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CLINICAL HEMATOLOGY
Part I (Anemias)

*Methodological recommendations
for students and doctors*

КЛІНІЧНА ГЕМАТОЛОГІЯ
Частина I (Анемії)

*Методичні вказівки
для студентів і лікарів*

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1. BLOOD SYSTEM

Functional anatomy and physiology of blood system

Blood flows throughout the body in the vascular system, and consists of plasma and three cellular components:

- red cells, which transport oxygen from the lungs to the tissues
- white cells, which protect against infection
- platelets, which interact with blood vessels and clotting factors to maintain vascular integrity and prevent bleeding.

Blood cells characteristics

Red blood cells or erythrocytes are anucleate, biconcave discoid cells filled with hemoglobin, the major protein that binds oxygen. The erythrocytes transport the respiratory gases oxygen and carbon dioxide. Mature red cells circulate for about 120 days.

White cells or leucocytes in the blood consist of granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (monocytes and lymphocytes).

Neutrophils are the most common white blood cells in the blood of adults. Their main function is to recognise, ingest and destroy foreign particles and microorganisms. A large storage pool of mature neutrophils exists in the bone marrow. Every day some 10^{11} neutrophils enter the circulation, where cells may be freely circulating or attached to endothelium in the marginating pool. These two pools are equal in size; factors such as exercise or catecholamines increase the number of cells flowing in the blood. Neutrophils spend 6–10 hours in the circulation before being removed, principally by the spleen. Alternatively, they pass into the tissues and either are consumed in the inflammatory process or undergo apoptotic cell death and phagocytosis by macrophages.

Eosinophils represent 1–6% of the circulating white cells. They persist in the circulation for 8–12 hours, and can survive in tissue for an additional 8–12 days in the absence of stimulation. Eosinophils are phagocytic and their granules contain a peroxidase capable of generating reactive oxygen species and proteins involved in the intracellular killing of protozoa and helminths. They are also involved in allergic reactions.

Basophils are less common than eosinophils, representing less than 1 % of circulating white cells. Their life span is about 3–10 days. These cells are involved in hypersensitivity reactions. Mast cells resemble basophils but are only found in the tissues.

Monocytes are the largest of the white cells, which circulate for a few hours and then migrate into the tissue where they become macrophages, Kupffer cells or antigen-presenting dendritic cells. The former phagocytose debris, apoptotic cells and microorganisms.

Lymphocytes are derived from pluripotent haematopoietic stem cells in the bone marrow. There are two main types: T cells (which mediate cellular immunity) and B cells (which mediate humoral immunity). Lymphoid cells which migrate to the thymus develop into T cells, whereas B cells develop in the bone marrow. The majority of lymphocytes (approximately 80 %) in the circulation are T cells. Lymphocyte subpopulations can be defined with specific functions and their lifespan can vary from a few days to many years.

Platelets or thrombocytes are formed in the bone marrow from megakaryocytes. Up to 3 000 platelets then fragment off from each megakaryocyte into the circulation in the marrow sinusoids. The formation and maturation of megakaryocytes are stimulated by thrombopoietin produced in the liver. Platelets circulate for 8–10 days before they are destroyed in the reticulo- endothelial system. Some 30% of peripheral platelets are normally pooled in the spleen and do not circulate. The main function of platelets is participating in clot formation.

Hematopoiesis

Hematopoiesis, the process by which bone marrow stem cells develop all of the cell types present in the blood, is an enormous undertaking in which approximately 4×10^{11} cells are produced each day, a number that can increase ten-fold or more in times of heightened demand.

Hematopoietic stem cells represent one in 10^5 to 10^6 marrow cells (only ~0.01 % of the total marrow cells), are not morphologically distinguishable from other progenitors or small lymphocytes, but can be purified to homogeneity using physical characteristics and combinations of monoclonal antibodies to cell surface proteins. The two critical characteristics of a hematopoietic stem cell are its ability to differentiate into all blood cell types and its ability to self-renew.

In a normal adult human 100 to 1 000 multipotential stem cells undergo division each day, and after 25 to 30 cell doublings, during which time they progressively lose developmental potential, approximately 1×10^9 cells emerge, each committed to a single blood cell lineage (erythrocyte, neutrophil, eosinophil, basophil, monocyte, platelet, T lymphocytes, B lymphocytes, natural killer cell).

These latter, committed progenitors cells then continue to divide and differentiate into mature blood cell types through the combined action of several lineage specific and nonspecific transcription factor-induced gene expression programs.

The resulting primitive progenitor cells cannot be identified morphologically, so they are named according to the types of cell (or colony) they form during cell culture experiments. CFU–GM (colony-forming unit–granulocyte, monocyte) is a stem cell that produces granulocytic and monocytic lines, CFU–E produces erythroid cells, and CFU–Meg produces megakaryocytes and ultimately platelets.

The regulation of the numbers of each cell type is carefully controlled by paracrine and endocrine hematopoietic growth factors, which exert antiapoptotic, proliferative, and differentiating effects on hematopoietic stem, progenitor, and maturing blood cells. Some, such as granulocyte macrophage–colony stimulating factor (GM–CSF), interleukin-3 (IL-3) and stem cell factor (SCF), act on a wide number of cell types at various stages of differentiation. Others, such as erythropoietin (Epo), granulocyte–colony stimulating factor (G–CSF) and thrombopoietin (Tpo), are lineage-specific. Many of these growth factors are now synthesised by recombinant DNA technology and used as treatments.

During development, haematopoiesis occurs in the liver and spleen and subsequently in red bone marrow in the medullary cavity of all bones. In childhood, red marrow is progressively replaced by fat (yellow marrow), so that in adults normal haematopoiesis is restricted to the vertebrae, pelvis, sternum, ribs, clavicles, skull, upper humeri and proximal femora.

2. ANEMIA (general)

Key concepts

1. Anemia is a reduction below normal in the concentration of hemoglobin and/or erythrocytes in the body that results in a reduction of the oxygen-carrying capacity of the blood.
2. Common signs and symptoms of anemia include fatigue, lethargy, dizziness, shortness of breath, headache, edema, and tachycardia.
3. A standard initial laboratory evaluation for anemia includes a complete blood count (evaluation of the serum hemoglobin and hematocrit concentration, red blood cell count, white blood cell count, platelets), measurement of the red blood cell size and shape.
4. The goal of anemia therapy is to increase hemoglobin, which will improve red cell oxygen-carrying capacity, alleviate symptoms, and prevent anemia complications.
5. The underlying cause of anemia (e.g., blood loss; iron, folic acid, or B12 deficiency; or chronic disease) must be determined and used to guide therapy.

Normal erythropoiesis

The oxygen required by tissues for aerobic metabolism is supplied by the circulating mass of mature erythrocytes (red blood cells). The mature red cell is 8 fl in diameter, anucleate, discoid in shape, and extremely pliable in order to traverse the microcirculation successfully.

It is lacking a nucleus but filled with haemoglobin, which delivers oxygen to the tissues from the lungs.

Normal red cell production results in the daily replacement of 0.8–1 % of all circulating red cells in the body, since the average red cell lives 100–120 days.

The organ responsible for red cell production is called the erythron. The erythron is a dynamic organ made up of a rapidly proliferating pool of marrow erythroid precursor cells and a large mass of mature circulating red blood cells. The size of the red cell mass reflects the balance of red cell production and destruction.

Haemoglobin is a protein specially adapted for oxygen transport. It is composed of four globin chains, each surrounding an iron-containing porphyrin pigment molecule termed hem.

The circulating red blood cell population is continually renewed by the erythroid precursor cells in the marrow, under the control of both humoral and cellular growth factors. This cycle of normal erythropoiesis is a carefully regulated process. Oxygen sensors within the kidney detect minute changes in the amount of oxygen available to tissue and by releasing erythropoietin (EPO) are able to adjust erythropoiesis to match tissue requirements.

The critical elements of erythropoiesis – EPO production, iron availability, the proliferative capacity of the bone marrow, and effective maturation of red cell precursors – are used for the initial classification of anemia.

Definition

Anemia is the clinical hematological syndrome, which is characterized by decreased level of hemoglobin and erythrocytes in blood and reduced O₂ delivery to tissue.

The World Health Organization (WHO) defines anemia as a hemoglobin level less than 130 g/L and erythrocytes count less than $4 \times 10^{12}/L$ in males and, accordingly less than 120 g/L and $3.5 \times 10^{12}/L$ in females.

Epidemiology of anemia

Around 30 % of the total world population is anemic and half of these, some 600 million people, have iron deficiency. The prevalence rates are especially high in developing countries where dietary insufficiency and intestinal parasites are prevalent.

Anemia accounts for around 841,000 deaths annually worldwide. Africa and parts of Asia bear 71 % of the global mortality burden; North America represents only 1.4 % of the total morbidity and mortality associated with iron deficiency.

Anemia is generally more common in women, particularly during their reproductive years (ages 17–49 years), when anemia occurs in over 12 %, but in less than 2 % of men.

Anemia of chronic inflammation is believed to be the second most common cause of anemia, after iron deficiency. It is the most common type of anemia encountered among hospitalized patients. The wide spectrum of underlying

diseases includes acute and chronic infections, inflammatory and autoimmune diseases, cancers, and chronic kidney diseases.

Thalassemias (inherited anemias due to abnormal hemoglobin) are the most common genetic disorders in the world, affecting nearly 200 million people worldwide. Hemoglobinopathies are especially common in areas in which malaria is endemic. This clustering of hemoglobinopathies is assumed to reflect a selective survival advantage for the abnormal RBC, which presumably provide a less hospitable environment during the obligate RBC stages of the parasitic life cycle. Very young children with α -thalassemia are *more* susceptible to infection with the nonlethal *Plasmodium vivax*. Thalassemia might then favor a natural protection against infection with the more lethal *P. falciparum*.

The classic type of B₁₂-deficiency – pernicious anemia – is estimated to affect 0.1 % of the general population and 1.9 % of those over 60, accounting for 20–50 % of B₁₂ deficiency in adults. It has been shown from a literature review that the prevalence of pernicious anemia was higher in North European, especially for Scandinavian countries and African descent, in which high awareness towards the disease and better diagnostic tools might partly play a role in it.

In Europe and North America, the annual incidence of aplastic anemia is 2 per 1 million persons, and in Asia, 4 to 7 per 1 million. No age group is exempt, and although the syndrome occurs most often in young adults, the age distribution of newly diagnosed patients is bimodal, with peaks at 15 to 25 years and at 60 to 65 years.

Classification of anemia

The classifications of anemia by the size of the red cells, hemoglobin content and peculiarities of cell growth and destruction indicate the likely cause. Etiologic classification helps in management of the anemia.

1. Initial classification by peculiarities of cell growth and destruction
 - Marrow production defects “hypoproliferation” (reticulocyte production index <2)
 - Red cell maturation defects “ineffective erythropoiesis” (reticulocyte production index <2)
 - Decreased red cell survival “blood loss/hemolysis” (reticulocyte production index >2.5)
2. Classification by cell size
 - Normocytic (mean cell volume 80-100 fl)
 - Microcytic (mean cell volume <80 fl)
 - Macrocytic (mean cell volume >100 fl)
3. Classification by hemoglobin content
 - Normochromic (color index 0.8–1.05)
 - Hypochromic (color index <0.8)
 - Hyperchromic (color index >1.05)

4. Classification by etiology and pathogenesis

- Iron-deficiency anemia
- Anemia of chronic disease
- B12 and folic acid deficiency anemia
- Hemolytic anemias
- Anemia due to acute blood loss
- Aplastic anemia

Red blood cell indices

A routine complete blood count (CBC) is required as part of the evaluation and includes the hemoglobin, hematocrit, and red cell indices: the mean cell volume (MCV) in femtoliters, mean cell hemoglobin (MCH) in picograms per cell, and mean concentration of hemoglobin per volume of red cells (MCHC) in grams per liter.

Mean cell volume (MCV) in femtoliters (fl) = (hematocrit \times 10)/(red cell count $\times 10^{12}/l$) NORMAL VALUE: 90 \pm 8 fl.

Mean cell hemoglobin (MCH) in picograms per cell (pg) = (hemoglobin (g/dl) \times 10)/(red cell count $\times 10^{12}/l$) NORMAL VALUE: 27–31 pg.

Mean cell hemoglobin concentration (MCHC) in grams per liter (g/l) = (hemoglobin \times 10)/hematocrit NORMAL VALUE: 32–36%.

Color index (CI) = hemoglobin (g/dl)/red cell count $\times 10^{12}$ NORMAL VALUE: 0.8–1.05.

Reticulocyte production index (RPI) = reticulocyte count \times hematocrit/normal hematocrit. NORMAL VALUE: 2–2.5

A normal MCV and CI (normocytic normochromic anemia) suggests either hemolysis, acute blood loss, aplastic anemia or the anemia of chronic disease.

A low MCV and CI (microcytic hypochromic anemia) suggests iron deficiency or thalassemia.

A high MCV and CI (macrocytic hyperchromic anemia) suggests vitamin B₁₂ or folate deficiency or myelodysplasia.

An accurate reticulocyte count is key to the initial classification of anemia. Reticulocytes are red cells that have been recently released from the bone marrow (1–2 days). They are identified by staining with a supravital dye that precipitates the ribosomal RNA. Normally, the reticulocyte count ranges from 1 to 2 % and reflects the daily replacement of 0.8–1.0 % of the circulating red cell population. A corrected reticulocyte count provides a reliable measure of effective red cell production. With anemia, the percentage of reticulocytes may be increased while the absolute number is unchanged. To correct for this effect, the reticulocyte percentage is multiplied by the ratio of the patient's hemoglobin or hematocrit to the expected hemoglobin/hematocrit for the age and sex of the patient.

A high RPI suggests hemolysis or acute blood loss.

A low RPI suggests marrow damage, iron-, folate-, B₁₂-deficiency, decreased stimulation due to chronic diseases, drug toxicity.

As a complement to the red cell indices, the blood smear also reveals variations in cell size (anisocytosis) and shape (poikilocytosis).

Some clinically significant findings in the blood smear are:

- Spherocytes (hereditary spherocytosis, autoimmune hemolytic anemia)
- Irregularly contracted cells (glucose-6-phosphate dehydrogenase deficiency, oxidant damage from chemicals or drugs)
- Sickle cells and boat-shaped cells (sickle cell disease)
- Target cells (hemoglobinopathies, hereditary xerocytosis)
- Stomatocytes (hereditary stomatocytosis)
- Acanthocytes (acanthocytes)
- Basophilic stippling (lead poisoning, pyrimidine 5'-nucleotidase deficiency)
- Red cell fragments (schistocytes) (microangiopathic hemolytic anemia, mechanical hemolytic anemia)

Nonspecific clinical manifestations of anemia

Anemia is most often recognized by abnormal screening laboratory tests. Regardless of the cause of anemia, the clinical features depend on the rate of development and the compensatory ability of the cardiovascular and pulmonary system to adjust to tissue hypoxia.

Many individuals with mild anemia have no complaints. Others may complain of fatigue as well as dyspnea and palpitations, particularly following exercise. Patients with severe anemia are often symptomatic at rest and are unable to tolerate significant exertion. The patient may complain of a rapid, pounding sensation in the precordium. Patients with compromised myocardial reserve may develop complaints due to cardiac failure.

The symptoms of severe anemia often extend beyond the cardiac or circulatory system. Patients sometimes experience dizziness and headache and, less often, syncope, tinnitus, or vertigo. Many are irritable and have difficulty sleeping or concentrating. Because blood flow is shunted away from the skin, patients may complain of increased sensitivity to cold. In like manner, gastrointestinal symptoms such as indigestion, anorexia, or even nausea are attributable to the shunting of blood away from the splanchnic bed. Females commonly develop abnormal menstruation, either amenorrhea or increased bleeding. Males may experience impotence or loss of libido.

Pallor is the most commonly encountered physical finding in patients with anemia. This sign is due to the shunting of blood away from the skin and other peripheral tissues, permitting enhanced blood flow to vital organs. The usefulness of pallor as a physical finding is limited by other factors that affect the appearance of the skin. Blood flow to the skin may undergo wide fluctuations.

Moreover, the skin's thickness and texture vary widely among individuals. Those with a fair complexion may appear pale even though they are not anemic, while pallor is difficult to detect in deeply pigmented individuals. The amount of melanin in the epidermis is an important determinant of skin color. Pallor may be difficult to detect in patients who have increased melanin pigmentation due to Addison's disease or hemochromatosis.

Nevertheless, even in blacks, the presence of anemia may be suspected by the color of the palms or of noncutaneous tissues such as oral mucous membranes, nail beds, and palpebral conjunctivas. When the creases of the palm are as pale as the surrounding skin, the patient usually has a hemoglobin of less than 70 g/L.

In addition to tachycardia, a systolic ejection murmur is often heard over the precordium, particularly at the pulmonic area. In addition, a venous hum may be detected over the neck vessels. These findings disappear when the anemia is corrected.

A comprehensive history and physical examination are important in the assessment of the anemic patient. More specifically, dietary habits, drug histories, surgical procedures, and occupation should be documented. Careful questioning about blood loss, menses, gastrointestinal symptoms, and history of pregnancy may provide useful information.

Diagnosis of anemia

A detailed medical and medication history along with hematologic and biochemical tests, including a full blood screen, are essential for identifying the type of anemia and in many cases directing the treatment. As the nutritional anemias progress in stages (normal, negative nutrient balance, nutrient depletion, nutrient deficiency, anemia), monitoring early indicators of depletion may prevent the progression to overt anemia. Risk factors for certain vitamin deficiencies and reported symptoms often suggest the possible cause of anemia or alert the physician to the potential for anemia.

Treatment of anemia (general)

The main principle is to initiate treatment of mild to moderate anemia only when a specific diagnosis is made. Rarely, in acute setting, anemia may be so severe that red cell transfusions are required before a specific diagnosis is available. Whether the anemia is of acute or gradual onset, the selection of the appropriate treatment is determined by the documented cause of the anemia.

Often, the cause of the anemia is multifactorial (for example, a combination of anemia of chronic disease with iron-deficiency anemia). In every circumstance, it is important to evaluate the patient's iron status fully before and during the treatment of any anemia.

Red cell transfusions can be used for treatment of any severe anemia irrespective to cause. The indication for red cell transfusion for anemia is decreasing of hemoglobin level less than 70 g/l.

Red blood cell transfusion

Worldwide, more than 75 million units of *whole blood* are estimated to be donated every year.

A unit of blood is collected as a donation of 450 mL \pm 10 % into a citrate anticoagulant that also contains phosphate and dextrose. Whole blood provides both oxygen-carrying capacity and volume expansion. It is the ideal component for patients who have sustained acute hemorrhage of >25 % total blood volume loss. The red cell and hemoglobin content is variable and dependent on the donor's hematocrit and the precise volume bled. Whole blood is stored at 4 \pm 2 °C to diminish red cells' utilization of adenosine triphosphate and to preserve their viability, which should be at least 70 % at the end of a shelf life of 35 days. In Western countries, whole blood is rarely used because within a few hours or days, some coagulation factors (especially factors V and VIII) and platelets decrease in quantity or lose viability. Fresh whole blood avoids these problems, but it is typically used only in emergency settings (i.e., military).

Red cell concentrate (packed red blood cells) increases oxygen-carrying capacity in the anemic patient. Adequate oxygenation can be maintained with a hemoglobin content of 70 g/L in the normovolemic patient without cardiac disease; however, comorbid factors may necessitate transfusion at a higher threshold. In this product most of the plasma is removed and replaced with a solution of glucose and adenine in saline to maintain viability of red cells. If no cardiovascular disease, transfuse to maintain Hb at 70–90 g/L. If known or likely to have cardiovascular disease, maintain Hb 90–100 g/L.

Blood and blood components for transfusion must be compatible with the same blood type as the patient. Obtaining an accurate ABO/Rh grouping for a patient is the most significant serologic test performed before transfusion. When type-specific blood and components are unavailable or emergency circumstances do not allow their identification or use, type O-negative red cells should be used. Group O is the only choice for group O recipients and is the alternative choice for groups A, B, and AB.

Death directly attributable to transfusion is rare, at <0.5 per 100 000 transfusions. However, relatively minor symptoms of transfusion reactions (fever, itch or urticaria) occur during about 1 % of transfusions, usually in patients who have had repeated transfusions.

Transfusion reactions may result from immune and nonimmune mechanisms. Immune-mediated reactions (hemolysis, fever, allergy, purpura) are often due to preformed donor or recipient antibody; however, cellular elements may also cause adverse effects. Nonimmune causes of reactions (fluid overload, hypothermia, hyperkalemia, iron overload, hypotension) are due to the chemical and physical properties of the stored blood component and its additives.

Transfusion-transmitted viral infections are increasingly rare due to improved screening and testing.

3. IRON-DEFICIENCY ANEMIA

Definition

Iron-deficiency anemia is a disorder, characterized by decreased iron supply to erythroid marrow and impaired red blood cell production leading to poorly hemoglobinized new cells released into circulation.

Iron deficiency occurs when the body's iron stores are insufficient for the normal formation of hemoglobin, iron-containing enzymes, and other functional iron compounds such as myoglobin and those of the cytochrome system. Iron deficiency can be classified according to its severity: normal stores; iron store depletion (low serum ferritin); decreased serum iron (low serum iron, increased total iron-binding capacity); and anemia (reduced hemoglobin with microcytic, hypochromic erythrocytes). Erythrocytes of patients with mild, early-stage iron deficiency often appear to be normal in color and size (i.e., normochromic, normocytic).

Iron metabolism

Iron is an essential element for many physiologic processes, including erythropoiesis, tissue respiration, and several enzyme-catalyzed reactions. The average adult body contains 3 to 5 g elemental iron, distributed into two major components: functional iron and storage. Functional iron exists predominantly as hemoglobin (1.5–3 g) in circulating erythrocytes, with lesser amounts in iron containing proteins such as myoglobin and cytochromes (0.4 g), 3 to 7 mg bound to transferrin in plasma, and the remainder in storage iron in the form of ferritin or hemosiderin.

The ability of iron to donate or accept electrons readily through conversion between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states makes it a critical component of the hemoglobin and myoglobin porphyrin rings that transport oxygen as well as cytochromes and various other vital enzymes. Free iron is extremely toxic owing to its capacity to catalyze the formation of free radicals, which lead to cellular damage. Therefore, the majority of body iron that is not stably incorporated into porphyrin rings is associated with proteins.

Transferrin is the major protein associated with circulating plasma iron, and ferritin is the major protein associated with stored intracellular iron both in the cytoplasm and in mitochondria. Because of the potent conservation mechanisms of iron recycling through the reticuloendothelial macrophage system, only an average of about 1 to 2 mg of iron is normally lost per day, largely through mucosal sloughing, desquamation, and, in females of reproductive age, menstruation.

The average Western diet contains about 20 mg/day, and the efficiency of iron absorption in the duodenum is usually sufficient to maintain the amount of iron required for homeostatic balance. Dietary iron is present as two major pools: heme iron and nonheme iron. Heme iron, found only in meats, is two to three times more absorbable than nonheme iron, found in plant-based and iron-

fortified foods. The stomach's acidity promotes reduction of iron from the ferric state (Fe^{3+}) to the ferrous state (Fe^{2+}), which is better absorbed. Patients with achlorhydria secondary to age or gastrectomy tend to absorb nonheme iron poorly.

Storage iron (0.3–1.5 g), in the form of ferritin and hemosiderin, is located mainly in the parenchymal cells of the liver, the reticuloendothelial cells of the spleen, and bone marrow, and it replenishes functional iron. Iron stores account for one third of body iron in healthy men. Iron stores are more variable and are generally lower in children and women of childbearing potential. Low iron stores are an early sign of iron deficiency and may help differentiate between iron deficiency anemia and other causes of anemia.

Etiology

- **Dietary:** starvation, poverty, vegetarianism, religious practice, food fadism
- **Blood loss:** esophageal varices, peptic ulcer, drug-induced gastritis, carcinomas of stomach and colon, ulcerative colitis, hemorrhoids, renal or bladder lesions (hematuria), hookworm infestation, other organ bleeding (hemoptysis, epistaxis), frequent blood donation, athletic training, widespread bleeding disorder, in women – menstruation, postmenopausal bleeding, pregnancy
- **Malabsorption:** celiac disease, partial and total gastrectomy, chronic inflammation
- **Increased requirements:** rapid growth (as in childhood and adolescence), pregnancy; erythropoiesis
- **Impaired iron transport** (transferrin deficiency due to protein malabsorption, rarely – in genetic disorder known as atransferrinemia)

Pathogenesis

The major role of iron in mammals is to carry O_2 , as part of hemoglobin. O_2 is also bound by myoglobin in muscle. Iron is a critical element in iron-containing enzymes, including the cytochrome system in mitochondria. Without iron, cells lose their capacity for electron transport and energy metabolism. In erythroid cells, hemoglobin synthesis is impaired, resulting in anemia and reduced O_2 delivery to tissue.

Three pathogenic factors are implicated in the anemia of iron deficiency. First, hemoglobin synthesis is impaired as a consequence of reduced iron supply. Second, there is a generalized defect in cellular proliferation. Third, survival of erythroid precursors and erythrocytes is reduced, particularly when the anemia is severe.

Clinical manifestations

Iron deficiency precedes the manifestations of anemia. Most people with iron deficiency have minimal anemia and are asymptomatic. Progression to iron deficiency anemia is often insidious, although mildly lowered hemoglobin concentrations generally decrease work capacity. The development of symptoms

depends on the rate of iron loss and the body's ability to compensate. Symptoms generally become evident when the blood hemoglobin concentration falls 100 g/L, although some patients remain asymptomatic even with hemoglobin concentrations of 70 g/L).

Iron-deficiency anemia produces the signs and symptoms common to all anemias: pallor, palpitations, tinnitus, headache, irritability, weakness, dizziness, easy fatigability, and other vague and nonspecific complaints. The prominence of these signs depends on the degree and rate of development of the anemia. Because iron deficiency often is of insidious onset and prolonged duration, adaptive circulatory and respiratory responses may minimize these manifestations, permitting a surprising tolerance of low hemoglobin concentrations. With greater severity, anemia becomes increasingly debilitating as work capacity and tolerance of physical exertion are restricted and eventually can produce cardiorespiratory failure and even death.

Iron deficiency may produce clinical manifestations independent of anemia. The abnormalities seem to result from depletion of functional iron compounds in nonerythroid tissues, resulting in impaired proliferation, growth, and function. Epithelial tissues have high iron requirements due to rapid rates of growth and turnover and thus are affected in many patients with chronic iron deficiency. Glossitis, angular stomatitis, postcricoid esophageal web or stricture (which may become malignant), and gastric atrophy may develop. The combination of glossitis, a sore or burning mouth, dysphagia, and iron deficiency is called the Plummer-Vinson syndrome. The prevalence of these abnormalities seems to vary geographically, which suggests that environmental or genetic factors are involved.

Determining whether gastric atrophy is the cause or the consequence of iron deficiency may be difficult, particularly in older patients. In some patients, pernicious anemia and iron deficiency coexist. Changes in the lingual or buccal mucosa have been suggested as factors causing or contributing to the pica that develops in many patients with iron deficiency. Pagophagia, a variant of pica in which ice is the substance obsessively consumed, is a behavioral abnormality that is considered to be a highly specific symptom of iron deficiency, resolving within a few days to 2 weeks after beginning iron therapy. Other types of pica involving a variety of nonfoods, such as clay, starch, paper, and dirt, or an assortment of foods may occur in iron deficiency but are not as clearly linked to a lack of iron or as frequently responsive to iron therapy. In *koilonychia*, the fingernails are thin, friable, and brittle, and the distal half of the nail is a concave or "spoon" shape resulting from impaired nail bed epithelial growth. This condition is considered virtually pathognomonic of iron deficiency but occurs in a small minority of patients. Blue sclerae, a condition in which the sclerae have a definite or striking bluish hue, were recognized in 1908 by Osler as being associated with iron deficiency and have been reported to be a highly specific and sensitive indicator of iron deficiency. The bluish tinge is believed to result from thinning of the sclera, which makes the choroid visible. This

thinning has been postulated to result from impairment of collagen synthesis by iron deficiency. Iron deficiency has been postulated to have other nonhematologic consequences associated with impaired immunity and resistance to infection, diminished exercise tolerance and work performance, and a variety of behavioral and neuropsychological abnormalities.

Diagnosis

The diagnosis of iron deficiency anemia is made by laboratory testing. Because microcytic hypochromic RBCs are a *sine qua non* of this type of anemia, initial screening consists of a determination of *hemoglobin* levels, *mean corpuscular volume*, *erythrocyte hemoglobin content*, and *reticulocyte count*.

The definitive diagnosis of iron deficiency anemia is made by tests that measure total body iron stores: the absence of iron stores that can be mobilized is unique to this microcytic hypochromic anemia.

The *serum ferritin* level is the most reliable, noninvasive, and cost-effective indicator that is routinely available in most clinical laboratories.

Plasma iron and *total iron binding capacity* (TIBC) are measures of iron availability; hence they are affected by many factors besides iron stores. Plasma iron has a marked diurnal and day-to-day variation and becomes very low during an acute phase response but is raised in liver disease and haemolysis. Levels of *transferrin*, the binding protein for iron, are lowered by malnutrition, liver disease, an acute phase response and nephrotic syndrome, but raised by pregnancy or the oral contraceptive pill. A low *transferrin saturation* is consistent with iron deficiency but is less specific than a ferritin measurement.

In difficult cases it may still be necessary to examine a *bone marrow aspirate* for iron stores. Normally, when the marrow smear is stained for iron, 20–40 % of developing erythroblasts-called sideroblasts-will have visible ferritin granules in their cytoplasm. This represents iron in excess of that needed for hemoglobin synthesis. In states in which release of iron from storage sites is blocked reticulo-endothelial iron will be detectable, and there will be few or no sideroblasts.

Differential diagnosis

Specific entities to be considered in the differential diagnosis of hypochromic microcytic disorders include:

- anemia of chronic disease
- sideroblastic anemia (rare type of anemia in which iron is available but cannot be incorporated into hemoglobin)
- thalassemia

In all of these disorders, body iron stores are normal or increased.

Treatment

The preferred route of iron administration is oral. Oral iron is most readily absorbed in the absence of food, especially in the setting of decreased stomach acid production owing to atrophic gastritis, gastric surgery, or chronic suppression of gastric acid with an H₂ antagonist or proton pump inhibitor.

The major obstacle to oral iron replacement is unacceptable side effects, chiefly epigastric discomfort or nausea; diarrhea or constipation also occurs in some patients. Reducing the dose often eliminates nausea and epigastric discomfort.

Despite the development of a number of orally effective iron-containing compounds, the original salt, ferrous sulfate (325 mg three times daily), remains the most useful. Although some newer oral iron preparations, such as ferrous gluconate (300 mg two or three times daily) or ferrous fumarate (325 mg two or three times daily), may induce fewer GI side effects per milligram of iron, they are also less well absorbed, so there is no net advantage to these costlier formulations except for patients who cannot tolerate ferrous sulfate. Given both the low toxicity and the low cost of oral iron replacement, a therapeutic trial is an alternative means of confirming a diagnosis of iron deficiency anemia.

In situations in which primary blood loss is uncontrollable, iron cannot be absorbed owing to severe malabsorption, or oral iron is not tolerated despite concerted efforts to minimize side effects, parenteral iron is an effective alternative treatment.

Intramuscular dosing is limited to 100 mg/injection, so intravenous administration is recommended. Sodium ferric gluconate (given intravenously at a dose of 125 mg over 10 minutes) is the preferred form of parenteral iron owing to the low incidence of adverse reactions.

One limitation of sodium ferric gluconate is that the maximum dose deliverable in a single injection is approximately 125 mg, and a total dose of 500 to 2000 mg is usually required for adequate repletion. Although large doses of iron dextran can be delivered in a single intravenous injection, this is currently reserved for situations in which rapid iron replacement is required because of the life-threatening anaphylactic and delayed adverse reactions that occur in 0.6 and 2.5 % of cases, respectively. If iron dextran is to be given intravenously, premedication with diphenhydramine and a slow test-dose injection of 30 to 40 mg diluted in normal saline are recommended.

The response to iron repletion therapy is usually quite rapid, with elimination of symptoms within a few days. Increased reticulocytosis usually begins within 4 to 5 days, and the hemoglobin level often rises within 1 week and reaches a normal level after 6 weeks of therapy if adequate iron replacement is achieved. The goal of therapy, which is to reach a serum ferritin level of greater than 50 mg/L, usually takes 4 to 6 months. Therapy must be continued after adequate replacement is achieved if the underlying cause of iron deficiency is not reversible. Because of the avidity of transferrin receptor-rich erythroid precursors for transferrin-bound iron, the serum ferritin level usually does not rise until hemoglobin levels reach normal.

An incomplete or lack of response to oral iron therapy, as determined by failure to normalize the hemoglobin level, usually means either that iron replacement has not been adequate (most commonly due to noncompliance with oral iron because of its side effects) or that iron deficiency is not the primary cause of the anemia (e.g., coexisting anemia of chronic disease).

Less common causes of failure to respond to oral iron include iron malabsorption (e.g., celiac disease, atrophic gastritis) or blood loss in excess of iron replacement.

Transfusion therapy is reserved for individuals who have symptoms of anemia, cardiovascular instability, and continued and excessive blood loss from whatever source and who require immediate intervention. The management of these patients is less related to the iron deficiency than it is to the consequences of the severe anemia. Not only do transfusions correct the anemia acutely, but the transfused red cells provide a source of iron for reutilization, assuming they are not lost through continued bleeding. Transfusion therapy will stabilize the patient while other options are reviewed.

Prognosis

In most cases, iron deficiency anemia can be corrected rapidly by either oral or parenteral replacement, but the long-term prognosis ultimately depends on the clinical course of the underlying cause. It is critical that the patient undergo a full evaluation to determine the underlying cause of the iron deficiency, especially because an occult gastrointestinal lesion, often malignant, may be present, particularly in patients older than 50 years

4. ANEMIA OF CHRONIC DISEASE

Definition

Anemia of chronic disease refers to anemia that occurs in the setting of a chronic disease state, usually one associated with elevated levels of inflammatory cytokines.

Etiology

Anemias of chronic disease can be divided into three categories:

1. Chronic inflammation

Anemia which encompasses inflammation, infection, tissue injury, and conditions (such as cancer) associated with the release of proinflammatory cytokines – is one of the most common forms of anemia seen clinically.

With chronic inflammation, the primary disease will determine the severity and characteristics of the anemia. For example, many patients with cancer also have anemia that is typically normocytic and normochromic. In contrast, patients with long-standing active rheumatoid arthritis or chronic infections such as tuberculosis will have a microcytic, hypochromic anemia. In both cases, the bone marrow is hypoproliferative, but the differences in red cell indices reflect differences in the availability of iron for hemoglobin synthesis.

2. Renal disease

Progressive chronic kidney disease (CKD) is usually associated with a moderate to severe hypoproliferative anemia; the level of the anemia correlates with the stage of CKD. Red cells are typically normocytic and normochromic, and reticulocytes are decreased. The anemia is primarily due to a failure of EPO production by the diseased kidney and a reduction in red cell survival.

3. Endocrine and nutritional deficiencies (hypometabolic states)

Patients who are starving, particularly for protein, and those with a variety of endocrine disorders that produce lower metabolic rates, may develop a mild to moderate hypoproliferative anemia.

The difference in the levels of hemoglobin between men and women is related to the effects of androgen and estrogen on erythropoiesis. Testosterone and anabolic steroids augment erythropoiesis; castration and estrogen administration to males decrease erythropoiesis. Patients who are hypothyroid or have deficits in pituitary hormones also may develop a mild anemia.

Anemia may be more severe in Addison's disease, depending on the level of thyroid and androgen hormone dysfunction; however, anemia may be masked by decreases in plasma volume.

Mild anemia complicating hyperparathyroidism may be due to decreased EPO production as a consequence of the renal effects of hypercalcemia or to impaired proliferation of erythroid progenitors.

Decreased dietary intake of protein may lead to mild to moderate hypoproliferative anemia; this form of anemia may be prevalent in the elderly. The anemia can be more severe in patients with a greater degree of starvation. Deficiencies in other nutrients (iron, folate) may also complicate the clinical picture but may not be apparent at diagnosis.

A mild hypoproliferative anemia may develop in patients with chronic liver disease. In alcoholic liver disease, nutritional deficiencies are common and complicate the management.

Pathogenesis

There are three major mechanisms of anemia of chronic inflammation, and all are believed to result from the effects of abnormal levels of inflammatory cytokines:

- Dysregulated iron homeostasis, manifested by low serum iron (hypoferremia) in the presence of normal or elevated serum ferritin levels and abundant reticuloendothelial macrophage iron stores. The functional consequence is a limited availability of iron for erythroid progenitor cells and resultant restriction of erythropoiesis. Pro-inflammatory stimuli, including lipopolysaccharides, interferon- γ , and tumor necrosis factor- α decrease iron uptake by the reticuloendothelial cells.
- Because hepcidin is an iron-regulated, acute phase reactant peptide that blocks both iron uptake in the gut and iron release from hepatocytes and

macrophage stores, its upregulation by lipopolysaccharides and interleukins results in another mechanism of anemia.

- The inhibition of erythroid progenitor expansion is third pathophysiologic feature of anemia of chronic inflammation. Interferon- γ is the most potent inhibitory factor of erythropoiesis, but similar inhibition is believed to be mediated by IL-1, TNF- α , and interferon- β . These mediators of inflammation act to increase erythroid progenitor apoptosis, downregulate erythropoietin receptors, and antagonize pro-hematopoietic factors.

Clinical manifestations

The clinical manifestations in patients with anemia of chronic inflammation are usually dominated by the underlying disease process. The anemia in this condition is usually mild, with hemoglobin levels in the range of 80 to 100 g/l. However, supervening blood loss, absolute iron deficiency, or other aggravating factors can produce life-threatening anemia. Even mild to moderate anemia contributes to the debilitating effects of the underlying disease, adversely impacting performance status and quality of life. Moreover, the presence of anemia is associated with a poorer overall prognosis in many of the underlying chronic diseases, although correction of anemia has not been directly demonstrated to improve survival.

Diagnosis

Although anemia of chronic inflammation usually manifests as a normochromic normocytic process, between 20 and 50% of cases are associated with microcytic RBC indices.

Serum ferritin is the best single laboratory marker for assessing iron storage, and it is almost invariably normal or elevated in anemia of chronic disease. If both the serum iron and the transferrin saturation are reduced, reflecting dysregulation of iron homeostasis, the diagnosis of anemia of chronic disease can be made in the appropriate clinical setting after the exclusion of other causes of anemia, such as coexistent blood loss, thalassemia, and drug-induced suppression of erythropoiesis. In the presence of inflammation, however, up to 30% of patients with true iron deficiency have serum ferritin levels greater than 100 μ g/L, potentially obscuring the diagnosis of iron deficiency.

Examination of the bone marrow for reticulo-endothelial macrophage iron stores (hemosiderin) and erythroblasts containing iron granules (sideroblasts) can provide definitive evidence of absent iron stores in the setting of anemia of chronic inflammation. A low serum erythropoietin level is also useful in supporting a diagnosis of anemia of chronic inflammation, but only when the hemoglobin level is less than 100 g/l.

Differential diagnosis

Differential diagnosis is based mainly on the presence of underlying pathology and level of plasma ferritin. It considers next diseases:

- iron-deficiency anemia
- sideroblastic anemia
- thalassemia

Treatment

The most effective treatment for anemia of chronic disease is successful treatment of the underlying inflammatory disease process, whether it is an acute or chronic infection, treatable cancer, renal failure, or rheumatoid arthritis.

Even if definitive treatment is not possible, quality of life and perhaps prognosis can improve if symptomatic anemia is treated directly. Unfortunately, anemia of chronic inflammation remains undertreated, even in developed countries.

Blood transfusion offers the immediate resolution of anemia, but it is indicated chiefly when the anemia is life threatening or seriously limits the patient's functioning. These situations almost always involve supervening blood loss or some other acute process that compounds the anemia of chronic disease.

If iron replacement is needed for anemia of chronic inflammation, parenteral iron administration is usually required to replenish stores because of the block in intestinal absorption.

Erythropoietin therapy is currently approved for use in patients with chronic kidney disease, those with HIV infection, and cancer patients who are undergoing myelosuppressive treatment.

Prognosis

The overall prognosis of anemia of chronic inflammation is determined almost exclusively by the course of the underlying disease. It is well established that the degree of anemia correlates well with the severity of the underlying disease process and therefore with levels of inflammatory cytokines. In the absence of a supervening process, anemia of chronic inflammation is not life threatening, and treatment of the anemia per se has not been proved to affect overall survival.

B₁₂ AND FOLIC ACID DEFICIENCY ANEMIA (MEGALOBLASTIC)

Megaloblastic anemias, a group of disorders characterized by a distinct morphologic pattern in hematopoietic cells, are commonly due to a deficiency of vitamin B₁₂ (cobalamin) or folates.

A deficiency in cobalamin or folate results in the common biochemical feature of a defect in DNA synthesis, along with lesser alterations in RNA and protein synthesis, leading to a state of unbalanced cell growth and impaired cell division.

This is morphologically expressed as larger-than-normal “immature” nuclei with finely particulate chromatin, whereas the relatively unimpaired RNA and protein synthesis results in large cells with greater “mature” cytoplasm and cell volume. The microscopic appearance of this nuclear-cytoplasmic asynchrony (or dissociation) is morphologically described as *megaloblastic*. Megaloblastic hematopoiesis commonly presents with anemia, the most easily recognized manifestation of a global defect in DNA synthesis in all proliferating cells (especially of the gastrointestinal and reproductive tracts).

METABOLISM OF VITAMIN B₁₂ AND FOLIC ACID

Vitamin B₁₂, also known as cobalamin, occurs in synthetic and biologically active forms. It is a cobalt-containing vitamin that cannot be synthesized by mammalian tissue. Therefore, it must be obtained via dietary intake (mainly from meat, fish, eggs and milk) or supplementation. Some bacterial synthesis of B₁₂ occurs in the large bowel and the cecum, but there is no absorption at these sites.

The daily requirement for humans is 0.4 to 2.4 µg, and higher in pregnant and lactating mothers. Some diets, such as vegan, macrobiotic, or weight-reduction diets that drastically restrict food selection, may not meet the minimum daily needs. The total body stores amount to 2 to 5 mg, mainly in the liver. Thus, B₁₂ deficiency takes years to develop.

Vitamin B₁₂, particularly at the usual low levels in foods, is well absorbed from the gastrointestinal tract by an orderly sequence of events involving different binding proteins: R-proteins, intrinsic factor (IF), and transcobalamin. The R-proteins, a group of high-affinity, B₁₂-binding glycoproteins, are produced predominantly by leukocytes and are present in a variety of biologic secretions, including gastric fluid, plasma, saliva, tears, milk, and bile. Their function is not fully understood. Although cobalamin can bind to R-proteins or IF, at the low gastric pH, binding to gastric R-proteins is favored. The cobalamin remains bound to R-proteins in the upper small intestine until pancreatic proteases, such as trypsin, partially degrade the complex, releasing B₁₂, which then binds to IF. IF, a specific B₁₂-binding glycoprotein, is synthesized and secreted by the parietal cells of the stomach. Its secretion parallels hydrochloric acid secretion. IF is released at the cell surface, and the vitamin is taken up by the enterocyte. Approximately 4 hours later, B₁₂ exits the cells bound to transcobalamin.

Another mechanism for B₁₂ absorption involves diffusion and not IF. This mechanism is biologically important only when large amounts are ingested and generally provides only small quantities of the vitamin. This mechanism is being explored as a potential method of providing oral B₁₂ therapy to people with low levels of IF (pernicious anemia).

The daily cellular needs for B₁₂ are low, and much of what is ingested is stored in the liver. Vitamin B₁₂ is conserved in the body by enterohepatic recycling. Biliary excretion of B₁₂ is much higher than excretion in urine or feces.

Folates are produced by plants and bacteria; hence dietary leafy vegetables (spinach, broccoli, lettuce), fruits (bananas, melons) and animal protein (liver, kidney) are a rich source. Folate needs depend on metabolic and cell turnover rates. In general, the minimum daily requirement is 65 to 400 µg daily. The average amount stored in the body is 5 to 10 mg, one half of which is found in the liver. With folate depletion, deficiency leading to anemia generally occurs within 6 months.

Active absorption of dietary folate occurs mainly in the proximal part of the small intestine. Amounts of folic acid beyond the daily needs are excreted almost entirely as metabolites by the kidney.

Etiology

Causes of vitamin B12 deficiency:

- *Dietary deficiency* (vegans, poor people)
- *Gastric factors* (hypochlorhydria in elderly patients or following gastric surgery)
- *Pernicious anaemia* (an autoimmune disorder in which the gastric mucosa is atrophic, with loss of parietal cells causing intrinsic factor deficiency)
- *Small bowel factors* (pancreatic enzymes insufficiency, bacterial overgrowth, fish tapeworm infection, Crohn's disease)

Causes of folate deficiency:

- *Poor intake of vegetables*
- *Malabsorption* (celiac disease, Crohn's disease)
- *Increased demand* (cell proliferation due to hemolysis or pregnancy)
- *Drugs* (methotrexate, contraceptive pill, certain anticonvulsive drugs)

Pathogenesis

The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow. All conditions that give rise to megaloblastic changes have in common a disparity: in the rate of synthesis or availability of the four immediate precursors of DNA: purines, thymine and cytosine, pyrimidines.

Clinical manifestations

The finding of macrocytosis (increased mean corpuscular volume [MCV]) on a routine complete blood count may be the first clinical manifestation. In other patients, the findings may be dominated by the condition causing the deficiency of cobalamin or folate, such as malabsorption, alcoholism, or malnutrition.

The main clinical features in more severe cases are those of anemia.

The clinical manifestations of folate deficiency may also include:

- *hematologic* (pancytopenia with megaloblastic bone marrow – low leukocyte count may predispose to infections, thrombocytopenia sometimes leads to bruising),

- *cardiopulmonary* (ischemic heart disease, cerebrovascular diseases and pulmonary thromboembolism secondary to anemia and raised plasma homocysteine levels in vitamin B₁₂ deficiency),
- *gastrointestinal* (anorexia, glossitis, angular cheilosis, malabsorption, mild hepatosplenomegaly),
- *dermatologic* (reversible hyperpigmentation of the skin, premature graying, unconjugated jaundice),
- *genital* (megaloblastosis of the cervical epithelium, recurrent fetal loss infertility)
- *psychiatric* (primarily a flat affect) symptoms.

If patients have additional neurologic findings, other diseases that predispose to folate deficiency (e.g., alcoholism with thiamine deficiency) or associated cobalamin deficiency must be considered. Because megaloblastosis secondary to either folate or cobalamin deficiency results in functional folate deficiency, the hematologic manifestations of both deficiencies, including pancytopenia with megaloblastic bone marrow, are indistinguishable. However, only cobalamin deficiency results in a patchy demyelinating process, which is expressed clinically as cerebral abnormalities and subacute combined degeneration of the spinal cord. This widespread demyelination begins in the dorsal columns in the thoracic segments of the spinal cord and then spreads contiguously to involve the corticospinal tracts and later the spinothalamic and spinocerebellar tracts. Diminished vibratory sensation and proprioception of the lower extremities are the most common early objective signs. Neuropathic involvement of the legs precedes that of the arms. Upper motor neuron signs may be modulated by the subsequent involvement of peripheral nerves. Loss of sphincter and bowel control or involvement of cranial nerves, such as optic neuritis, may be accompanied by other dysfunction of the cerebral cortex, including dementia, psychoses, and disturbances of mood. Folate deficiency in adults does not give rise to significant neurologic findings.

Hematologic manifestations, neurologic manifestations, or both may dominate the clinical picture.

Diagnosis

Early diagnosis of B₁₂ deficiency relies on identifying risk factors for deficiency, obtaining a complete medical, dietary, and medication history, and assessing appropriate clinical laboratory tests. The goal is to prevent development of anemia or neurologic symptoms by early recognition and treatment of B₁₂ deficiency.

Measurement of plasma B₁₂ concentrations is simple and inexpensive and is considered the standard test for diagnosing B₁₂ deficiency.

Once B₁₂ deficiency is determined, assessment of the cause (malabsorption vs. other) guides treatment selection. Antigastric parietal cell or anti-IF antibodies can be measured to provide information about a patient's ability to absorb B₁₂.

A Schilling test (with or without IF) was an alternative method of assessing B₁₂ malabsorption, once provided invaluable information on the locus and mechanism of cobalamin malabsorption, but was discontinued in 2003.

Anemia is a late presentation of B₁₂ deficiency that may be avoided with early detection and correction of B₁₂ depletion. Hematologic tests, such as a blood smear and the red cell indices, help differentiate the cause of anemia. Macrocytosis (MCV >100 fL) often occurs with B₁₂ deficiency, but it also occurs with other conditions, such as liver disease, myxedema, acute myelogenous leukemia, acquired sideroblastic anemia, aplastic anemia, hemolytic anemia, posthemorrhagic states, splenectomy. Evaluating the smear for megaloblastic changes, such as neutrophil hypersegmentation and oval-shaped erythrocytes, generally differentiates a B₁₂ or folate deficiency from other causes.

As with B₁₂ deficiency, early diagnosis and prevention of anemia depend on identification and treatment of high-risk patients. Serum or erythrocyte folate concentrations may be determined to assess folate status.

In the later stages of folate deficiency, a blood smear and hematologic evaluation often show macrocytosis with megaloblastosis. Anemia occurs only when tissue levels are depleted, so a normal serum folate concentration does not exclude deficiency.

In florid hematologic disease with or without neurologic disease suggestive of cobalamin or folate deficiency, the identification of nucleated red cells with megaloblastic changes in the peripheral smear - which reflects the morphology in the bone marrow - can clinch the diagnosis of megaloblastosis. If not found, a bone marrow aspirate can be invaluable in making the rapid diagnosis of megaloblastosis.

Differential diagnosis

Macrocytosis in the peripheral smear can be found in number of diseases:

- Response to hemolysis
- Response to blood loss
- Post-splenectomy
- Liver disease
- Aplastic anemia
- Myelodysplastic syndrome
- Hypothyroidism
- Chronic obstructive pulmonary disease

Treatment

Vitamin B₁₂ deficiency is treated with cobalamin 1 000 mg i.m. in five doses 2 or 3 days apart, followed by maintenance therapy of 1000 mg every 3 months for life.

The reticulocyte count will peak by the 5th–10th day after therapy and may be as high as 50 %. The haemoglobin will rise by 10 g/L every week. The

response of the marrow is associated with a fall in plasma potassium levels and rapid depletion of iron stores. If an initial response is not maintained and the blood film is dimorphic (i.e. shows a mixture of microcytic and macrocytic cells), the patient may need additional iron therapy. A sensory neuropathy may take 6–12 months to correct.

Oral folic acid 5 mg daily for 3 weeks will treat acute deficiency and 5 mg once weekly is adequate maintenance therapy. Prophylactic folic acid in pregnancy prevents megaloblastosis in women at risk, and reduces the risk of fetal neural tube defects.

Prophylactic supplementation is also given in chronic haematological disease associated with reduced red cell lifespan (e.g. autoimmune haemolytic anaemia or haemoglobinopathies).

Prognosis

The general response to cobalamin replacement is a dramatic improvement in well-being, with alertness, a good appetite, and resolution of a sore tongue. Megaloblastic hematopoiesis reverts to normal within 12 hours and resolves by 48 hours; the only persistent findings may be giant metamyelocytes in the bone marrow and hypersegmented neutrophils in the blood for up to 14 days.

The reticulocyte count peaks by days 5 to 8, followed by a rise in the red cell count, hemoglobin level, and hematocrit. By the end of the first week, the white blood cell count rises, sometimes with a transient left shift, as does the platelet count; both normalize by approximately 2 months.

HEMOLYTIC ANEMIAS

Definition

Hemolytic anemias – is group of inherited and acquired diseases, which is characterized by hemolytic syndrome – excessive destruction of erythrocytes.

Depending on localization of hemolysis anemias can be with intracellular (extravascular) hemolysis (in the macrophages) and with intravascular hemolysis (in the lumen of vessels)

The anemia is of greatest clinical concern when the rate of RBC destruction exceeds that of erythropoiesis. The hemolytic process may occur chronically or manifest as an acute episode, depending on the etiologic mechanism. Acute hemolysis is generally a more clinically threatening event. Many anemias have a hemolytic component due to the production of defective or damaged RBCs (e.g., megaloblastic anemias, thalassemias, sickle cell anemia).

Etiology

Hemolytic anemias can be categorized as either inherited or acquired disorders. Inherited hemolytic anemias include defective globin synthesis, erythrocyte membrane defects, and erythrocyte enzyme deficiencies. Acquired hemolytic disorders are those caused by some extrinsic event and do not

involve a genetic component. Typically, the acquired hemolytic anemias are either immune-mediated, due to physical stress on the RBC, or are induced by certain infections.

Classification of common hemolytic anemias

I. Inherited

1. *Globin synthesis defect*

Sickle cell anemia

Thalassemia

2. *Erythrocyte membrane defect*

Hereditary spherocytosis

Hereditary elliptocytosis

3. *Erythrocyte enzyme defect*

Glucose- 6-phosphate dehydrogenase deficiency (favism)

Pyruvate kinase deficiency

II. Acquired

1. *Immune-mediated*

Autoimmune hemolytic anemia

Drug-induced immune hemolytic anemia

2. *Non-immune hemolytic anemia*

Paroxysmal nocturnal hemoglobinuria

Physical trauma

Infection (malaria)

Exogenous substances

Pathogenesis

The average RBC life span is 120 days, but during severe hemolytic episodes this can be reduced to as low as 5 to 20 days. RBCs are hemolyzed either within the circulation (intravascular hemolysis) or taken up by the RES and destroyed (extravascular hemolysis). Intravascular hemolysis may be caused by trauma to the RBC, complement fixation to the RBC (immune-mediated), or exposure to exogenous substances. Under normal circumstances, however, most RBC catabolism occurs extravascularly by the RES in the liver and spleen.

Following lysis of the RBC, hemoglobin is released into the blood, where it is bound by the plasma protein haptoglobin. Free heme molecules are bound by the plasma protein hemopexin. The hemoglobin-haptoglobin complex is rapidly cleared from the circulation by the RES, and the heme component is metabolized to unconjugated (indirect) bilirubin. In the liver, this is linked with glucuronic acid, forming conjugated (direct) bilirubin, which passes from the bile duct into the intestine. Fecal bacteria then metabolize conjugated bilirubin to urobilinogen, which is primarily excreted in the feces. Iron from heme catabolism is stored as ferritin or hemosiderin.

During hemolysis, if the haptoglobin binding capacity is exceeded, unbound hemoglobin levels increase, resulting in hemoglobinemia. In this case, free hemoglobin is filtered through the glomerulus and is usually reabsorbed by the proximal tubules. In severe intravascular hemolysis, the reabsorptive capacity is exceeded, causing hemoglobinuria. When the liver's conjugating capacity is exceeded during moderate or severe hemolysis, unconjugated (indirect) bilirubin serum levels increase.

Clinical manifestations (common for hemolytic anemias)

The clinical presentation of a patient with anemia is greatly influenced in the first place by whether the onset is abrupt or gradual, and HAs are no exception. A patient with autoimmune HA or with favism may be a medical emergency, whereas a patient with mild hereditary spherocytosis or with cold agglutinin disease may be diagnosed after years. This is due in large measure to the remarkable ability of the body to adapt to anemia when it is slowly progressing. At the clinical level, the main sign is jaundice; in addition, the patient may report discoloration of the urine. In many cases of HA, the spleen is enlarged, because it is a preferential site of hemolysis; and in some cases, the liver may be enlarged as well. Fever, chills, chest pain, tachycardia, and backache may occur if the intravascular hemolysis is particularly rapid.

In all severe congenital forms of HA, there may also be skeletal changes due to overactivity of the bone marrow (although they are never as severe as they are in thalassemia).

Diagnosis (common for hemolytic anemias)

The laboratory features of HA are related to hemolysis per se and the erythropoietic response of the bone marrow. Hemolysis regularly produces an increase in unconjugated bilirubin and aspartate aminotransferase (AST) in the serum; urobilinogen will be increased in both urine and stool. If hemolysis is mainly intravascular, the telltale sign is hemoglobinuria (often associated with hemosiderinuria); in the serum, there is hemoglobin, lactate dehydrogenase (LDH) is increased, and haptoglobin is reduced. In contrast, the bilirubin level may be normal or only mildly elevated. The main sign of the erythropoietic response by the bone marrow is an increase in reticulocytes.

The increased number of reticulocytes is associated with an increased mean corpuscular volume (MCV) in the blood count. On the blood smear, this is reflected in the presence of macrocytes; there is also polychromasia, and sometimes one sees nucleated red cells. In most cases, a bone marrow aspirate is not necessary in the diagnostic workup; if it is done, it will show erythroid hyperplasia. In practice, once an HA is suspected, specific tests will usually be required for a definitive diagnosis of a specific type of HA.

Treatment (common for all hemolytic anemias)

Blood transfusion is needed as in all forms of anemia when the hematocrit becomes low. Hydration is, in general, useful to help prevent toxicity to the kidney tubule from the free hemoglobin. Specific therapy is discussed with each disease below.

Specific features of some hemolytic anemias

Sickle-cell anemia

Sickle-cell disease results from a single glutamic acid to valine substitution at position 6 of the beta globin polypeptide chain. It is inherited as an autosomal recessive trait.

Etiology and pathogenesis. Homozygotes only produce abnormal beta chains that make hemoglobin S (HbS, termed SS), and this results in the clinical syndrome of sickle-cell disease.

Heterozygotes produce a mixture of normal and abnormal beta chains that make normal HbA and HbS (termed AS), and this results in the clinically asymptomatic sickle-cell trait.

When hemoglobin S is deoxygenated, the molecules of hemoglobin polymerise to form pseudocrystalline structures known as 'tactoids'. These distort the red cell membrane and produce characteristic sickle-shaped cells.

Clinical features. Sickling is precipitated by hypoxia, acidosis, dehydration and infection. Irreversibly sickled cells have a shortened survival and plug vessels in the microcirculation. This results in a number of acute syndromes, termed 'crises', and chronic organ damage.

Vaso-occlusive crisis. Plugging of small vessels in the bone produces acute severe bone pain. This affects areas of active marrow: the hands and feet in children (so-called dactylitis) or the femora, humeri, ribs, pelvis and vertebrae in adults. Patients usually have a systemic response with tachycardia, sweating and a fever. This is the most common crisis.

- ***Sickle chest syndrome.*** This may follow on from a vaso-occlusive crisis and is the most common cause of death in adult sickle disease. Bone marrow infarction results in fat emboli to the lungs which cause further sickling and infarction, leading to ventilatory failure if not treated.

- ***Sequestration crisis.*** Thrombosis of the venous outflow from an organ causes loss of function and acute painful enlargement. In children the spleen is the most common site. Massive splenic enlargement may result in severe anaemia, circulatory collapse and death. Recurrent sickling in the spleen in childhood results in infarction and adults may have no functional spleen. In adults the liver may undergo sequestration with severe pain due to capsular stretching.

- ***Aplastic crisis.*** Infection of adult sicklers with human erythrovirus results in a severe but selflimiting red cell aplasia. This produces a very low hemoglobin which may cause heart failure. Unlike in all other sickle crises, the reticulocyte count is low.

Investigations. Patients with sickle-cell disease have a compensated anaemia, usually around 60–80 g/L. The blood film shows sickle cells, target cells and features of hyposplenism. A reticulocytosis is present. The definitive diagnosis requires hemoglobin electrophoresis to demonstrate the absence of HbA, 2–20 % HbF and the predominance of HbS. Both parents of the affected individual will have sickle-cell trait.

Management. All patients with sickle-cell disease should receive prophylaxis with daily folic acid, and penicillin V to protect against pneumococcal infection which may be lethal in the presence of hyposplenism. These patients should be vaccinated against pneumococcus and, where vaccine is available, *Haemophilus influenzae* and hepatitis B.

Vaso-occlusive crises are managed by aggressive rehydration, oxygen therapy, adequate analgesia (which often requires opiates) and antibiotics. Blood transfusion may be used in a sequestration or aplastic crisis.

Some agents are able to increase synthesis of HbF and this has been used to reduce the frequency of severe crises. The oral cytotoxic agent hydroxycarbamide has been shown to have clinical benefit with acceptable side-effects in children and adults who have recurrent severe crises.

Relatively few allogeneic stem-cell transplants from HLA-matched siblings have been performed but this procedure appears to be potentially curative.

Prognosis. Even with standard medical care, approximately 15 % of patients die by the age of 20 years and 50 % by the age of 40 years.

Talassemia

Thalassemia is an inherited impairment of hemoglobin production, in which there is partial or complete failure to synthesize a specific type of globin chain.

Etiology and pathogenesis. In alpha-thalassemia, disruption of one or both alleles on chromosome 16 may occur, with production of some or no alpha globin chains. In beta-thalassemia, defective production usually results from disabling point mutations causing no or reduced beta chain production.

Failure to synthesize beta chains (beta-thalassemia) is the most common type of thalassemia, most prevalent in the Mediterranean area.

Heterozygotes have thalassemia minor, a condition in which there is usually mild anemia and little or no clinical disability, which may be detected only when iron therapy for a mild microcytic anemia fails. Homozygotes (thalassemia major) either are unable to synthesize haemoglobin A or at best produce very little; after the first 4–6 months of life they develop profound hypochromic anaemia.

Reduced or absent alpha chain synthesis is common in Southeast Asia. There are two alpha gene loci on chromosome 16 and therefore each individual carries four alpha gene alleles.

- If one is deleted, there is no clinical effect.
- If two are deleted, there may be a mild hypochromic anaemia.

- If three are deleted, the patient has haemoglobin H disease.
- If all four are deleted, the baby is stillborn (hydrops fetalis).

Haemoglobin H is a beta-chain tetramer, formed from the excess of beta chains, which is functionally useless, so that patients rely on their low levels of HbA for oxygen transport.

Clinical features. The clinical manifestations (phenotype expression) of the thalassemia syndromes are extremely variable and depend on the degree of globin chain imbalance. The predominant features are a hypochromic microcytic anemia, with jaundice and hepatosplenomegaly. The most common complication is the development of hypersplenism due to severe splenomegaly. Other complications include gallstones, leg ulcers, increased risk of infection, folic acid deficiency, and increased risk of venous thrombosis, mainly following splenectomy. Extramedullary hematopoiesis, as a compensatory mechanism, leads to the formation of erythropoietic tissue masses that primarily affect the spleen, liver, lymph nodes, and vertebrae.

Investigations. The diagnosis of thalassemia may be required in a patient with a suspicious clinical picture or to identify a heterozygote subject as part of a family study or population screening program. The general diagnostic approach is common to any form of thalassemia, regardless of presentation. Individuals with a mean cell volume (MCV) below 80 fL and mean cell hemoglobin (MCH) below 27 pg with normal iron parameters need further investigation. The number of red blood cells is usually higher than normal. In the presence of anemia with thalassemic red cell changes, the next step is the evaluation of hemoglobin fractions (HbA, HbA₂, HbF, or Hb variants) by electrophoresis.

Management. No specific treatment is required for α - or β -thalassemia heterozygotes (carriers, thalassemia minor), but they should receive appropriate genetic counseling.

For beta-thalassemia major several options available:

- Blood transfusion to maintain Hb >100 g/L
- Folic acid 5 mg daily
- Iron chelation therapy (deferoxamine)
- Splenectomy (if splenomegaly causes mechanical problems)
- Allogeneic bone marrow

Treatment of hemoglobin H disease is similar to that of beta-thalassemia of intermediate severity, involving folic acid supplementation, transfusion if required and avoidance of iron therapy.

Hereditary spherocytosis and elliptocytosis

Spherocytosis is usually inherited as an autosomal dominant condition, although 25 % of cases have no family history and represent new mutations. The incidence is approximately 1:5 000 in developed countries.

Inheritance of elliptocytosis may be autosomal dominant or recessive. It is less common than hereditary spherocytosis in Western countries, with an incidence of 1:10 000, but is more common in equatorial Africa and parts of Southeast Asia.

Etiology and pathogenesis. The membrane-cytoskeleton complex is so integrated that, not surprisingly, an abnormality of almost any of its components will be disturbing or disruption, causing structural failure, which results ultimately in hemolysis. These abnormalities are almost invariably inherited mutations; thus, diseases of the membrane-cytoskeleton complex belong to the category of inherited hemolytic anemias. When the normal red cell structure is disturbed, usually by a quantitative or functional deficiency of one or more proteins in the cytoskeleton, cells lose their elasticity. Each time such cells pass through the spleen, they lose membrane relative to their cell volume.

This results in an increase in mean cell hemoglobin concentration (MCHC), abnormal cell shape and reduced red cell survival due to extravascular hemolysis.

Clinical features. Classic triad of hereditary spherocytosis is anemia, jaundice, and splenomegaly. Chronic hemolysis leads to the formation of bilirubinate gallstones, the most frequently reported complication in hereditary spherocytosis patients.

Other complications of HS include aplastic, hemolytic, and megaloblastic crises. Aplastic crises occur after virally induced bone marrow suppression and present with anemia, jaundice, fever, and vomiting. The most common etiologic agent in these cases is parvovirus B19. Hemolytic crises, usually associated with viral illnesses and occurring before 6 years of age, are generally mild and present with jaundice, increased spleen size, and a decrease in hematocrit. Megaloblastic crises occur in HS patients with increased folate demands, such as the pregnant patient, growing children, or patients recovering from an aplastic crisis.

The clinical presentation of hereditary elliptocytosis is heterogeneous, ranging from asymptomatic carriers to patients with severe, life-threatening anemia. Most hereditary elliptocytosis patients are asymptomatic and are diagnosed incidentally during testing for unrelated conditions.

Investigations. Patients with hereditary spherocytosis may present at any age, usually with anemia, hyperbilirubinemia, or an abnormal blood smear. In evaluating a patient with suspected HS, particular attention should be paid to the family history, including questions about anemia, jaundice, gallstones, and splenectomy. Initial laboratory investigation should include a complete blood count with a peripheral smear, reticulocyte count, direct antiglobulin test (Coombs' test), and serum bilirubin.

When the peripheral smear or family history is suggestive of hereditary spherocytosis, an incubated osmotic fragility test should be obtained.

Typical hereditary spherocytosis patients have blood smears with easily identifiable spherocytes lacking central pallor. Osmotic fragility is tested by adding increasingly hypotonic concentrations of saline to red cells. Normal erythrocytes are able to increase their volume by swelling, but spherocytes, which are already at maximal volume for surface area, burst at higher saline concentrations than normal. Cigar-shaped elliptocytes on peripheral blood smear are the hallmark of hereditary elliptocytosis.

Management. Folic acid prophylaxis, 5 mg once weekly, should be given for life. If anemia is severe – blood transfusions may be required. Consideration may be given to splenectomy, which improves but does not normalise red cell survival. Potential indications include moderate to severe haemolysis with complications (anaemia and gallstones), although splenectomy should be delayed until after 6 years of age in view of the risk of sepsis.

Therapy is rarely needed in patients with hereditary elliptocytosis. In rare cases, occasional red blood cell transfusions may be required.

Glucose- 6-phosphate dehydrogenase (G6PD) deficiency (favism)

G6PD deficiency is the most common inherited disorder of erythrocyte metabolism, affecting more than 400 million people worldwide. The high prevalence of G6PD deficiency is thought to be due to genetic selection because G6PD-deficient erythrocytes have a selective advantage against invasion by the malaria parasite *Plasmodium falciparum*.

Etiology and pathogenesis. G6PD is pivotal in the hexose monophosphate shunt pathway. The enzyme is a heteromeric structure made of catalytic subunits which are encoded by a gene on the X chromosome. The deficiency therefore affects males and rare homozygotic females, but it is carried by females.

Clinical features. The vast majority of people with G6PD deficiency remain clinically asymptomatic throughout their lifetime. The most clinically significant syndrome of G6PD deficiency is acute hemolytic anemia. Presenting symptoms include irritability, fever, nausea, abdominal pain, and diarrhea within 48 hours of oxidant exposure (fava beans, infection, certain drugs). Hemoglobinuria, jaundice, and anemia ensue. The spleen and liver may be enlarged and tender. Cases with severe anemia may precipitate congestive heart failure.

Investigations. The suspicion of G6PD deficiency can be confirmed by semiquantitative methods often referred to as screening tests, which are suitable for population studies and can correctly classify male subjects, in the steady state, as G6PD normal or G6PD deficient. However, in clinical practice, a diagnostic test is usually needed when the patient has had a hemolytic attack; this implies that the oldest, most G6PD-deficient red cells have been selectively destroyed, and young red cells, having higher G6PD activity, are being released into the circulation. Under these conditions, only a quantitative test can give a definitive result.

Management. The best treatment for the individual with acute hemolytic anemia is careful prescription of medications and avoidance of inciting agents. Outside of acute hemolytic episodes, these patients do not require any special therapy.

Acute hemolytic episodes are managed with particular attention to hematologic, cardiopulmonary, and renal complications of hemolysis.

Pyruvate kinase deficiency

This is the second most common red cell enzyme defect. It results in deficiency of ATP production and a chronic hemolytic anemia. It is inherited as an autosomal recessive trait. The extent of anemia is variable. Enzyme activity is only 5–20% of normal. Red blood cell transfusion support may be necessary.

Autoimmune hemolytic anemia (AIHA)

This results from increased red cell destruction due to red cell autoantibodies. The antibodies may be IgG or M, or more rarely IgE or A. Autoimmune hemolytic anemia is uncommon. The estimated overall annual incidence is about 1 case per 100,000 population; after age 60 years, the annual incidence reaches 10 per 100,000. The disorder can occur at any age, but most patients are older than 40 years.

Pathogenesis. If an antibody avidly fixes complement, it will cause intravascular haemolysis, but if complement activation is weak, the hemolysis will be extravascular. Antibody-coated red cells lose membrane to macrophages in the spleen and hence spherocytes are present in the blood. The optimum temperature at which the antibody is active (thermal specificity) is used to classify immune hemolysis:

- *Warm antibodies* bind best at 37 °C and account for 80 % of cases. The majority are IgG.
- *Cold antibodies* bind best at 4 °C but can bind up to 37 °C in some cases. They are usually IgM and bind complement. They account for the other 20 % of cases.

Clinical features. AIHA is a serious condition; without appropriate treatment, it may have a mortality of approximately 10%. The onset is often abrupt and can be dramatic. The hemoglobin level can drop, within days, to as low as 40 g/l; the massive red cell removal will produce jaundice; and sometimes the spleen is enlarged.

Autoimmune hemolytic anemia is a well-known complication of chronic lymphocytic leukemia and non-Hodgkin's B-cell lymphomas. It can also occur occasionally in ulcerative colitis, rheumatoid arthritis, and various carcinomas. Autoimmune hemolytic anemia occurs in about 10 % of cases of systemic lupus erythematosus; it may be the initial clinical manifestation of the disease or occur later.

Investigation. There is evidence of hemolysis and spherocytes on the blood film. The diagnosis is confirmed by the direct Coombs or antiglobulin test. The patient's red cells are mixed with Coombs reagent, which contains antibodies against human IgG/M/complement. If the red cells have been coated by antibody in vivo, the Coombs reagent will induce their agglutination and this can be detected visually.

Management. If the hemolysis is secondary to an underlying cause, this must be treated and any offending drugs stopped.

It is usual to treat patients initially with prednisolone 1 mg/kg orally. If the hemolysis fails to respond to corticosteroids or can only be stabilised by large doses, then splenectomy should be considered. This removes a main site of red cell destruction and antibody production, with a good response in 50–60 % of cases. If splenectomy is not appropriate, alternative immunosuppressive therapy with azathioprine or cyclophosphamide may be considered.

Drug-Induced Immune Hemolytic Anemia

Many drugs or drug metabolites have the potential to elicit antidrug antibodies.

Drugs that form covalent bonds with proteins in the red cell membrane can bind antidrug antibodies to the red cell surface, causing a positive direct antiglobulin test (Coombs test) and, in some cases, initiating antibodymediated destruction of red cells.

Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is an acquired form of hemolytic anemia in which a somatic mutation in a hematopoietic stem cell renders erythrocytes susceptible to the lytic components of complement, thereby causing intravascular hemolysis in the absence of an anti-red blood cell antibody. The median age at diagnosis is about 40 years; median survival after diagnosis is approximately 20 years. It is a rare disorder with an estimated prevalence in the population of 1 in 100 000 to 1 in 1 000 000. Episodes of intravascular haemolysis result in haemoglobinuria, most noticeable in early morning urine which has a characteristic red-brown colour. The disease is associated with an increased risk of venous thrombosis in unusual sites such as the liver or abdomen. Management is supportive with transfusion and treatment of thrombosis. Recently the anti-complement C5 monoclonal antibody eculizumab was shown to be effective in reducing haemolysis.

Infection

Plasmodium falciparum malaria may be associated with intravascular haemolysis; when severe, this is termed blackwater fever due to the associated haemoglobinuria.

Physical trauma

Physical disruption of red cells may occur in a number of conditions and is characterised by the presence of red cell fragments on the blood film and markers of intravascular hemolysis:

- *Mechanical heart valves*. High flow through incompetent valves or periprosthetic leaks through the suture ring holding a valve in place result in shear stress damage.
- *March hemoglobinuria*. Vigorous exercise, such as prolonged marching or marathon running, can cause red cell damage in the capillaries in the feet.
- *Thermal injury*. Severe burns cause thermal damage to red cells characterised by fragmentation and the presence of microspherocytes in the blood.
- *Microangiopathic haemolytic anemia*. Fibrin deposition in capillaries can cause severe red cell disruption. It may occur in a wide variety of conditions: disseminated carcinomatosis, malignant or pregnancy-induced hypertension, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and disseminated intravascular coagulation.

Exogenous substances

Dapsone and sulfasalazine cause hemolysis by oxidative denaturation of hemoglobin. Arsenic gas, copper, chlorates, nitrites and nitrobenzene derivatives may all cause hemolysis.

ANEMIA DUE TO ACUTE BLOOD LOSS

Acute blood loss has a direct impact on the integrity of the blood volume and oxygen supply to tissues. Sudden, severe hemorrhage can induce hypovolemic shock, cardiovascular failure, and death.

Etiology and pathogenesis

Blood loss causes anemia by two main mechanisms: (1) by rapid loss of red cells (acute posthemorrhagic anemia); and (2) if the loss of blood is protractible, it gradually depletes iron stores, eventually resulting in iron deficiency anemia.

Blood loss can be external (e.g., after trauma) or internal (e.g., from bleeding in the gastro tract, rupture of an ectopic pregnancy, subarachnoid hemorrhage). In any of these cases, after the sudden loss of a large amount of blood, there are three clinical/pathophysiologic stages.

1. At first, the dominant feature is hypovolemia, which poses a threat particularly to organs that normally have a high blood supply, like the brain and the kidneys; therefore, loss of consciousness and acute renal failure are major threats. It is important to note that at this stage an ordinary blood count will not show anemia, because the hemoglobin concentration is not affected.
2. Next, as an emergency response, baroreceptors and stretch receptors will cause release of vasopressin and other peptides, and the body will shift

fluid from the extravascular to the intravascular compartment, producing hemodilution; thus, the hypovolemia gradually converts to anemia. The degree of anemia will reflect the amount of blood lost. If after 3 days the hemoglobin is, for example, 7 g/dL, it means that about half of the entire blood has been lost.

3. Provided bleeding does not continue, the bone marrow response will gradually ameliorate the anemia.

Clinical manifestations

Clinical presentation of a blood loss anemia will vary according to the site and severity of blood loss. Massive hemorrhage from the gastrointestinal tract or an external trauma site will be immediately obvious. The rate and magnitude of the blood loss may also be apparent. Slower bleeding internally can be harder to diagnose. Important features in the clinical presentation include the cause, location, magnitude, and duration of the blood loss.

Sudden loss of a large volume of blood has an immediate impact on the patient's cardiovascular status. There are changes in clinical picture depending on degree of blood loss volume (calculated for a 70-kg adult with a blood volume of 5 000 mL):

< 1000 ml – anxiety.

1 000–1 500 ml – anxiety, exercise intolerance, may faint on standing. On examination: orthostatic hypotension, exertional tachycardia.

1 500–2 000 ml – anxiety, syncope when sits or stands. On examination: orthostatic hypotension, tachycardia at rest.

>2000 ml – patient is anxious, restless, often confused, and may be short of breath. On examination: hypovolemic shock, fall in supine blood pressure, tachycardic with cool, clammy skin.

Diagnosis

Laboratory findings will also vary according to the size and rate of blood loss. The behavior of the complete blood count (CBC) is a good example. Although it would seem to make sense that the hemoglobin level (hematocrit) would provide a good measure of red blood cell loss, it is not necessarily the case. Hemoglobin and hematocrit measurements are volume ratios where the quantity of red blood cells depends on the volume of diluent plasma. Immediately after an acute, severe hemorrhage, there will be little change in the hemoglobin (hematocrit) until the plasma volume has time to expand. Without fluid administration, this process takes more than 24 hours. If a volume expander is given, the change in hemoglobin level correlates as much with the volume transfused as with the volume of blood lost.

Severe hemorrhage may be accompanied by other changes in the CBC. The granulocyte count can increase dramatically to levels of $20 \times 10^9/l$ or higher, and immature white cell forms can appear in circulation. This situation reflects a demargination of granulocytes secondary to the patient's catecholamine

response and a release of granulocytes from the marrow storage pool. With hypovolemic shock and hypoxia, very immature cells, including metamyelocytes, myelocytes, and nucleated red blood cells, can also appear in circulation. A rise in the platelet count to levels exceeding $1\,000 \times 10^9/l$ can also occur in the days after a severe hemorrhage.

The diagnosis of acute posthemorrhagic anemia (APHA) is usually straightforward, although sometimes internal bleeding episodes (e.g., after a traumatic injury), even when large, may not be immediately obvious. Whenever an abrupt fall in hemoglobin has taken place, whatever history is given by the patient, APHA should be suspected.

Supplementary history may have to be obtained by asking the appropriate questions, and appropriate investigations (e.g., a sonogram or an endoscopy) may have to be carried out.

Treatment

First steps in stabilizing a severely hemorrhaging patient are the essential components of first aid. Most important, any obvious bleeding sites should be controlled. The next important step is to establish reliable intravenous access and begin an aggressive program of fluid and volume expander administration. Choice of volume expanders will depend on the type and severity of the bleeding, symptoms and signs of hypovolemia, and patient age. Electrolyte solutions (Ringer's lactate and normal saline) and purified colloid preparations (5% albumin solution, purified plasma protein fraction) are immediately available, whereas the administration of red blood cells, platelets, and fresh frozen plasma will involve some delay.

Iron plays a key role in the erythroid marrow response to a blood loss anemia. A normal adult male with 1 000 mg of reticuloendothelial stores can acutely increase red blood cell production to two to three times normal. This production level cannot be sustained, however, if the blood loss exceeds the amount of stored iron. At this point, the patient must receive iron therapy.

Prognosis

Prognosis varies significantly depending on severity of hemorrhage and effectiveness of urgent treatment.

APLASTIC ANEMIA AND MYELOYDYSPLASIA

Definition

Aplastic anemia is a life-threatening syndrome characterized by failure of the bone marrow to produce peripheral blood cells and their progenitors. Diverse diseases and environmental factors can cause this syndrome, but its hallmark is bone marrow hypocellularity and hypoplasia of the erythroid, myeloid, and megakaryocyte lines.

Etiology and pathogenesis

Marrow aplasia in a few patients (10 %) can be attributed to an inherited bone marrow failure syndrome, but most cases are acquired and caused by factors other than an inherited gene mutation. In all cases, it is clear that the causative factors, genetic or environmental, injure pluripotent hematopoietic stem cells. This is in contrast to the case of the lineage-restricted disorders, wherein the causative agents and factors suppress the growth and development of unipotent progenitor cells committed to that particular lineage. Radiation, viral diseases (Epstein-Barr virus, hepatitis of unclear type “non-A, -B, -C, -E, or -G”), cytotoxic drugs, and chemicals (benzene) are known causes of aplastic anemia, but the most common form of acquired aplastic anemia is immunologically mediated, and evidence is emerging that some marrow failure states attributed to viral infection or to idiosyncratic drug reactions may also result from immune suppression of hematopoiesis.

Clinical manifestations

Aplastic anemia can appear abruptly or insidiously. Bleeding is the most common early symptom; a complaint of days to weeks of easy bruising, oozing from the gums, nose bleeds, heavy menstrual flow, and sometimes petechiae will have been noticed. With thrombocytopenia, massive hemorrhage is unusual, but small amounts of bleeding in the central nervous system can result in catastrophic intracranial or retinal hemorrhage. Symptoms of anemia are also frequent, including lassitude, weakness, shortness of breath, and a pounding sensation in the ears. Infection is an unusual first symptom in aplastic anemia (unlike in agranulocytosis where pharyngitis, anorectal infection, or frank sepsis occurs early). A striking feature of aplastic anemia is the restriction of symptoms to the hematologic system, and patients often feel and look remarkably well despite drastically reduced blood counts. Systemic complaints and weight loss should point to other etiologies of pancytopenia.

Petechiae and ecchymoses are typical, and retinal hemorrhages may be present. Pallor of the skin and mucous membranes is common except in the most acute cases or those already transfused. Infection on presentation is unusual but may occur if the patient has been symptomatic for a few weeks. Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia.

Diagnosis

The evaluation of pancytopenic patients first requires examination of the peripheral blood smear. If there are morphologic or clinical signs of vitamin B₁₂ or folic acid deficiency (e.g., hypersegmented neutrophils and oval macrocytes), those disorders should be ruled out because a bone marrow aspiration and biopsy would not be required in those conditions.

The biopsy best assesses overall bone marrow cellularity and provides the most sensitive evidence for some infiltrative processes. The aspirated sample can be examined microscopically for the presence of abnormal cells but also

provides cells for cytogenetic analyses (which can provide evidence supporting hypoplastic myelodysplasia and acute leukemia).

Differential Diagnosis

Most classic aplastic anemia patients have immunologically mediated disease. Notwithstanding strong evidence in support of this mechanism from some research laboratories over the past 30 years, there exists no validated screening tool that can either rule in or rule out immune-mediated disease, a fact that is complicated by the evidence that some cases of drug- and virus-induced disease are also immunologically mediated. Therefore, idiopathic autoimmune aplastic anemia remains a diagnosis of exclusion.

Severe aplastic anemia is readily detected and easily distinguished from most other forms of hypoproliferative anemia (iron-deficiency, anemia of chronic disease, thalassemia, sideroblastic anemia). The one exception is myelodysplasia with a severely hypoplastic marrow. With this exception, the involvement of all cell lines in the marrow both confirms the diagnosis and often explains the reason for the marrow failure.

Treatment

All patients will require blood product support and aggressive management of infection. The curative treatment for patients under 30 years of age with severe idiopathic aplastic anaemia is allogeneic bone marrow transplantation if there is an available donor. In older patients, immunosuppressive therapy with ciclosporin and antithymocyte globulin gives 5-year survival rates of 75 %. Such patients may relapse or other clonal disorders of hematopoiesis may evolve, such as paroxysmal nocturnal haemoglobinuria, myelodysplastic syndrome (this syndrome consists of a group of clonal hematopoietic disorders which represent steps in the progression to the development of leukaemia) and even acute myeloid leukaemia.

Prognosis

The prognosis of severe aplastic anemia managed with supportive therapy only is poor and more than 50 % of patients die, usually in the first year. Those with a compatible sibling donor should proceed to transplantation as soon as possible; they have a 75–90 % chance of long-term cure.

Further reading:

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CLINICAL HEMATOLOGY

Part I (Anemias)

*Methodological recommendations
for students and doctors*