

**California Association  
for  
Medical Laboratory Technology**

**Distance Learning Program**

**ERYTHROPOIETIN AND BLOOD PRODUCTION**

by

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Course Number: DL-969

1.0 CE/Contact Hour

Level of Difficulty: Basic

CAMLT is approved by the California Department of Health Services as a  
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CAMLT urges you to earn your CE units early!

## OBJECTIVES:

At the end of the course the participant will be able to answer the following questions:

- What results of the anemia evaluation in a chronic kidney disease (CKD) patient indicate use of erythropoietin?
- What is erythropoietin and how does it act?
- What are the maturation stages of erythropoiesis in the bone marrow?
- Erythropoietin is recommended for use in what conditions?

**CASE STUDY:** A 48 year old male with chronic kidney disease is on dialysis. The physician has been monitoring monthly hemoglobin and hematocrit. The latest results showed hemoglobin of 10.6 g/dl and a hematocrit of 32%. In order to determine if the patient is a candidate for erythropoietin (brand names – Epogen and Procrit) therapy, the physician then orders an anemia evaluation. The following anemia evaluation is recommended by the Anemia Work Group of the National Kidney Foundation (1).

- Hemoglobin and hematocrit
- Red blood cell count and RBC indices
- Reticulocyte count
- Iron parameters:
  - Serum iron
  - Total iron binding capacity (TIBC)
  - Percent transferrin saturation (TSAT) (serum iron x 100 divided by TIBC)
  - A test for occult blood in stool (stool guaiac)

## INTRODUCTION:

Erythropoietin (abbreviated as EPO or Epoietin), produced primarily by the kidneys, is the principal factor responsible for the regulation of red blood cell production. In 1989 recombinant human EPO was approved for treatment of humans by the U.S. Federal Drug Administration.

One of the principal uses of EPO is control of anemia in chronic kidney disease patients. In order to understand the function of EPO in controlling anemia it is necessary to understand red blood cell production (erythropoiesis) in the bone marrow.

This distance learning course will cover erythropoiesis and the function and clinical uses of EPO.

## HEMATOPOIESIS:

The process by which blood is formed is called hematopoiesis. Formed elements in the peripheral blood of an adult are the result of several generations of cells that begin development in the bone marrow and are released into the blood when they are mature or are needed. However, blood is not always formed only in the bone marrow but can be produced in different areas of the body, especially during different phases of growth and development. In the child blood is formed in all bones of the body including the shafts of the long bones such as the humerus and femur. In the adult blood is formed in the bone marrow in the skull, sternum, clavicles, ribs, vertebrae, and pelvis but only in the proximal ends of the humerus and femur. The blood forming marrow is red whereas the marrow in other areas of the bone has been replaced by fat cells and is termed yellow marrow. Blood may also be formed in other regions in the adult (such as the spleen) in response to blood loss and during disease processes.

Developing hematopoietic cells of red bone marrow, fat cells of yellow bone marrow, reticuloendothelial cells, nutrient arteries, veins, and nerves lie between shelves of cancellous bone tissue within a bone. Cords of hematopoietic cells surround venous sinuses that empty into a central vein in the center of a region. Endothelial cells lining these sinuses have very small apertures, or openings, between adjacent cells. When hematopoietic cells are ready to enter the blood, they alter their shape and pass through these small openings to enter the venous sinuses and eventually the central vein which drains toward the peripheral circulation.

The origin of all types of formed elements in the blood is the pluripotential (totipotential) stem cell (PSC) in the red bone marrow. This cell is called pluripotential or totipotential because it has the capability of becoming any one of the hematopoietic cell lines. It is related through embryogenesis to the other types of connective tissue precursors. The pluripotential hematopoietic stem cell can divide and produce more pluripotential stem cells or it can differentiate. Cellular differentiation in the bone marrow is an apparently irreversible process in which a cell becomes a specific type that normally does not go backwards in development.

During hematopoiesis, a pluripotential stem cell differentiates to become a lymphoid stem cell or a myeloid stem cell. The term colony-forming unit refers to a progenitor (stem) cell that is destined to become one or more cell lines. A CFU-L (colony-forming unit – lymphoid) is a committed lymphoid stem cell that differentiates into either thymus cell precursors that eventually become T lymphocytes or bone marrow precursors that eventually become B lymphocytes. A CFU-GEMM (colony-forming unit – granulocyte, erythroid, monocyte, megakaryocyte) is a myeloid stem cell that is also committed but remains more versatile in that it can give rise to granulocytes, erythrocytes, monocytes and megakaryocytes. Each of these cell lines further develops from a cell line specific CFU that has differentiated from the CFU-GEMM. These specific CFUs differentiate into blast forms that can be identified in the bone marrow using a microscope, hematologic stains and special markers. As cells develop in each cell line from the blast form to the mature form, changes occur most remarkably in their physical characteristics, biochemical components, and surface membrane proteins (markers). These features aid the clinician in evaluating morphology and numbers of specific cell types within the marrow.

## ERYTHROPOIESIS:

The CFU-E is the committed cell that gives rise to the erythrocytic line of development. When describing the stages of red blood cell development, three different nomenclatures have been used. They include the erythroblast, normoblast, and rubriblast nomenclatures. The erythroblast and rubriblast nomenclatures are used in this discussion. The earliest recognizable form in the red cell developmental series that is visible with a microscope is the proerythroblast (rubriblast). The subsequent stages of development are the basophilic erythroblast (prorubricyte), polychromatophilic erythroblast (rubricyte), orthochromatic erythroblast (metarubricyte), polychromatophilic erythrocyte (diffusely basophilic erythrocyte), and erythrocyte. Once a cell is committed to red blood cell development, it takes about a week to develop from the proerythroblast stage to a mature erythrocyte. Fourteen to sixteen erythrocytes can result from each proerythroblast (rubriblast) after cellular division, differentiation and maturation.

During the proerythroblast, basophilic erythroblast, polychromatophilic erythroblast and orthochromatic erythroblast stages of development, each red cell precursor contains a nucleus. However during the orthochromatic erythroblast (metarubricyte) stage of development, the nucleus degenerates. It becomes pyknotic and is extruded from the cell. This process of nuclear extrusion results in a polychromatophilic erythrocyte (diffusely basophilic erythrocyte) also known as a reticulocyte. After a few days, the reticulocyte is ready to leave the marrow. The

reticulocyte circulates for an additional 24-48 hours before it loses its RNA and its ability to synthesize hemoglobin. At this time the mature erythrocyte is fully developed and will circulate for approximately 120 days.

As the erythrocyte ages, changes occur that eventually signal the end of its usefulness and trigger its subsequent removal from the blood in the spleen. Some of these changes include loss of lipid in the membrane, decreased surface area of the cell, decreased production of energy through deficient ATP production and changes in the ion transport mechanisms in the cell membrane. When the cell is no longer capable of functioning properly, a macrophage that lines a splenic sinus removes the aging erythrocyte by phagocytosis. If cells are severely damaged or deformed, macrophages in the liver may also assist in removal of the cells from circulation.

Morphological characteristics: As erythrocytes develop from immature to mature cells, several changes occur that assist in the morphological identification of each cell stage. Staining a blood or bone marrow film with a Romanowsky stain facilitates demonstration of these cellular changes. As cells proceed through differentiation, they generally decrease in overall size. The N:C ratio is defined as the ratio of the volume of the nucleus in a cell compared to the volume of the cytoplasm in that same cell. As erythrocytes develop, the N:C ratio decreases from a 4:1 in the proerythroblast stage to 1:1 in the orthochromatic erythroblast stage and finally 0:1 when the cell loses its nucleus. The nucleus stains darker blue and the chromatin is more condensed in appearance as the cell matures. A nucleolus within the nucleus is present in the proerythroblast and basophilic erythroblast stages but is usually gone by the polychromatophilic erythroblast stage. The cytoplasm in developing erythrocytes changes from blue in the earliest stages to bluish-gray to red as hemoglobin accumulates in the cell. Cytoplasmic granules are never present during any stage of erythrocyte development.

The mature erythrocyte is a biconcave disc that looks like a doughnut without the center removed. It is about 7-8  $\mu\text{m}$  in diameter and 2  $\mu\text{m}$  thick. Because the cell contains very little hemoglobin in the thin area in the middle of the cell, the erythrocyte is characterized by a darker-staining outer portion and a central pallor region on a Wright stained blood film.

#### ERYTHROPOIETIN:

Erythropoietin is a glycoprotein with a molecular weight of approximately 30,000. It is produced primarily by endothelium of peritubular capillaries in the kidney. Lower levels of EPO, about 10% of the total, are produced by hepatocytes surrounding the central vein in the liver. Macrophages in the bone marrow and astrocytes in the CNS may also make small amounts of EPO.

Erythropoietin binds to a surface receptor present on erythroid progenitors and precursors in the bone marrow. Although relatively few receptors are found on early erythroid forming cells, the number of receptors increases with differentiation until a peak of about 1,100 receptors per cell is reached. The principal function of EPO is to act in concert with other factors to stimulate the proliferation and maturation of responsive bone marrow erythroid precursors. EPO affects expansion of progenitor cells by repressing apoptosis (programmed cell death) and by acting as a mitogen to increase production. EPO along with other factors also decreases the maturation time in the bone marrow.

The normal regulation of erythropoiesis is a feedback loop. The primary stimulus for increased EPO synthesis is tissue hypoxia caused by decreased blood  $\text{O}_2$  availability. This hypoxia signal is received primarily in the kidney, which responds by increasing production and secretion of EPO. The EPO is transported to the bone marrow where it promotes proliferation and differentiation of red cells. As a result of this increased red cell production, the blood's oxygen carrying capacity increases, the stimulus of hypoxia is reduced, and EPO production is decreased to maintain a steady state.

In order for EPO to increase blood production, the other substances required for erythropoiesis must be present in adequate amounts. These include iron for hemoglobin, Vitamin B12 and folic acid for DNA synthesis. When EPO is administered in the presence of adequate building blocks, red cell expansion is seen by an increase in reticulocytes by the third day. The equivalent of one unit of blood can be produced by the seventh day and 5 units by 28 days.

When EPO was tested in normal individuals who initially had adequate iron stores it was found that they had difficulty maintaining prolonged red cell expansion due to the depletion of iron. Therefore intravenous iron supplementation is recommended when EPO is used for long term treatment. Additional Vitamin B12 and folate may also be needed.

#### CLINICAL ASPECTS OF ERYTHROPOIETIN:

Any factor that impairs delivery of oxygen to tissues usually results in increased erythropoietin release and stimulation of erythropoiesis. Factors that cause decreased tissue oxygenation (hypoxia) include

- Hypoxic hypoxia: Decrease in oxygenation of blood in the lungs. This can be due to decreased oxygen in the ambient air as occurs in high altitude, or pulmonary conditions such as emphysema or chronic obstructive pulmonary disease.
- Anemic hypoxia: Decreased hemoglobin mass in anemia or due to blood loss by bleeding or red cell hemolysis.
- Other conditions include abnormal hemoglobins that have higher than normal oxygen affinity and congestive heart failure.

Hypoxic conditions which do not result in increase in erythropoietin include

- Chronic kidney disease in which the diseased kidneys are unable to produce erythropoietin.
- Anemia of chronic disease such as rheumatoid arthritis and AIDS and malignancies, in which inflammatory cytokines suppress the endogenous production of EPO.

#### ERYTHROPOIETIN THERAPY:

Medical treatment with erythropoietin is used in chronic kidney disease, anemia of chronic disease, HIV, and anemia associated with cancer radiation therapy and chemotherapy. Correcting anemia with EPO therapy has been shown to decrease morbidity and mortality and improve the quality of life in these patients. EPO may also be used to increase the red cell mass pre-surgery, particularly when trying to avoid transfusions, such as in Jehovah's Witness patients.

EPO has been used inappropriately by athletes to increase the red cell mass ("blood doping") in an attempt to improve performance by increasing the amount of oxygen the blood carries. The practice of blood doping by athletes has been outlawed. In the 1998 Tour de France several team doctors and personnel were caught with thousands of doses of EPO and other banned substances. This blatant use of banned substances caused about 50% of the teams to withdraw from the race, either for cheating or in protest. Until recently accurate testing has been difficult because the recombinant human EPO made in the lab is almost identical to the naturally occurring hormone and there are no firmly established normal ranges for EPO in the body. Previously sports governing bodies used the hematocrit to attempt to curtail the use of blood doping. They banned athletes if the hematocrit was over 50%, which meant athletes could cheat as long as they kept their hematocrit below this level. Now there is an accurate urine test that can detect the differences between normal and synthetic EPO. This test for recombinant EPO depends on the difference in sugar molecules contained in manufactured EPO compared to those in the naturally produced hormone. Electrophoresis shows different patterns between these compounds. This test is now the standard and was used in the 2004 Olympic Games.

Not only is blood doping unfair in athletic competition, but it is dangerous because of increased blood viscosity. Above a certain hematocrit whole blood can sludge and clog capillaries. During prolonged exercise water is shifted out of the blood to replace fluid lost in perspiration and respiration which further increases the hematocrit. Sludging of the blood can cause stroke or heart attack.

CASE STUDY: As an example of evaluation for EPO therapy, refer to the case at the beginning of the course.

The results of the patient's anemia evaluation were as follows:

Test	Patient	Normal
Hgb	10.6 g/dl	14-18 g/dl
Hct	32%	42-52%
RBC	$3.5 \times 10^6/\text{ul}$	$4.7-6.1 \times 10^6/\text{ul}$
MCV	91.4 fl	81-99 fl
MCHC	33.1 g/dl	33-37 g/dl
Retics	0.4%	0.5-1.5%
Serum Iron	110 ug/dl	65-165 ug/dl
TIBC	330 ug/dl	260-440 ug/dl
TSAT	33%	20-50%
Serum ferritin	120 ug/L	30-250 ug/L
Stool guaiac	negative	negative
TSH	1.7 mIU/L	0.4-4.0 mIU/L

The anemia evaluation is done to detect anemias that are not due to EPO deficiency. Correcting an easily reversible cause of anemia makes both clinical and economic sense before considering EPO therapy. The National Kidney Foundation (1) states, "The red blood cell indices, reticulocyte count and iron parameters are helpful to detect the cause of many anemias which are not due to EPO deficiency."

The anemia of CKD is generally normocytic and normochromic. The MCV, MCHC and RBC appearance in the peripheral blood smear are useful in detecting anemias caused by other conditions. Microcytosis (MCV <80 fl) and hypochromia (MCHC <30) may reflect iron deficiency or certain hemoglobinopathies. Macrocytosis (MCV >100 fl) may be associated with vitamin B12 or folate deficiency. Macrocytosis can also be associated with therapy that shifts immature, larger reticulocytes into the circulation. An elevated reticulocyte count (corrected for the degree of anemia) suggests that active hemolysis may be present, such as in acute renal failure due to the hemolytic uremic syndrome. Another cause of anemia is hypothyroidism which is common in the general population, and can cause a normochromic, normocytic anemia that can mimic the anemia due to EPO deficiency. The TSH is another test that could be considered if hypothyroidism is suspected.

The anemia of CKD should not be confused with the anemia of chronic disease. In the latter, inflammatory cytokines suppress the endogenous production of EPO and erythropoiesis directly. Measuring levels of circulating cytokines may indicate that the anemia is due to chronic disease.

Iron is critical for hemoglobin synthesis. Consequently, patients should be carefully evaluated for the availability of iron by measuring the serum iron and the TIBC. The serum iron and the percent TSAT reflect the amount of iron immediately available for hemoglobin

synthesis. The serum ferritin reflects total body iron stores. A low level of either of these indices may indicate the need for supplemental iron to support erythropoiesis. The presence of iron deficiency requires a search for the cause, which is usually blood loss. A stool guaiac test for occult blood is recommended to test for gastrointestinal bleeding in patients with iron deficiency.

The NKF's guidelines for EPO treatment: "If no cause for anemia other than CKD is detected based on the anemia evaluation, and the serum creatinine is  $\geq 2$  mg/dL, anemia is most likely due to EPO deficiency. In patients with non-renal anemia, serum EPO levels are usually elevated in an effort to compensate for the anemia. In patients with impaired kidney function and a normochromic, normocytic anemia, it is rare for the serum EPO level to be elevated. Therefore, measurement of EPO levels in such patients is not likely to guide clinical decision-making or EPO therapy."

The results of the Case Study patient's tests indicate that he has a normocytic, normochromic anemia with adequate iron stores and no indication of intestinal bleeding. Therefore he is a candidate for EPO therapy.

#### REFERENCES:

1. National Kidney Foundation, Guidelines for Anemia of Chronic Kidney Disease, [www.kidney.org/professionals/kdoqi/guidelines](http://www.kidney.org/professionals/kdoqi/guidelines)
2. Advanced Transfusion Practices and Blood Research, Johns Hopkins University, Cases of Interest for Physicians, Maximizing the Effectiveness of Erythropoietin Therapy, [http://atpcenter.glow.com/physicians/cases\\_of\\_interest\\_archive\\_detail.cfm?ID=19](http://atpcenter.glow.com/physicians/cases_of_interest_archive_detail.cfm?ID=19)
3. Braunwald E, Fauci AS, Dasper DL, et al.eds. *Harrison's Principles of Internal Medicine*, 2001: 653-659, 666.
4. Jenkins M, EPO, [www.rice.edu/~jenky/sports/epo.html](http://www.rice.edu/~jenky/sports/epo.html)

## Review Questions

Course #056-969

Choose the **one** best answer

1. CFU-L is a stem cell committed to production of
  - a. leukocytes
  - b. liver cells
  - c. lymphocytes
  - d. leukocidins
2. The origin of all types of formed elements in the blood is
  - a. CFU-GEMM
  - b. CFU-E
  - c. CFU-L
  - d. PSC
3. As red blood cells mature all but which of the following occurs?
  - a. nuclear size increases
  - b. cytoplasm color changes from blue to reddish
  - c. nuclear chromatin condenses
  - d. overall cell size decreases
4. Erythropoietin is produced primarily in the
  - a. liver
  - b. kidney
  - c. macrophages
  - d. astrocytes
5. The primary stimulus for erythropoietin secretion is
  - a. hypoxia in the bone marrow
  - b. decreased red cell mass
  - c. decreased oxygenation of kidney tissue
  - d. decreased oxygen in ambient air
6. Which of the following patients' test results indicate qualification for EPO therapy?
  - a. MCV = 83 fl, MCHC = 33 g/dl
  - b. MCV = 75 fl, MCHC = 29 g/dl
  - c. MCV = 102 fl, MCHC = 34 g/dl
  - d. TSH = 11 mIU/L (normal = 0.4-4.0 mIU/L)
7. Hypoxic conditions which do not cause an increase in erythropoietin include all but
  - a. rheumatoid arthritis
  - b. AIDS
  - c. emphysema
  - d. chronic kidney disease

8. The test for blood doping used in the 2004 Olympics depends on
  - a. a DNA analysis of manufactured EPO compared to natural EPO
  - b. increased blood levels of EPO
  - c. a hematocrit of over 50%
  - d. differences in sugars attached to recombinant EPO compared to natural EPO
  
9. A patient has a TSAT of 28%. This means that the patient
  - a. has decreased iron stores
  - b. is not a candidate for EPO therapy
  - c. has increased serum ferritin
  - d. has normal serum iron
  
10. A Hgb of 11.0 g/dL and a positive stool guaiac in an adult man indicate that
  - a. the expected MCV would be above normal
  - b. the patient has anemia due to intestinal bleeding
  - c. the patient is a candidate for EPO treatment
  - d. the patient probably has a decreased EPO level

**Course #969 ERYTHROPOIETIN AND BLOOD PRODUCTION**  
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 Please circle the one best answer for each question.

- |    |   |   |   |   |    |   |   |   |   |
|----|---|---|---|---|----|---|---|---|---|
| 1. | a | b | c | d | 6  | a | b | c | d |
| 2. | a | b | c | d | 7  | a | b | c | d |
| 3. | a | b | c | d | 8  | a | b | c | d |
| 4. | a | b | c | d | 9  | a | b | c | d |
| 5. | a | b | c | d | 10 | a | b | c | d |

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