California Association for Medical Laboratory Technology

Distance Learning Program

Erythrocyte Volume Disorders

Course # DL-015

by
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Approved for 2.0 CE
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Level of Difficulty: Intermediate

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COURSE NAME: ERYTHROCYTE VOLUME DISORDERS
COURSE # DL-015

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3. The difficulty of this Distance Learning course was consistent with the number of CE hours.
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Erythrocyte Volume Disorders

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Abstract

Erythrocytes or red blood cells (RBC) transport oxygen and carbon dioxide between the lungs and the tissue. Reduction in red blood cells or the hemoglobin content results in anemia, while increased production of red blood cells causes polycythemia. Anemia may be the results of low production of cells in the bone marrow or increased destruction of RBC in the bone marrow and/or circulation; polycythemia is a general term describing an increase in the rate of RBC production in the bone marrow and having too many red blood cells in the circulation, while the plasma volume remains constant.

This course provides a review on causes for reduction in RBC production in the bone marrow and increased RBC mass in the circulation. This course will not evaluate the causative agents of anemia which may influence RBC hemoglobin concentration. The content includes two case studies to discuss the acquired and congenital causes for low and high RBC production in the bone marrow. The following is the list of conditions that will be covered in this course

Low RBC Production
1. Hemorrhagic Anemia
2. Pernicious Anemia
3. Aplastic Anemia

High RBC Production
1. Polycythemia Vera
2. Compensatory Polycythemia
3. Relative Polycythemia
4. Erythrocytosis

Objectives:

1. Summarize the types of erythrocyte disorders due to decreased or increased RBC production.
2. Identify the symptoms associated with decreased or increased RBC production.
3. Describe the mechanisms that may lead to aplastic anemia.
4. Describe the mechanisms of pernicious anemia.
5. Describe the use of methylmalonic acid and homocysteine testing in the differential diagnosis of B12 and folate deficiency.
6. Describe the genetic and acquired causes for polycythemia.
7. Describe the general structure and function of telomeres and telomerase complex.
Case Study #1

A 38-year-old man was referred to a physician for cough and dyspnea. The patient was a police officer with a history of smoking. The initial examinations indicated the pulse was regular at 70/min, blood pressure 145/95 mm Hg, and temperature 36.9°C. Chest examination revealed poor bilateral expansion. Cardiovascular and neurological examinations were normal. The skin examination revealed a spotty skin hypopigmentation and flat white spots on back and upper torso which the patient indicated he has had since the age of 10. In addition, there was marked leukoplakia of the tongue, the fingernails demonstrated a mild clubbing, and the small toenails were dystrophic. The patient indicated his brother, his maternal grandfather and his maternal great uncle have similar skin and nail appearances. No female in their family has reported similar skin or nail issues.

Blood morphological analysis indicated pancytopenia and macrocytic RBCs with 1+ dacryocytes and occasional Howell-Jolly Body. The peripheral blood count was as follows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC:</td>
<td>1.2 x 10⁹/L (4.5-10.5)</td>
</tr>
<tr>
<td>RBC:</td>
<td>2.42 x 10¹²/L (4.30-5.72)</td>
</tr>
<tr>
<td>HGB:</td>
<td>7.1 g/dL (13.5-16.5)</td>
</tr>
<tr>
<td>HCT:</td>
<td>24% (39-48)</td>
</tr>
<tr>
<td>PLT:</td>
<td>8.0 x 10⁹/L (150-450)</td>
</tr>
<tr>
<td>MCV:</td>
<td>99 fl (80-96)</td>
</tr>
<tr>
<td>MCH:</td>
<td>29 pg (28-33)</td>
</tr>
<tr>
<td>MCHC:</td>
<td>29 g/dl (32-36)</td>
</tr>
<tr>
<td>Retics</td>
<td>0.7% (1.5-3)</td>
</tr>
</tbody>
</table>

The lymphocyte count was 1.1*10³/µl (normal 1.2–3.4*10³/µl) with CD4 20% (normal 28.5–60.5%) and CD8 64% (normal 11.1–38.4%); the CD4/CD8 ratio was 0.31 (normal >1). Routine biochemical and liver function tests were normal. The cholesterol level was 245 mg/dL (normal <200 mg/dL) and HDL and triglyceride levels were normal. Serum protein electrophoresis was normal without monoclonal bands. Serological tests for hepatitis B and C, HIV, rheumatoid factor, anti-nuclear and anti-DNA antibodies were negative. IgM was low (0.38 g/L; normal 0.60–2.50 g/L); IgA and IgG were normal. Urinalysis was normal. Genetic analysis indicated a mutation in \( DKC1 \) gene with 5C to T missense mutation in exon 1 of the \( DKC1 \) gene. This mutation involves the fifth nucleotide of exon 1, which corresponds to nucleotide 97 from the start of the \( DKC1 \) gene.

Case Study #2

A female visited her physician with complaint of malaise, lethargy, and lack of appetite. The patient indicated she has not been feeling well lately and has a constant feeling of bloating in her stomach after even a small meal. She also indicated that she has been developing a rash every time she takes a hot shower particularly on her face. In addition, she has been having a lower back pain for a while. Initial examination indicated a palpable spleen and abdominal/ hepatic tenderness. The imaging studies indicated enlarged liver and spleen. The initial complete blood count (CBC) results are as follows.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC:</td>
<td>11 x 10⁹/L (4.5-10.5)</td>
</tr>
<tr>
<td>RBC:</td>
<td>5.84 x 10¹²/L (4.30-5.72)</td>
</tr>
</tbody>
</table>
HGB:  16.9 g/dL  (13.5-16.5)
HCT:  51%   (39-48)
PLT:  450 x 10^9/L  (150-450)
MCV:  80 fl   (80-96)
Morphology: Normochromic Normocytic; Dacryocytes 1+

The physician ordered further studies:
Cytogenetics studies indicated a normal karyotype.
Genomic DNA analysis from patient's leukocytes identified JAK2 V617F mutation.
Genetic analysis indicated a somatic mutation pattern.

Discussion
RBC Production
The number of erythrocytes in a person's blood can vary significantly from the normal range, leading to various clinical conditions, generally classified as anemia when the production is low and polycythemia when the production is high.

Anemia
Anemia due to inadequate production of RBC is characterized by the low number of RBC per cubic millimeter in blood with symptoms of fatigue, lethargy, dyspnea, pallor of the skin and cardiac irregularities. The major causes of anemia due to inadequate RBC production include:

1. **Hemorrhagic anemia** results from acute or chronic blood loss due to such factors as gastrointestinal bleeding or heavy menstrual flow. The symptoms may include pallor, sensitivity to cold, fatigue, malaise, dizziness, tachycardia and dyspnea. Since anemia is often a symptom of another illness, it is important to identify the exact causes of hemorrhagic anemia to determine the best regimen of therapy. For instance, chronic blood loss due to gastroduodenal ulcer, gradually diminishes the body of its iron stores, and if the intake of iron is insufficient, iron deficiency anemia would result. With supportive treatment, a healthy bone marrow can restore the normal erythrocyte count to a normal range quickly. The three most common causes of chronic blood loss are excessive menstrual bleeding, gastroduodenal ulcer, and colon cancer. Depending the stage of hemorrhagic anemia, the RBC morphology may vary from normochromic normocytic (early stage) to hypochromic microcytic (chronic stage). The CBC results will demonstrate lower RBC count, hemoglobin concentration and hematocrit percentage. In the presence of a healthy bone marrow, in chronic cases, there will be an increase in reticulocytes percentage.

2. **Pernicious anemia (PA)** is a decrease in RBC production due to inadequate absorption of cobalamin (vitamin B12) in the ileum of small intestine. B12 is a cobalt containing coenzyme with essential roles in two biochemical reactions in the human body. In one reaction, B12 acts as a cofactor for methylmalonyl CoA mutase in converting methylmalonyl CoA to succinyl CoA in the mitochondria supplying the Krebs cycle. Inadequate B12 results in increased methylmalonyl CoA in the serum and can be used for differential diagnosis of B12 and folate deficiency (Figure 1A) (2). In the second biochemical reaction, B12 acts as a coenzyme for methionine synthase by transferring a methyl group from 5-methyltetrahydrofolate (5-methyl-THF) to homocysteine, generating methionine in the process (Figure 1B). If either folate or B12 are deficient, homocysteine levels increase because methionine synthase is unable to convert it to methionine. Following demethylation by methionine synthase, tetrahydrofolate continues in a series of
biochemical reactions that are required for the DNA synthesis. Thus, B12 or folate deficiency inhibit thymidine production, in turn reducing DNA transcription and cell proliferation\(^{(2)(4)}\).

![Diagram](image)

Figure 1. (A) Algorithm for the interpretation of serum vitamin B12, methylmalonic acid (MMA) and homocysteine (HCY) in the diagnosis of vitamin B12 and folate deficiency. (B) Vitamin B12 biochemical reaction with methionine synthase results in demethylation of 5-methyl-Tetrahydrofolate (5-methyl-THF), in turn converting the homocysteine to methionine (MET) and releasing THF to enter other biochemical reactions for deoxythymidine triphosphate synthesis.
One common cause of PA is due to the defective production of intrinsic factor (IF), which is produced by the lining of stomach to protect and facilitate B12 absorption in the ileum. IF is a glycoprotein produced by parietal cells in the gastric mucosa that binds directly to B12 after B12 is released from associated proteins by the action of gastric acid and pepsin in the stomach. The B12-IF complex travels to the ileum, and IF component of the molecule targets the receptor on ileal mucosa and facilitate B12 endocytosis. Unless the causes of PA are dietary or due to surgical complication, most PA patients have autoantibodies against gastric parietal cells or IF. In such instance, the serum B12 concentration is very low (<150 ng/L). Destructive autoimmune process diminishes IF function; as a result, the accumulation of 5-methyl-THF in the absence of B12 results in increased levels of methylated THF. This event is also known as folate trap (Figure 1B). The inhibition of homocysteine methylation to generate methionine also results in increased homocysteine in the blood. Increased homocysteine is associated with increased risk of cardiovascular disease.

The PA symptoms are similar to hemorrhagic anemia; however, it may also involve the nervous system characterized by symptoms such as tingling and numbness at extremities, loss of balance, muscle weakness, and mental confusion. For clinical diagnosis, if vitamin B12 is <150 ng/L, then intrinsic factor blocking antibody (IFBA) is performed. If IFBA is negative or indeterminate, then gastrin is performed. If vitamin B12 is 150 to 400 ng/L, then methylmalonic acid (MMA) is performed. If MMA is >0.40 nmol/mL, then IFBA is performed. Due to interruption of DNA synthesis, RBC precursors divide fewer times, giving the RBC macrocytic morphology. Depending on the extent of extramedullary hematopoietic activity, Howell-Jolly bodies, ovalocytes, dacryocytes and hypersegmented neutrophils are common morphological characteristics of B12 and folate deficiency.

3. **Aplastic Anemia (AP)** is characterized by a decrease in hematopoietic stems cells, pancytopenia, reticulocytopenia and bone marrow hypocellularity. AP can be acquired or congenital. In most acquired cases, the hematopoietic stem cells are the target of an immune process triggered by viral infection or toxins, which is mediated by cytotoxic T-cells, and cause hematopoietic stem cell death by apoptosis. Acquired aplastic anemia can be treated by immunosuppressive therapy and bone marrow transplantation.

A rare and congenital form of aplastic anemia is dyskeratosis congenita (DKC). DKC may be inherited in an X-linked or autosomal dominant pattern (OMIM number 305000; OMIM number 127550). The X-linked pattern is caused by mutations in the **DKC1** gene, which encodes dyskerin, a nucleolar protein that associates with the telomerase complex. The common symptoms of DKC include dystrophic fingernails and toenails; skin pigmentation, especially on the neck and chest; and oral leukoplakia. The blood analysis is characterized by pancytopenia and may include macrocytic RBC’s, similar to the results of the patient in Case Study #1. Howell Jolly bodies and dacryocytes may also be seen on blood smear. Involvement of **DKC1** has implicated the telomere-repair complex in the pathophysiology of DKC, and interestingly, cells from patients with DKC have short telomeres and low telomerase activity. Subsequently, mutations in the **TERC** gene on chromosome 3, which encodes the RNA component of the telomerase complex (TERT), were identified in the autosomal dominant form of DKC (5).

Telomeres are structural elements that cap the ends of chromosomes, protecting them from fusion and erosion. In human somatic cells, telomeres typically consist of thousands of tandem repeats of nucleotides TTAGGG. These repeats are gradually lost with cellular replication and
aging, and when the erosion of telomeric repeats reaches a critical limit, the cell undergoes arrested proliferation and senescence, shortened life span, apoptosis, and genomic instability. Maintenance of the integrity of telomeres requires the telomerase ribonucleoprotein complex, which consists of TERT and TERC, in addition to a multiplex of proteins (3). TERT copies a short region of TERC into telomeric DNA to extend the 3’ end of the chromosome and maintain chromosomal structural integrity (Figure 2).

The identification of genetic mutations in AP is by Polymerase-chain-reaction (PCR) amplification of genes encoding the telomerase complex — namely DKC1 and TERT — with DNA samples extracted from peripheral blood or bone marrow cells. PCR products are sequenced using automated genetic sequence analyzer to identify the mutations.

**High RBC Production**

**Polycythemia** is an increase in the circulating red blood cell mass. There are two distinct forms: Primary and Secondary polycythemia.

1. **Primary polycythemia**, also known as **Polycythemia Vera (PV)**, is a myeloproliferative disorder characterized by hyperplasia of erythropoietic tissues in the bone marrow, resulting an increase in RBC count irrespective of the presence or absence of erythropoietin. PV is usually a diagnosis of elimination, ruling out other causes of secondary polycythemia. The affected person has the same total blood volume, but the number of erythrocytes is increased, rendering the patient’s blood more viscous and increasing susceptibility to cardiovascular disease. In humans, this has been shown to be largely due to a mutation in Janus-activated kinase 2 (JAK2). JAK2 is a tyrosine kinase that is downstream to the erythropoietin receptor. It directs the signaling cascade, triggered by erythropoietin binding to its receptor on RBC precursors in the bone marrow, that, in turn, controls RBC production (Figure 3) (1). Erythropoietin results in dimerization of tyrosine kinase monomers, which in turn activate members of the signal transducer and activator of transcription (STAT) protein family by JAK2 phosphorylation. STAT act as intracellular transcription factors and mediate hematopoietic differentiation and proliferation activities by influencing anti-apoptotic and proliferative gene expression, ultimately resulting in cell accumulation (2). Normally, JAK2 is only activated by phosphorylation of tyrosine residues when erythropoietin binds to its receptor. However, more than 90% of PV cases are specific for valine to phenylalanine (V617F) substitution at amino acid 617 due to a mutation of guanine to thymine at exon 14 of the gene (2). V617F mutation drives erythropoiesis, either independently of erythropoietin or because RBC precursors become hypersensitive to normal concentrations of erythropoietin.
Clinical presentation of PV may include splenomegaly, bone marrow hyperplasia and pancytopenia. There may also be an increase in WBC and platelet counts. Excess WBC’s may manifest as a rash when exposed to warm temperature due to subcutaneous histamine release as with the patient in Case Study #2. Excess platelets render the patient susceptible to deep vein thrombosis, stroke and cardiovascular disease. The treatment for PV is primarily therapeutic phlebotomy with the goal of maintaining hematocrit below 45%. The chances of thrombotic events increase with patients who are only treated by phlebotomy; thus, low dose aspirin regimen

**Figure 3.** Erythropoietin (E) signaling pathway resulting in STAT activation is shown. After Erythropoietin targets its receptor, causing dimerization of monomers, JAK2 phosphorylation (P) occurs. JAK2 phosphorylation is followed by STAT activation and forming a dimer in the presence of homocysteine (H). STAT homodimer acts as a transcription factor, crosses the nuclear membrane and regulates hematopoietic cell survival, proliferation, and differentiation.
and myelosuppressive therapy may be required to control such complications and reduce the
chances of thrombosis.

2. **Secondary polycythemia** is a physiological condition resulting from a decreased oxygen
supply to the tissues caused by living at high altitudes, heart disease, circulatory insufficiency,
severe pulmonary disease, or the production of erythropoietin or erythropoietin-like compounds,
as in polycystic kidney disease or renal neoplasms. The following are some of the major causes
of secondary polycythemia.

**Compensatory polycythemia (CP)** results from chronic hypoxia often seen in smokers,
congenital heart disease, pulmonary emphysema, or prolonged residence at a high altitude. The
low blood oxygen content results in increased erythropoietin production, in turn increasing RBC
production to increase blood oxygen carrying capacities. Individuals with CP are asymptomatic
but will have increased in RBC count, hemoglobin concentration and hematocrit percentage.

**Relative polycythemia (RP)** is an increase in the number of erythrocytes in the blood per unit
volume because of a decrease in blood plasma volume. RP is often a result of severe dehydration
as seen in a serious case of diarrhea. Progressive loss of water alters the ratio of blood cells to
plasma and makes blood more concentrated. This type of polycythemia is a temporary condition,
and the ratio of erythrocytes to water in the blood returns to normal when the patient becomes
rehydrated. During the course of RP, there may be an increase in hematocrit, but RBC indices
remain normal.

**Erythrocytosis** is an increase in erythrocytes due to an increase in the level of erythropoietin.
This is due to conditions often seen in hepatocellular carcinoma, renal cancers, renal diseases and
renal cysts, which may stimulate erythropoietin production without the presence of hypoxia.
Epinephrine-producing neoplasms, such as pheochromocytoma, can also result in erythrocytosis.
In erythrocytosis, the patients have symptoms similar to PV, and the course of treatment will
depend on the underlying source causing the increase in erythropoietin.
1. Identify the mode of transmission for mutant *DKC1* gene in Case Study #1.
   a. Autosomal dominant
   b. Autosomal recessive
   c. X-linked
   d. Y-linked

2. What is the diagnosis for the patient in Case Study #1?
   a. Idiopathic aplastic anemia
   b. Chronic hemorrhage
   c. Dyskeratosis congenital
   d. Pernicious anemia

3. The patient in Case Study #1 has a teenage daughter. What is the probability she would develop the disease later in her life?
   a. 0%
   b. 25%
   c. 50%
   d. 100%

4. The symptoms of dyskeratosis congenital include all the following except:
   a. Leukoplakia
   b. Nail dystrophy
   c. Skin spots
   d. B12 deficiency

5. Peripheral blood morphology seen in dyskeratosis congenital includes all the following except:
   a. Howell Jolly bodies
   b. Heinz bodies
   c. Dacryocytes
   d. Macrocytosis

6. Telomerase reverse transcriptase function is:
   a. To act as an RNA template for gene transcription
   b. To increase the length of *DKC1* gene
   c. To repair the DNA at the end of chromosomes
   d. To repair single base mutations

7. Increase in serum methylmalonyl CoA in the presence of normal homocysteine indicates:
   a. Folate deficiency
   b. B12 deficiency
   c. B12 and folate deficiency
   d. Excludes B12 and folate deficiency
8. Folate trap due to B12 deficiency occurs during:
   a. 5-methyl THF crossing plasma membrane into cytoplasm
   b. Thymidine synthesis
   c. Homocysteine conversion into methionine and making THF
   d. Crossing the duodenal mucosa and generating 5-methyl THF

9. Telomeres:
   a. gradually increase in length during aging
   b. are tandem repeats of nucleotides TTAGGG at the end of chromosomes
   c. are tandem repeats of nucleotides TTAGGG in the middle of chromosomes
   d. contain many genes

10. Which of the following may result in low B12 absorption in the ileum?
    a. Low acidity in the stomach
    b. Low levels of intrinsic factor in stomach
    c. Autoimmune disease
    d. All of the above

11. What is the likely diagnosis in Case Study #2?
    a. Aplastic Anemia
    b. Pernicious Anemia
    c. Polycythemia Vera
    d. Chronic Myelogenous Leukemia

12. In Case Study #2, the physician orders a series of blood and bone marrow biopsy. The bone marrow biopsy results indicate:
    a. hypocellular marrow
    b. hypercellular marrow
    c. acellular marrow
    d. normal marrow

13. The patient’s coworker tells the patient that the prognosis is good and there is an effective treatment for her condition. The treatment is:
    a. Chemotherapy
    b. Phlebotomy
    c. Radiation
    d. Imatinib

14. The likely cause of patient’s condition in Case Study #2 is:
    a. Janus-activated kinase 2 mutation
    b. Translocation (9;22)
    c. Translocation (8;14)
    d. Translocation (12;21)
15. Which of the following is NOT a cause for secondary polycythemia?
   a. living at high altitudes,
   b. cardiovascular insufficiency
   c. pulmonary disease
   d. JAK2 mutation

16. Tobacco smokers are at higher chance of developing ________________.
   a. Compensatory Polycythemia
   b. Relative Polycythemia
   c. Erythrocytosis due to increased erythropoietin
   d. Polycythemia Vera

17. The cause of skin rash for patient in case study #2 is due to:
   a. Exposure to heat
   b. Histamine release
   c. Allergic reaction
   d. Sunburn

18. Increase in erythrocytes due to an increase in the level of erythropoietin is often seen in:
   a. Primary polycythemia
   b. Polycythemia Vera
   c. Compensatory polycythemia
   d. Erythrocytosis

19. Decrease in plasma volume may lead to ________________.
   a. Primary polycythemia
   b. Polycythemia Vera
   c. Compensatory polycythemia
   d. Relative polycythemia

20. The function of STAT is best described as:
   a. tyrosine kinase receptor
   b. JAK2 Inhibitor
   c. Transcription factor
   d. DNA repair complex
References