# Toxic Responses of the Blood

Blood plays a major role to **transport oxygen** from the lungs around the body and **carbon dioxide** from the cells to the lungs for exhalation, to **remove wastes** such as lactic acid from muscle cells, to deliver **nutrients** such as amino acids and glucose to cells around the body, to maintain homeostasis and to clot wounds and fight infection.

Blood contains many element in addition to other organs such as bone marrow which is the site of hematopoiesis (cell production), spleen which is responsible for clearance of defective cells, and lymph nodes.

Bone marrow contains stem cells which are immature precursors for **erythrocyte** (the red blood cell), **leukocyte** (the white blood cell) and **thrombocyte** (the platelet), each are produced approximately 1-3 millions/sec., RBCs life spam is 120 days.

The study of the adverse effects of exogenous chemicals on blood and blood-forming tissues is known as Hematotoxicology.

↓ RBCs → anemia.

↓ WBCs → leucopenia

↓ platelets (thrombocytes) → thrombocytopenia.

Hematotoxicity may be primary (direct), in which one or more blood components are affected directly, or secondary (indirect), in which the toxic effect is a consequence of other tissue injury or systemic disturbances.

## Toxicology of the Erythron

## The Erythrocyte

Erythrocytes [red blood cells (RBCs)] transport oxygen from the lungs to the peripheral tissues and carbon dioxide from tissues to the lung. Erythrocytes also are involved as a carrier and/or reservoir for drugs and toxins. Xenobiotics may affect the production, function, and/or survival of erythrocytes. Acute damage to RBCs or its Hb can result in anemia. Anemia results from decrease production and increase destruction of erythrocytes. The usual parameters of a complete blood count (CBC), including the RBC count, hemoglobin concentration, and hematocrit (PCV), can establish the presence of anemia. Increased destruction

usually is accompanied by an increase in reticulocytes (young erythrocytes).

Two related processes contribute to the increased number of reticulocytes in humans.

**First**, increased destruction is accompanied by a compensatory increase in bone marrow production, with an increase in the number of cells being released from the marrow and into the circulation. **Second**, during compensatory erythroid hyperplasia, the marrow releases reticulocytes earlier in their life span; thus, the reticulocytes persist for a longer period in the peripheral blood.

**Alterations in Red Cell Production**

Erythrocyte production is a continuous process that is dependent on frequent cell division and a high rate of hemoglobin synthesis. Adult hemoglobin (hemoglobin A) is a tetramer composed of two α -globin and two β-globin chains, each with a heme residue. The synthesis of heme requires the incorporation of iron into a porphyrin ring. Iron deficiency usually results from dietary deficiency or increased blood loss. Which may potentiate the risk of developing **iron deficiency anemia**. Defects in the synthesis of the porphyrin ring of heme can lead to **sideroblastic** anemia, with its characteristic accumulation of iron in bone marrow erythroblasts. The accumulated iron precipitates within mitochondria, causing the intracellular injury.

**Xenobiotics Associated with sideroblastic Anemia include:** ethanol, isoniazide, cycloserine, chloramphenicol, zinc and lead intoxication.

Many factors can affect blood toxicity:

1. Decrease folic acid or vitamin $B_{12}$ which are necessary to maintain the synthesis of DNA during RBCs production, their deficiency causes **megaloplastic** anemia. **E.g. of drugs cause folic acid deficiency:** methotrexate, phenytoin, primidine, carbamazepine and phenobarbital. **E.g. of drugs cause vitamin $B_{12}$ deficiency:** colchicin, neomycin, ethanol and omeprazol.

2. Chemical toxicity to the bone marrow may cause aplastic anemia which is inability of bone marrow to proliferate, this life threatening disorder is characterized by reticulocytopenia and bone marrow hypoplasia in addition to peripheral blood pancytopenia which is a decrease in the circulating number of RBCs, WBCs and platelets. This is caused by ionization, radiation, gold, mercury, chloramphenicol, penicillin, methicillin, streptomycin, allopurinol and tetracycline.
3. Bone marrow damage may have an allergic basis involve antibodies to a precursor cells and sensitizing chemicals, e.g. chloramphenicol induced bone marrow damage may be involved in immune mechanism

**Alteration in the respiratory function of hemoglobin:**

Hemoglobin consist of 4 chains (tetramer): 2 α globin and 2 β globin chains, with a heme residue.

Reversible binding of $O_2$ with Hb is called oxygenation.

**Oxygen dissociation curve:**

the normal oxygen dissociation curve has a sigmoid shape as a result of the cooperative interaction between the four globin chains in the Hb molecule. Fully deoxygenated Hb has low affinity for oxygen, interaction of oxygen with one heme-iron moiety induces a conformational changes which affect other globin chain lead to increase their affinity for $O_2$ .

when Hb molecule is fully saturated with $O_2$, all oxygen molecules are equivalent, a fall in $PO_2$ lead to the release of the first $O_2$ molecule, this facilitate the release of the second $O_2$ molecule from Hb molecule, this is responsible for the sigmoid shape of the curve.

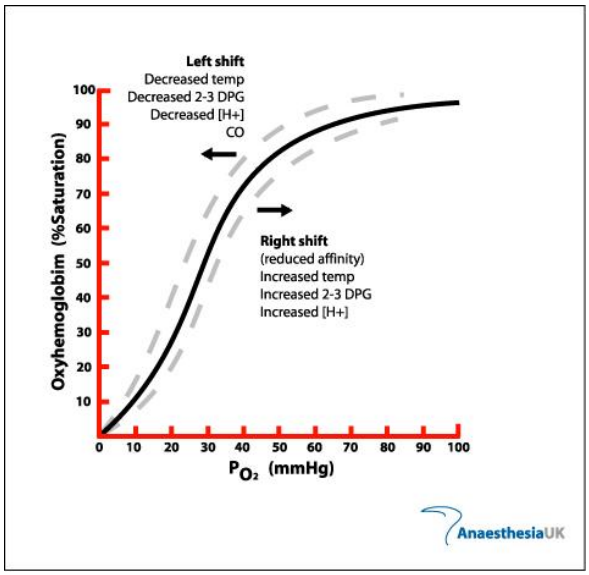


**Figure 2:**oxygen dissociation curve

**Factors affecting Hb-$O_2$ affinity:**

1) **Homotropic effect:** including methemoglobin formation (oxidation of heme iron to the ferric state) which is not capable of binding and transporting oxygen. This causes a leftward shift of oxygen dissociation curve, e.g. lidocaine, dapson, nitroglycerin, sulfonamide, metoclopramide, nitrate and potassium chlorate.
2) **Heterotropic effect:**
   - **a- PH:** decrease PH by ($CO_2$ or lactic acid) lead to decrease affinity and right shift of the oxygen dissociation curve so increase $O_2$ delivery to the tissue.
   - **b- Temperature:** increase temperature lead to decrease affinity and right shift in the curve.
   - **c- 2,3-biphosphoglycerate ( 2,3-BPG):** binding of 2,3-BPG to deoxyhemoglobin results in reduced affinity i.e. a right shift in $O_2$ dissociation curve. Adult Hb bind 2,3-BPG more tightly than does fetal Hb.

**Note:** right shift → ↑ $O_2$ delivery to tissue.
Left shift → ↓ $O_2$ delivery to tissue.

**Anemia:** it can result from increase RBCs destruction (hemolytic anemia)

**Manifestation of hemolysis:** ↓ RBCs life span (normal 120 days), ↓ count, ↓ hematocrit, ↓ Hb and ↑ plasma bilirubin.

## Nonimmune Hemolytic Anemia

### Microangiopathic Anemias

Is the intravascular fragmentation of erythrocytes, results from the formation of fibrin strands in the microcirculation slice the erythrocytes into fragments.

### Infectious Diseases

Erythrocytes are parasitized in malaria, leading to their destruction. Clostridial infections are associated with the release of hemolytic toxins that enter the circulation and lyse erythrocytes.

### Oxidative Hemolysis

Several mechanisms protect against oxidative injury in erythrocytes, including superoxide dismutase, catalase, and the glutathione pathway. Xenobiotics capable of inducing oxidative injury in erythrocytes and are capable of inhibiting the usual protective mechanisms, e.g. naphthalin, nitrofurantoin, aminosalicylic acid, dapson, sulfasalazine and phenol. The most common enzyme defect associated with oxidative hemolysis is glucose-6-phosphate dehydrogenase (G-6-PD) deficiency.

### Nonoxidative Chemical-Induced Hemolysis

Inhalation of gaseous arsenic hydride (arsine) can result in severe hemolysis, with anemia, jaundice, and hemoglobinuria. Lead poisoning is associated with defects in heme synthesis, a shortening of erythrocyte survival, and hemolysis.

## Immune Hemolytic Anemia

Immunologic destruction of erythrocytes is mediated by the interaction of IgG or IgM antibodies with antigens expressed on the surface of the erythrocyte. In the case of autoimmune hemolytic anemia, the antigens are intrinsic components of the patient's own erythrocytes. Some drugs, like penicillin appear to bind to the surface of the cell, with the "foreign" drug acting as a hapten and eliciting an immune response. The antibodies that arise in this type of response bind only to drug-coated erythrocytes. Other drugs include quinidine and α- methyldopa.

**Polycythemia:** it is abnormal increase in RBCs number, mostly polycythemia vera it is a disease associated with over production of RBCs due to unusual sensitivity of stem cells to erythropoietin. Polycythemia is a response to the hypoxia that is due to cardiovascular disease or anemic hypoxia. It is one of the manifestation of cobalt toxicity, the effect of cobalt is secondary to the action in the CNS that results in respiratory alkalosis which increases the affinity of Hb to $O_2$.

## Toxicology of the Leukon

## Components of Blood Leukocytes

The leukon consists of leukocytes, or white blood cells, including granulocytes (neutrophils, eosinophils, and basophils); monocytes; and lymphocytes.

## Toxic Effects on Granulocytes

**Effects on Proliferation:** Methotrexate, cytosine arabinoside, daunorubicin, cyclophosphamide, cisplatin, and the nitrosureas are toxic to resting and actively dividing cells, in which maximum effects usually are seen 7 to 14 days after exposure.

**Effects on Function:** Ethanol and glucocorticoids impair phagocytosis and microbe ingestion. Superoxide production, which is required for microbial killing is reduced in patients who use parenteral heroin.

**Idiosyncratic Toxic Neutropenia:** some agents can damage neutrophils and granulocyte precursors, induce agranulocytosis, which is

characterized by a profound depletion in blood neutrophils. This type of injury occurs in specifically conditioned individuals and therefore is termed idiosyncratic. Hematopoietic function usually is restored when the agent is detoxified or excreted.

**Mechanisms of Toxic Neutropenia:** In **immune-mediated** neutropenia, antigen-antibody reactions lead to the destruction of peripheral neutrophils, granulocyte precursors, or both. Xenobiotic can induce immunogenic cells to produce antineutrophil antibodies e.g. aminopyrine, ampicillin, phenytoin, lidocaine and quinidine. **Nonimmune-mediated** toxic neutropenia often shows a genetic predisposition. **Direct damage** may cause inhibition of granulopoiesis or neutrophil function e.g. isoniazide, rifampicin, ethambutol and allopurinol.

**Human Leukemias**

Leukemias are proliferative disorders of hematopoietic tissue that originate from individual bone marrow cells, as for e.g. after treatment with alkylating or other antineoplastic agents, benzene (from aromatic hydrocarbons) and Exposure to high-dose γ- or x-ray radiation.

**Toxic Effects on Platelets (thrombocytes)**

**The Thrombocyte:** Platelets are essential for the formation of a stable hemostatic plug in response to vascular injury. Platelets initially adhere to the damaged wall. Activation of a pathway of several factors permits fibrinogen and other adhesive molecules to form cross-links between nearby platelets, resulting in platelet aggregation. Xenobiotics may interfere with the platelet response by causing thrombocytopenia or interfering with platelet function.

**Thrombocytopenia**

thrombocytopenia may be due to decreased production or increased destruction of platelets. Thrombocytopenia is a common side effect of intensive chemotherapy, Exposure to xenobiotics may cause increased **immune-mediated** platelet destruction through any of several mechanisms. Some drugs, such as penicillin, function as haptens, binding

to platelet membrane components and eliciting an immune response. A **second** mechanism of immune thrombocytopenia is initiated by a change in a platelet membrane glycoprotein caused by the xenobiotic. This altered protein then elicits an antibody response.

**Heparin-induced thrombocytopenia** represents another mechanism of immune-mediated platelet destruction. This results in platelet aggregation instead of heparin's normal function of preventing clot formation, which can lead to a risk of thrombosis (pieces of clots falling off and lodging in the microvasculature, impairing circulation).

Major drug groups that affect platelet function include nonsteroidal anti-inflammatory agents; β-lactam-containing antibiotics; cardiovascular drugs, particularly beta blockers; psychotropic drugs; anesthetics; antihistamines. Other agents appear to interfere with the interaction between platelet agonists and their receptors (e.g., antibiotics, ticlopidine, clopidogrel), calcium channel blockers may inhibit platelet function.

**Toxic Effects on Fibrin Clot Formation**

**Decreased Synthesis of Coagulation Proteins**

Most proteins involved in the coagulation cascade are synthesized in the liver. Therefore, any agent that impairs liver function may cause a decrease in the production of coagulation factors. The common tests of the coagulation cascade—prothrombin time (PT) and activated partial thromboplastin time (aPTT)—may be used to screen for liver dysfunction and a decrease in clotting factors. Factors II, VII, IX, and X are dependent on vitamin K for their complete synthesis.

Anything that interferes with the absorption of vitamin K from the intestine may lead to a deficiency of these factors and a bleeding tendency. Conditions Associated with Abnormal Synthesis of Vitamin K–Dependent Coagulation Factors: **Warfarin and analogs, Rodenticides, Broad-spectrum antibiotics and Dietary deficiency**.

**Increased Clearance of Coagulation Factors:** include the formation of antibodies that react with coagulation proteins, forming an immune complex that is cleared rapidly from the circulation and resulting in

deficiency of the factor. these antibodies often inhibit the function of the coagulation factor.

**Toxicology of Agents Used to Modulate Hemostasis**

**Oral Anticoagulants:** The therapeutic window for oral anticoagulants (warfarin) is relatively narrow, The consequence of insufficient anticoagulant effect is an increased risk of thromboembolism, whereas the consequence of excessive anticoagulation is an increased risk of bleeding. Therapy with these agents must be monitored routinely with PT, with the results expressed in terms of the international normalized ratio (INR). Oral anticoagulants are absorbed readily from the gastrointestinal tract and bind to albumin in the circulation.

foods, can affect the response to oral anticoagulants by interference with biotransformation; interference with the absorption of warfarin from the gastrointestinal tract; displacement of warfarin from albumin in plasma, which temporarily increases the bioavailability of warfarin; diminished vitamin K availability; and inhibition of the reduction of vitamin K epoxide, which potentiates the effect of oral anticoagulants. **Side effects of oral anticoagulants:** warfarin-induced skin necrosis, long-term administration of warfarin has been associated with bone demineralization. Administration of warfarin, particularly during the first 12 weeks of pregnancy, is associated with congenital anomalies due to abnormal bone formation.

**Heparin:** Heparin is used widely for both prophylaxis and therapy for acute venous thromboembolism. The major complication associated with heparin therapy is bleeding. Long-term administration of heparin is associated with osteoporosis. Also, heparin administration may cause a transient rise in serum aminotransferases.

**Fibrinolytic Agents**: Fibrinolytic agents dissolve the pathogenic thrombus by converting plasminogen, to plasmin leading to systemic fibrinolysis, which is characterized by prolongation of PT, aPTT, and thrombin time. All these effects potentiate the risk of bleeding. Platelet inhibitors and heparin commonly are used in conjunction with fibrinolytic therapy to prevent recurrent thrombosis.

Antibody formation to streptokinase occurs commonly in association with streptococcal infections as well as exposure to streptokinase, and streptokinase-derived peptides.

**Inhibitors of Fibrinolysis:** Inhibitors of fibrinolysis commonly are used to control bleeding, Tranexamic acid and aminocaproic acid are small molecules that block the binding of plasmin to fibrin.

**CO poisoning:**

It is tasteless, odorless gas, it decreases $O_2$ transport capacity of the blood and produces anemic hypoxia. Its very dangerous agent at low concentration (0.1 % CO mixture will result in 50% of carboxy Hb and this depend on the diffusion capacity of the lung and alveolar ventilation which depend on the level of exercise).

Symptoms of CO exposure are headache, weakness, nausea, dizziness, vision disturbances, lactic acidosis, unconsciousness, coma and death.

CO-Hb is cherry-red color, so it cause abnormal color to skin and mucus membrane and nail. CO exposure can be treated by supporting ventilation artificially.

**Note:** 50% CO-Hb is more dangerous than 50% anemia, this is because:

1- 50% anemia requires 75 mmhg drop to deliver 5ml $O_2$/dl; while 50% COHb requires 85 mmhg.
2- Absence of cooperativity in COHb which is present in anemia.

**Methemoglobinemia:** methemoglobin is greenish brown to black in color, causing anemic hypoxia. It is the oxidized form of the heme-iron from ferrous to ferric state, with the result of metHb level that can not combine $O_2$ reversibly, result in increase affinity of oxy Hb to $O_2$ and left shift of $O_2$ dissociation curve and decrease delivery of $O_2$ to the tissues.

Methemoglobinemia can be induced by sod.nitrite, inorganic hydroxyl amine, nitrate, lidocaine, dapson, nitroglycerin, metoclopramide and sulfonamide.

excess nitrite first generate metHb, then form a nitrite-metHb complex which is important for spectrophotometric determination of methb. Normal level of metHb is less than 1% of total Hb, this percent is maintained by metHb reduction which is either by:

**1-** methb reductase or diaphorase which has been identified as cytochrom $b_5$ , with intracellular NADH as a cofactor.
2- or by NADPH-methylene blue system.

So failure of these mechanisms lead to increase metHb, cyanosis (5-10 % metHb)

**Histotoxic hypoxia:** in which there is normal $O_2$ supply and normal $PO_2$ , but the cell can not utilized $O_2$, e.g. of agents causing this type of hypoxia: hydrogen sulfide and hydrogen cyanide.

**Cyanide:**

It is very dangerous toxicant causing histotoxic hypoxia by interruption with electron transport (inhibiting cytochrom chain), thus inhibing mitochondrial enzyme with subsequent respiratory arrest and death early after inhalation and slowly after ingestion.

Cyanide present in amygdaline (cyanogenic glycoside in almond), peach and in sodium nitroprusside.

**Treatment:** CN has high affinity to $Fe^{+3}$, so giving agents that produce $Fe^{+3}$ such as metHb, which compete with cytochrom to CN so we give:

1- Amylnitrite (by inhalation) which give 5% MetHb it has fast action (do not use nitrate inhalation due to slow action), but 5% MetHb is not adequate for treatment.
2- Sod.nitrite (injection) which raise metHb to 25-30%

$HbO_2 \xrightarrow{NO_2}$ MetHb $\xrightarrow{CN}$ CNMetHb, but this compound (cyanometHb) is rapidly dissociated and liberate CN again so we give:

3- sod.thiosulfate injection: which mediate conversion of CN to less toxic thiocyanate which excreted in urine.

$$CNMetHb \xrightarrow{S_2O_3} SCN + SO_3 + Hb$$

**Heinz bodies hemolytic anemia:**

Hb consist of exposed free cysteines, so by oxidation of these groups, denatured Hb is produced with decreased solubility (sulf-Hb), this hb aggregate and bind to the cell membrane to form inclusion (Heinz body) so Heinz body are dark staining granules in RBC, consisting of denatured Hb or sulf-hb, these body lie on or near the interior surface of RBC membrane, so SH-group on Sulf-Hb form disulfide (-S=S-) bind with the membrane surface leading to impairment of the membrane surface function which lead to hyperpermeability and hemolysis. This hemolysis is produced by aniline, nitrobenzene, phenol, propylene glycol and ascorbic acid.