanized monoclonal antibody against iNKT cells, have been shown to be effective at depleting iNKT cells in animal models. Currently, NKTT120 has been granted fast-track status by the FDA to undergo clinical trials [36].

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