**Agranulocytosis** is a group of pathologic disorders, characterized by severe leukopenia (leukocyte count < 1.5 x 10⁹/L) with neutropenia, leading to high susceptibility to bacterial and fungal infections.

**Pathophysiology**

Agranulocytosis may be broadly divided into 2 groups: hereditary disease due to genetic mutations and acquired disease.

**Hereditary disease due to genetic mutations**

Many hereditary disorders are due to mutations in the gene encoding neutrophil elastase, or *ELA2*. Several alleles are involved. The most common
mutations are intronic substitutions that inactivate a splice site in intron 4. Genes other than *ELA2* are also involved.

The table below summarizes the genetic conditions; these are uncommon conditions. A strong family history of recurrent infections, usually beginning in childhood, is strongly indicative of a genetic defect.

**Genetic Conditions in Agranulocytosis**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic neutropenia</td>
<td>Autosomal</td>
<td><em>ELA2</em></td>
<td>Alternate 21-day cycling of neutrophils and monocytes</td>
</tr>
<tr>
<td>Kostman syndrome</td>
<td>Autosomal</td>
<td>Unknown</td>
<td>Stable neutropenia, no MDS or AML</td>
</tr>
<tr>
<td>Severe congenital neutropenia</td>
<td>Autosomal</td>
<td><em>ELA2</em>(35-84%)</td>
<td>Stable neutropenia, MDS or AML</td>
</tr>
<tr>
<td></td>
<td>dominant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autosomal</td>
<td><em>Gfi1</em></td>
<td>Stable neutropenia, circulating myeloid progenitors, lymphopenia</td>
</tr>
<tr>
<td></td>
<td>dominant</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sex linked</td>
<td><em>Wasp</em></td>
<td>Neutropenic variant of Wiskott-Aldrich syndrome</td>
</tr>
<tr>
<td></td>
<td>Autosomal</td>
<td><em>G-CSFR</em></td>
<td>G-CSF – refractory neutropenia, no AML or MDS</td>
</tr>
<tr>
<td>Hermansky-Pudlak syndrome type 2</td>
<td>Autosomal</td>
<td><em>AP3B1</em></td>
<td>Severe congenital neutropenia, platelet dense-body defect, oculocutaneous albinism</td>
</tr>
<tr>
<td></td>
<td>recessive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chediak-Higashi syndrome</td>
<td>Autosomal</td>
<td><em>LYST</em></td>
<td>Neutropenia, oculocutaneous albinism, giant lysosomes, impaired platelet function</td>
</tr>
<tr>
<td></td>
<td>recessive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barth syndrome</td>
<td>Sex linked</td>
<td><em>TAZ</em></td>
<td>Neutropenia, often cyclic; cardiomyopathy, methylglutaconic</td>
</tr>
<tr>
<td>Aciduria</td>
<td>Cohen syndrome</td>
<td>Autosomal recessive</td>
<td>COH1</td>
</tr>
</tbody>
</table>

AML = acute myeloid leukemia; G-CSF = granulocyte colony-stimulating factor; MDS = myelodysplastic syndrome.

In agranulocytosis, neutrophil count is sharply reduced in the blood. Agranulocytosis may be inherited neutropenia, such as Kostmann’s syndrome (syn: severe infantile agranulocytosis) and benign familial neutropenia; or acquired agranulocytosis. There are two types of development of acquired agranulocytosis:

1) Premature antibody destruction of granulocytes (immune agranulocytosis). Immune agranulocytosis appears in hypersensibilization to drugs (hapten-mediated agranulocytosis), infections (infectious-allergic agranulocytosis) or autoimmunization in systemic diseases (autoimmune agranulocytosis);

2) Cessation of neutrophil production in connection with impairment of precursor cells (unipotential cells). Thus, myelotoxic agranulocytosis develops. Similar injury may occur in irradiation, intake of antineoplastic agents and neoplasm of the bone marrow.

Acquired agranulocytic disease may be due to drugs, chemicals, autoimmunity, infectious agents, or other causes.

Bone marrow and peripheral blood are the organ systems affected. Agranulocytosis is characterized by inadequate production of neutrophils, excessive destruction of neutrophils, or both. The resulting infections tend to involve the oral cavity, mucous membranes, and skin. Systemic life-threatening sepsis may ensue. The most common infecting organisms are staphylococci, streptococci, gram-negative organisms, and anaerobes. Fungi are also commonly involved as secondary infective agents.
The occurrence of infection depends on the degree and duration of neutropenia. When the ANC is persistently fewer than 100/µL for longer than 3-4 weeks, the incidence of infection approaches 100%.

**Causes**

1) The most common cause of agranulocytosis is exposure to drugs or chemicals. About one half of patients have a history of medication or chemical exposure. The patient's history must be carefully taken to elicit this information.

- Any chemical or drug that can depress the bone marrow and cause hypoplasia or aplasia is capable of causing agranulocytosis. Some drugs do this to everyone if they are administered in large enough doses. Other agents seem to cause idiosyncratic reactions that affect only certain susceptible individuals.

- The mechanisms that cause neutropenia are not completely understood. In many cases, neutropenia occurs after prolonged exposure, resulting in decreased neutrophil production by hypoplastic bone marrow. In other cases, repeated but intermittent exposure is needed. This suggests an immune mechanism, although this idea has not been proven.

- A drug may act as a hapten and induce antibody formation. This mechanism operates in cases due to gold, aminopyrine, and antithyroid drugs. The antibodies destroy the granulocytes and may not require the continued presence of the drug for their action. As an alternative, the drug may form immune complexes that attach to the neutrophils. This mechanism operates with quinidine.

- Another mechanism is direct inhibition of myelopoiesis. Valproic acid, carbamazepine, and beta-lactam antibiotics act by this mechanism. In bone marrow cultures, these agents inhibit granulocyte colony formation in a dose-related fashion.

- Direct damage to the bone-marrow microenvironment or myeloid precursors plays a role in most other cases.

2) Many drugs associated with agranulocytosis have been reported to the US Food and Drug Administration (FDA) under its adverse reactions reporting requirement.
Many agents are also reported to a registry maintained by the American Medical Association (AMA). The reported drugs were used alone, in combination with another drug known to be potentially toxic, or with another drug without known toxicity. Several drugs are salient because of their high frequency of association with agranulocytosis. They include the following:

- Phenothiazine
- Antithyroid drugs (thiouracil and propylthiouracil)
- Aminopyrine
- Phenylbutazone
- Chloramphenicol
- Sulfonamides

3) Drugs reported to be associated with agranulocytosis include the following:

1. Analgesics
   - Acetaminophen
   - Aminopyrine
   - Dipyrrone

2. Cardiovascular drugs
   - Captopril
   - Hydralazine
   - Methyldopa
   - Pindolol
   - Procainamide
   - Propranolol
   - Quinidine

3. Antibiotics
   - Cephalosporins
   - Clindamycin
   - Chloramphenicol
   - Doxycycline
   - Gentamicin
• Griseofulvin
• Isoniazid
• Metronidazole
• Nitrofurantoin
• Penicillins
• Rifampin
• Streptomycin
• Sulfonamides
• Vancomycin

4. Diuretics
• Acetazolamide
• Bumetanide
• Chlorothiazide
• Hydrochlorothiazide
• Chlorthalidone
• Methazolamide
• Spironolactone

5. Anticonvulsants
• Carbamazepine
• Mephenytoin
• Phenytoin
• Primidone
• Trimethadione

6. Hypoglycemic agents
• Chlorpropamide
• Tolbutamide

7. Antihistamines
• Brompheniramine
• Cimetidine
• Tripelennamine
• Ranitidine
• Thenalidine

8. Phenothiazines
• Chlorpromazine
• Clozapine
• Desipramine
• Prochlorperazine
• Promazine
• Thioridazine
• Trifluoperazine
• Trimeprazine

9. Anti-inflammatory drugs
• Fenoprofen
• Gold salts
• Ibuprofen
• Indomethacin
• Phenylbutazone

10. Neuropharmacologic agents
• Chlordiazepoxide
• Clozapine
• Desipramine
• Meprobamate
• Metoclopramide
• Prochlorperazine
• Promazine

11. Antimalarials
• Amodiaquine
• Dapsone
• Hydroxychloroquine
• Pyrimethamine
12. Miscellaneous drugs
- Quinine
- Allopurinol
- Colchicine
- D-Penicillamine
- Ethanol
- Levamisole
- Levodopa

13. Antithyroid agents
- Carbimazole
- Methylthiouracil
- Propylthiouracil

4) Viral infections often lead to mild or moderate neutropenia. Agranulocytosis is uncommon but may occur. The most common organisms are Epstane-Barr virus, hepatitis B virus, yellow fever virus, cytomegalovirus, and influenza.

5) Many overwhelming infections, both viral and bacterial, may cause severe neutropenia.

6) Autoimmune neutropenia is the neutrophil analogue of autoimmune hemolytic anemia idiopathic thrombocytopenic neutropenia. It should be considered in the absence of any of the common causes. Antineutrophil antibodies have been demonstrated in these patients.

7) Cyclic neutropenia is characterized by periodic bouts of agranulocytosis associated with infection.
   - Cyclic neutropenia has a periodicity of about 21 days (range, 12-35 d).
   - Granulocyte precursors disappear from the marrow before each neutrophil nadir in the cycle because of accelerated apoptosis of myeloid progenitor cells.
   - Some cases may be genetically determined with an autosomal recessive inheritance. Other cases may be due to an autosomal dominant inheritance.

8) Several uncommon causes of severe neutropenia include Kostmann syndrome chronic severe neutropenia, and myelokathexis.
- Kostmann syndrome (severe congenital neutropenia) is most often caused by a recessive inheritance and found in remote, isolated populations with a high degree of consanguinity. Autosomal dominant and sporadic cases have also been reported, most often due to mutations in the G-CSF receptor. A risk of conversion to MDS/AML with monosomy 7 exists after treatment with G-CSF.

- Chronic severe neutropenia has an underlying unknown cause.

- Myelokathexis occurs in early infancy and is associated with recurrent infections. The condition is due to accelerated apoptosis and decreased expression of bcl-x in neutrophil precursors.

**Symptoms of Agranulocytosis**

The list of signs and symptoms mentioned in various sources for Agranulocytosis includes the 6 symptoms listed below:

- Fever
- Sore throat
- Painful mouth ulcer
- Anal ulcer
- Reduced immune response
- Prone to bacterial infections

**Clinic features**

Main signs are ulcerative-necrotic changes in the mucous membranes of the URT, GIT and ulcerative-necrotic tonsillitis. Agranulocytosis may be acute or subacute with fever, regional lymphadenopathy and complications (fungal infections). In severe case, jaundice, hematuria and cylinduria may be found in patients with agranulocytosis.

**Investigations**

1. CBC: leukopenia (< 2 x 10⁹/L), granulocytes 1 - 4 %, relative lymphocytosis (until 90 %). For example, RBC - 3.8 x 10¹²/L, Hb - 121 g/L, CI - 0.95, thrombocytes - 179 x 10⁹/L, WBC - 0.9 x 10⁹/L: stab cells - 2, segmented cells - 3, lymphocytes - 93, monocytes - 2, ESR - 21mm/h.

2. Myelogram: Partial or full hypoplasia of granulocytic lines.

3. Immunologic studies: presence of Ig against WBC and dysimmunoglobulinemia.

**Laboratory Studies**

- Perform a complete blood cell (CBC), including a manual differential in evaluating cases of agranulocytosis. Careful evaluation of the peripheral
blood smear provides information about red blood cell (RBC) and platelet morphology.

- Examine bone marrow smears and biopsy samples with techniques including flow cytometry.
- Microbiologic cultures of blood, wounds, and body fluids are indicated in febrile patients.
- Tests for antineutrophil antibodies should be performed in patients with a history suggestive of autoimmune neutropenia and in those with no other obvious explanation for the agranulocytosis.
- In congenital neutropenia and cyclic neutropenia, genetic analysis should be done to correctly classify the condition.

**Imaging Studies**

- No specific imaging study establishes the diagnosis of agranulocytosis.
- As part of the workup for localization of infection, appropriate radiographs (eg, chest images) are indicated.
- Other imaging studies are determined by the specific circumstances of each case.
Sternal puncture, indications and diagnostic able.

Bone marrow examination is performed in adults either from sternum or posterior iliac crest. Marrow may be simply aspirated or a bone marrow biopsy (trephine) performed.

A small amount of bone marrow is removed during a bone marrow aspiration. The procedure is uncomfortable, but can be tolerated by both children and adults. The marrow can be studied to determine the cause of anemia, the presence of leukemia or other malignancy, or the presence of some "storage diseases" in which abnormal metabolic products are stored in certain bone marrow cells.

The latter cannot be obtained safely from the sternum and increasingly both aspirate and biopsy are performed from the posterior iliac crest. A biopsy is superior for assessing marrow cellularity and infiltration. Bone marrow examination is performed under local anaesthesia and can easily be undertaken as an outpatient procedure. Both aspiration and trephine biopsy can be carried out by the same needle but often separate needles are used.
**Procedures**

- Bone marrow aspiration and biopsy

**Histologic Findings**

- The peripheral blood smear shows a marked decrease or absence of neutrophils.
- The bone marrow may show myeloid hypoplasia or absence of myeloid precursors.
• In many cases, the bone marrow is cellular with a maturation arrest at the promyelocyte stage.
• On occasion, the marrow may be hypercellular.

**Differential diagnosis**

It is carried out with hypoplastic anemia, in which the main clinical signs are pancytopenia, hypoplasia of the bone marrow and acute leukemia. Anemia is accompanied with ostealgia and blasts. A more detailed differential diagnosis is concluded in table.

Ø Myelodysplastic Syndrome
Ø Acute lymphoblastic leukemia
Ø AML
Ø Human immunodeficiency virus (HIV) infection
Ø Large granular lymphocyte leukemia
Ø Shwachman-Diamond syndrome

**Management**

1. Abolish all early drug prescriptions. Remove possible causes of agranulocytosis.
2. Empiric antibiotics, antifungals and antiviral agents (taking into account individual endurance and indications).
4. Pentoxil®, leucogen and methyluracil.
5. Colony-stimulating factors.
6. Detoxification.

**Differential diagnosis of aplastic anemia, agranulocytosis and acute leukemia**

<table>
<thead>
<tr>
<th>Signs</th>
<th>Aplastic anemia</th>
<th>Agranulocytosis</th>
<th>Acute leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic syndrome</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>-</td>
<td>-</td>
<td>Moderately enlarged</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>-</td>
<td>-</td>
<td>Moderately enlarged</td>
</tr>
<tr>
<td>Infectious complications</td>
<td>May be</td>
<td>Often</td>
<td>Often</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>Usually absent</td>
<td>Often</td>
<td>Often (hyperplastic)</td>
</tr>
<tr>
<td>Fever</td>
<td>Usually absent</td>
<td>Present in infection</td>
<td>+</td>
</tr>
<tr>
<td>Character of anemia</td>
<td>Normochromic, hyporegenerative</td>
<td>-</td>
<td>Normochromic, normoregenerative</td>
</tr>
<tr>
<td>WBC</td>
<td>Leukopenia</td>
<td>Expressed leukopenia</td>
<td>Different quantity, often leukocytosis</td>
</tr>
<tr>
<td>WBC count</td>
<td>Absolute neutropenia</td>
<td>Significantly absolute neutropenia</td>
<td>Blasts, leukemic failure</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>↓</td>
<td>Normal</td>
<td>↓</td>
</tr>
<tr>
<td>Blastemia</td>
<td>-</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>All lines are suppressed</td>
<td>↓ granulocytes</td>
<td>Blast metaplasia</td>
</tr>
</tbody>
</table>

**TREATMENT**

**Medical Care**

Medical care is based on the etiology of the agranulocytosis. In most cases in which drug exposure is involved, the most important step is to discontinue the offending agent. If the identity of the causative agent is not known, stop administration of all drugs until the etiology is established.

1. Start specific antibiotic therapy to combat infections. This often involves the use of third-generation cephalosporins or equivalents.
2. Treat areas of stomatitis and skin infections with local cleaning, antisepsis, and dental care. These infections should be managed by someone who has experience in the treatment of infections in neutropenic patients.

3. Control oral and gingival lesion pain with saline and hydrogen peroxide rinses and local anesthetic gels and gargles.

4. The availability of the recombinant neutrophil cytokine, filgrastim (ie, G-CSF), has altered the management of agranulocytosis.
   - When administered before infection is established, filgrastim shortens the period to recovery and the duration of infection.
   - The agent is especially indicated in the management of congenital neutropenia, idiopathic severe chronic neutropenia, and cyclic neutropenia when serious infections are involved.
   - If the condition is mild, with only neutropenia without a serious infection, filgrastim may be withheld.

5. Granulocyte transfusions have undergone a cycle of popularity followed by disfavor, although they may be useful in patients with life-threatening infections whose conditions are not responding to antibiotics.
   - These transfusions are accompanied by many complications, including severe febrile reactions.
   - The use of granulocyte transfusions remains controversial.

6. In cases caused by heavy metals such as gold, chelation with British anti-Lewisite (dimercaprol) may be needed.

**Surgical Care**

1. Surgical care is generally not indicated for patients with agranulocytosis.

2. Systemic lupus associated with autoimmune agranulocytosis may respond to splenectomy. 

3. Splenectomy has also been used in Felty syndrome, but the response is often short lived.
Consultations

1. Patients with agranulocytosis are seriously ill, and several consultations are indicated.
2. A hematologist reviews the bone-marrow slides and peripheral blood smears to confirm the diagnosis and to assist in G-CSF dosing and evaluation.
3. An infectious disease specialist advises and assists in the selection of appropriate antibiotics.

Diet

1. All foods must be thoroughly cooked. Raw fruits and vegetables may contain large numbers of bacteria and should be avoided.
2. In patients with periodontitis and stomatitis, a soft or full liquid diet is indicated. Spicy and acidic foods should be avoided until recovery is complete.

Activity

Patient activity is permitted as tolerated.

Medication

Antibiotics are used to treat infections. The antibiotics of choice are those shown by culture and sensitivity studies to be the most effective for the organism causing the infection. If no causative organism is identified, use empirical broad-spectrum antibiotic coverage. Granulocyte growth factors and general supportive care should also be provided. Cytokines (growth factors) are used to stimulate production of neutrophils by acting on precursor cells.

Colony-Stimulating Factors

Colony-stimulating factors are used to stimulate production of neutrophils by acting on precursor cells.
Filgrastim (Neupogen)


Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. NEUPOGEN® is the Amgen Inc. trademark for Filgrastim, which has been selected as the name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF).

NEUPOGEN® is a 175 amino acid protein manufactured by recombinant DNA technology. NEUPOGEN® is produced by Escherichia coli (E coli) bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. NEUPOGEN® has a molecular weight of 18,800 daltons. The protein has an amino acid sequence that is identical to the natural sequence predicted from human DNA sequence analysis, except for the addition of an N-terminal methionine necessary for expression in E coli. Because NEUPOGEN® is produced in E coli, the product is nonglycosylated and thus differs from G-CSF isolated from a human cell.

NEUPOGEN® is a sterile, clear, colorless, preservative-free liquid for parenteral administration containing Filgrastim at a specific activity of $1.0 \pm 0.6 \times 10^8$ U/mg (as measured by a cell mitogenesis assay). The product is available in single use vials and prefilled syringes. The single use vials contain either 300 mcg or 480 mcg Filgrastim at a fill volume of 1.0 mL or 1.6 mL, respectively. The single use prefilled syringes contain either 300 mcg or 480 mcg Filgrastim at a fill volume of 0.5 mL or 0.8 mL, respectively. See table below for product composition of each single use vial or prefilled syringe.

What are the possible side effects of filgrastim (Neupogen)?

Get emergency medical help if you have any of these signs of an allergic reaction: hives; difficulty breathing; swelling of your face, lips, tongue, or throat.

Stop using filgrastim and call your doctor at once if you have a serious side effect such as:
• sudden or severe pain in your left upper stomach spreading up to your shoulder;
• rapid breathing or feeling short of breath; or
• signs of infection such as fever, chills, sore throat, flu symptoms, easy bruising or bleeding (nosebleeds, bleeding gums), loss of appetite, nausea and vomiting, mouth sores, unusual weakness.

Less serious side effects may include:
• diarrhea, constipation;
• bone pain;
• muscle aches;
• hair loss;
• headache, tired feeling;
• mild skin rash; or
• itching, swelling, or redness where the medicine was injected.

SIDE EFFECTS: Aching in the bones and muscles may occur. Taking a non-aspirin pain reliever such as acetaminophen may help with this pain. Ask your doctor or pharmacist for more details. Nosebleeds or injection site reactions such as
redness, swelling, itching, lumps or bruising may also occur. If any of these effects persist or worsen, notify your doctor or pharmacist promptly.

Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects.

Tell your doctor immediately if any of these rare but very serious side effects occur: easy bleeding/bruising, bloody urine, bloody vomit, fast/irregular heartbeat, fever, muscle pain, joint pain, fast breathing, trouble breathing.

Rarely, possibly fatal damage to the spleen may occur. Seek immediate medical attention if you experience the following side effects: stomach/abdominal pain, and/or shoulder pain.

A very serious allergic reaction to this drug is unlikely, but seek immediate medical attention if it occurs. Symptoms of a serious allergic reaction may include: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

PRECAUTIONS: Before using filgrastim, tell your doctor or pharmacist if you are allergic to it; or to other medications made in a similar manner (biotechnology-produced proteins using E. coli); or if you have any other allergies. This product may contain inactive ingredients (such as dry natural rubber/latex in the needle cover on the prefilled syringe), which can cause allergic reactions or other problems. Talk to your pharmacist for more details.

Before using this medication, tell your doctor or pharmacist your medical history, especially of: sickle cell disease, heart disease, spleen problems, other blood disorders (e.g., myelodysplastic syndrome, congenital neutropenia), certain skin disorders (e.g., psoriasis).

If you are scheduled to have radiation therapy, tell your doctor you are taking filgrastim. This medication should not be given during the time you are receiving radiation therapy.

This medication should be used only when clearly needed during pregnancy. Discuss the risks and benefits with your doctor.
It is not known whether this drug passes into breast milk. Consult your doctor before breast-feeding.

**Adult**

5 mcg/kg SC qd; titrate to effect; continue until ANC = 1000/µL; continuous administration may be required for chronic conditions

**Pediatric**

Administer as in adults.

**Pegfilgrastim (Neulasta)**

Long-acting filgrastim created by the covalent conjugate of recombinant G-CSF (ie, filgrastim) and monomethoxypolyethylene glycol. As with filgrastim, acts on hematopoietic cells by binding to specific cell-surface receptors, activating and stimulating production, maturation, migration, and cytotoxicity of neutrophils.

Neulasta (pegfilgrastim) is a covalent conjugate of recombinant methionyl human G-CSF (filgrastim) and monomethoxypolyethylene glycol. Filgrastim is a water-soluble 175 amino acid protein with a molecular weight of approximately 19 kilodaltons (kD). Filgrastim is obtained from the bacterial fermentation of a strain of E coli transformed with a genetically engineered plasmid containing the human G-CSF gene. To produce pegfilgrastim, a 20 kD monomethoxypolyethylene glycol molecule is covalently bound to the N-terminal methionyl residue of filgrastim. The average molecular weight of pegfilgrastim is approximately 39 kD.

Neulasta is supplied in 0.6 mL prefilled syringes for subcutaneous injection. Each syringe contains 6 mg pegfilgrastim (based on protein weight) in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), polysorbate 20 (0.02 mg), sodium (0.02 mg), and sorbitol (30 mg) in Water for Injection, USP.

Get emergency medical help if you have any of these **signs of an allergic reaction**: hives; difficulty breathing; swelling of your face, lips, tongue, or throat. Stop using pegfilgrastim and call your doctor at once if you have a serious side effect such as:
• sudden or severe pain in your left upper stomach spreading up to your shoulder;
• severe dizziness, skin rash, or flushing (warmth, redness, or tingly feeling);
• rapid breathing or feeling short of breath;
• signs of infection such as fever, chills, sore throat, flu symptoms, easy bruising or bleeding (nosebleeds, bleeding gums), loss of appetite, nausea and vomiting, mouth sores, unusual weakness; or
• bruising, swelling, pain, redness, or a hard lump where the injection was given.

Less serious side effects may include:
• bone pain;
• pain in your arms or legs; or
• bruising, swelling, pain, redness, or a hard lump where the injection was given.

SIDE EFFECTS: Bone pain may occur. Taking a non-aspirin pain reliever such as acetaminophen may help with this pain. Ask your doctor or pharmacist for more details. Injection site reactions such as redness, swelling, itching, lumps, or bruising may also occur. If any of these effects persist or worsen, notify your doctor promptly.

Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects.

Get medical help right away if any of these rare but very serious side effects occur: breathing problems (e.g., trouble breathing, shortness of breath, fast breathing).

Rarely, possibly fatal damage to the spleen may occur. Get medical help right away if you experience the following side effects: stomach/abdominal pain and/or shoulder pain.

A very serious allergic reaction to this drug is unlikely, but get medical help right away if it occurs. Symptoms of a serious allergic reaction may include: rash,
fast heartbeat, itching/swelling (especially of the face/tongue/throat), severe
dizziness, trouble breathing.

PRECAUTIONS: Before using pegfilgrastim, tell your doctor or pharmacist
if you are allergic to it or to filgrastim; or to other medications made in a similar
way (biotechnology-produced proteins using E. coli); or if you have any other
allergies. This product may contain inactive ingredients (such as dry natural
rubber/latex in the needle cover on the prefilled syringe), which can cause allergic
reactions or other problems. Talk to your pharmacist for more details.
Before using this medication, tell your doctor or pharmacist your medical history,
especially of: sickle cell disease, spleen problems.

If you are scheduled only for radiation therapy, pegfilgrastim may still help
your body fight off infections. Discuss the risks and benefits with your doctor.

This medication should be used only when clearly needed during pregnancy.
Discuss the risks and benefits with your doctor.

Adult
6 mg SC once

Pediatric
<45 kg: Not established>45 kg: Administer as in adults.

Further Inpatient Care
- If septic shock occurs, move the patient to the intensive care unit
  (ICU).
- Intubation may be required.

Further Outpatient Care
- Observation and CBC monitoring at increasing intervals of patients
  with agranulocytosis after recovery

Transfer
- If septic shock occurs, the patient should be transferred to the ICU.

Deterrence/Prevention
1. Caution patients to avoid any drug that was previously implicated in
   causing them agranulocytosis.
2. When prescribing new drugs to a patient with a history of a relatively high incidence of associated agranulocytosis, frequently obtain CBCs in the initial period.
   - The exact frequency depends on the specific drug and the time course of neutropenia association.
   - At the first sign of a drop in the ANC, the drug should be discontinued.

**Complications**
- A metastatic abscess formation may result from infections, even if the infection was successfully resolved.

**Prognosis**
- If treated promptly and vigorously, patients with drug-induced agranulocytosis have a good prognosis.
- Agranulocytosis secondary to viral infections is usually self-limited, and patients with such conditions have a good prognosis.

**Patient Education**
- Patients should be educated to avoid drugs that have caused them agranulocytosis.
- Patients should be educated about the importance of follow-up CBC testing when a new drug with a high propensity to cause neutropenia is introduced.
- In the workplace, people must be educated to follow regulations from the Occupation Safety and Health Administration (OSHA) that cover safety precautions when they deal with toxic substances.

**Miscellaneous**

**Medicolegal Pitfalls**
- Failure to appropriately monitor blood cell counts
- Failure to administer appropriate antibiotics
- Failure to diagnose leukemia or other life-threatening diseases that may have a similar presentation

**Special Concerns**
Obtain a detailed history in patients with agranulocytosis, with particular emphasis on medication use. The inquiry must extend back in time to include discontinued medications.

Over-the-counter drugs must be included in the inquiry, because patients often do not consider these agents to be medications.

Any possible occupational or accidental exposure to toxic chemicals or physical agents must be excluded.

Agranulocytosis should be differentiated from other syndromes of bone-marrow failure, including pancytopenia and aplastic anemia. Leukemia should be excluded.

**Haemolytic crisis.**

Hemolysis is the premature destruction of erythrocytes, and it leads to hemolytic anemia when bone marrow activity cannot compensate for the erythrocyte loss. Clinical presentation depends on whether the onset of hemolysis is gradual or abrupt and on the severity of erythrocyte destruction. A patient with mild hemolysis may be asymptomatic. In more serious cases, the anemia can be life threatening, and patients can present with angina and cardiopulmonary decompensation. The clinical presentation also reflects the underlying cause for hemolysis. For example, sickle cell anemia is associated with a painful occlusive crisis (see the image below).
Peripheral blood smear with sickled cells at 1000X magnification. Image courtesy of Ulrich Woermann, MD.

**Pathophysiology**

Hemolysis is the final event triggered by a large number of hereditary and acquired disorders. The etiology of premature erythrocyte destruction is diverse and can be due to conditions such as intrinsic membrane defects, abnormal hemoglobins, erythrocyte enzymatic defects, immune destruction of erythrocytes, mechanical injury, and hypersplenism. Hemolysis is associated with a release of hemoglobin and lactic acid dehydrogenase (LDH). An increase in indirect bilirubin and urobilinogen is derived from released hemoglobin.

A patient with mild hemolysis may have normal hemoglobin levels if increased production matches the rate of erythrocyte destruction. Alternatively, patients with mild hemolysis may experience marked anemia if their bone marrow erythrocyte production is transiently shut off by viral (parvovirus B19) or other infections, resulting in uncompensated destruction of erythrocytes (aplastic hemolytic crisis, in which a decrease in the erythrocytes occurs in a patient with ongoing hemolysis). Skull and skeletal deformities can occur with a marked increase in hematopoiesis, expansion of bone in infancy, and early childhood disorders such as sickle cell anemia or thalassemia.
Polychromasia.

Spherocytes. One arrow points to a spherocyte; the other, to a normal RBC with a central pallor.

**Frequency**

International

Hemolytic anemia represents approximately 5% of all anemias.

**Mortality/Morbidity**
The overall incidence of death is low in cases of hemolytic anemia. However, older patients and patients with cardiovascular impairment are at an increased risk. Morbidity is dependent on the etiology of the hemolysis and the underlying disorder such as sickle cell anemia or malaria.

- Tachycardia and dyspnea symptoms occur when the onset of hemolysis is abrupt and the anemia is severe.
- Angina and heart failure symptoms can occur in patients with underlying cardiovascular disease and severe uncompensated hemolysis.
- **Hemosiderosis, leg ulcers, folate deficiency, and gallstones** can also occur.

**Race**

Most of the disorders that lead to hemolysis are not specific to any race.

- Sickle cell disorders are found primarily in Africans, African Americans, some Arabic peoples, and Aborigines in southern India.
- Several variants of glucose-6-phosphate dehydrogenase (G6PD) deficiency exist. The A variant is generally found in West Africans and African Americans. Approximately 10% of African Americans carry at least 1 copy of the gene for this variant. The Mediterranean variant occurs in individuals of Mediterranean descent and in some Asians.

**Sex**

- Most cases of hemolytic anemia are not sex specific.
- Autoimmune hemolytic anemia (AIHA) is slightly more likely to occur in females than in males.
- G6PD deficiency is an X-linked recessive disorder. Males are usually affected, and females are carriers.

**Age**

- Hemolytic anemia can occur in persons of any age.
- Hereditary disorders are usually evident early in life.
- Autoimmune hemolytic anemia is more likely to occur in middle-aged and older individuals.
Clinical

Symptoms of hemolytic anemia are diverse and are due to the anemia, the extent of compensation, previous treatment, and the underlying disorder.

- Patients with minimal or long-standing hemolytic anemia can be asymptomatic, so hemolysis is often found incidentally during routine laboratory testing.
- In intravascular hemolysis, iron deficiency due to chronic hemoglobinuria can exacerbate anemia and weakness.
- Tachycardia, dyspnea, angina, and weakness occur in patients with severe anemia. Cardiac function is sensitive to anoxia. Angina and evidence of cardiac decompensation occurs if anemia is severe or if the onset is rapid.
- Gallstones may cause abdominal pain. Bilirubin stones can develop in patients with persistent hemolysis.
- Bronze skin color and diabetes result from hemochromatosis due to multiple transfusions or erroneously administered iron therapy.
- Hemoglobinuria produces dark urine. It can occur in patients with intravascular hemolysis and has similar results to a transfusion of ABO-incompatible blood.
- Patients with thrombotic thrombocytopenic purpura (TTP) may experience fever, neurologic signs, renal failure, petechiae, and hemolysis because of the widespread occlusion of small vessels.
- Leg ulcers may develop in patients with sickle cell anemia and other hemolytic disorders as a result of decreased red blood cell deformity and endothelial changes.
- Penicillin, quinine, quinidine, L-dopa, and other agents may cause immune hemolysis (see Medical Care).
- Oxidant drugs (see Diet) and stress from infections can trigger hemolysis in patients with G6PD deficiency.
- Fava beans can induce hemolysis in susceptible individuals with the Mediterranean variant of G6PD deficiency.
A patient who needs a transfusion but does not show evidence of blood loss or bone marrow suppression may have hemolytic anemia.

**Physical**

The physical examination in an individual with hemolytic anemia can reveal signs of anemia, erythrocyte destruction, complications of hemolysis, and evidence of an underlying disease.

1. General pallor and pale conjunctivae and fingernails indicate anemia but are not specific for hemolytic anemias.
2. Tachycardia, tachypnea, and hypotension due to anoxia and decreased vascular volume usually occur in severe anemias but are not specific for hemolytic anemias.
3. Jaundice may occur because of a modest increase in indirect bilirubin in hemolysis. The rise is not specific for hemolytic disorders and may occur in liver disease, biliary obstruction, and hereditary liver disorders. Bilirubin levels are rarely greater than 4 mg/dL in hemolysis unless complicated by hepatic disease or cholelithiasis.
4. Splenomegaly
   - Splenomegaly occurs in hereditary spherocytosis and other hemolytic anemias, but it is not present in other hemolytic disorders such as G6PD deficiency.
   - The presence of splenomegaly suggests underlying disorders such as chronic lymphocytic leukemia (CLL), some lymphomas, and systemic lupus erythematosus (SLE).
5. Leg ulcers
6. Right upper abdominal quadrant tenderness may indicate gallbladder disease.
7. Bleeding and petechiae indicate thrombocytopenia due to Evans syndrome or thrombotic thrombocytopenic purpura if neurologic signs are also present.
8. Butterfly malar rash and arthritis suggest SLE.
9. **Lymphadenopathy with splenomegaly may indicate an underlying chronic CLL.**

**Causes**

More than 200 causes for hemolysis exist. Only the main categories and some examples of hemolytic disorders are considered in this article.

1. Hereditary disorders include erythrocyte membrane and enzymatic defects and hemoglobin abnormalities. Some hereditary disorders include the following:
   - G6PD deficiency
   - Hereditary spherocytosis
   - Sickle cell anemia

2. Acquired hemolytic conditions can be due to immune disorders, toxic chemicals and drugs, antiviral agents (eg, ribavirin) physical damage, and infections. They can include the following:
   - Autoimmune hemolytic anemia (AIHA) may result from warm or cold autoantibody types; rarely, mixed types occur. Most warm autoantibodies are immunoglobulin (Ig) G and can be detected with the direct Coombs test, which is also known as the direct antiglobulin test (DAT).
   - AIHA may occur after allogeneic hematopoietic stem cell transplantation. The 3-year cumulative incidence in this population has been reported at 4.44%.
   - Microangiopathic anemia is found in patients with disseminated intravascular coagulation (DIC) or hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. Fragmented erythrocytes (schistocytes) also occur with defective prosthetic cardiac valves.

3. Autoimmune hemolytic anemia and hereditary spherocytosis are classified as examples of extravascular hemolysis because the red blood cells are destroyed in the spleen and other reticuloendothelial organs.

4. Intravascular hemolysis occurs in hemolytic anemia due to prosthetic cardiac valves, G6PD deficiency, thrombotic thrombocytopenic purpura,
disseminated intravascular coagulation, and paroxysmal nocturnal hemoglobinuria (PNH).

HEMOLYTIC ANEMIAS

Hemolytic anemias are a group of hereditary and acquired diseases, characterized by an increase in the rate of intravascular or extravascular RBC destruction. Intravascular hemolysis is lysis of RBC in the circulation and release of their contents into plasma; whereas extravascular hemolysis occurs in the RES, especially the spleen.

Criteria of hemolysis

1. Elevated reticulocyte count in the peripheral blood (more than 5%);
2. Significant normoblastosis in the myelogram;
3. Raised serum unconjugated (indirect) bilirubin;
4. Raised serum iron (except PNH);
5. Elevated plasma LDH;
6. Shortened RBC life span detected by radioactive chromium (Cr), technetium (Tc) and indium (In).

HEREDITARY HEMOLYTIC ANEMIAS

HEREDITARY MICROSpherOCYTOSIS

Hereditary microspherocytosis (syn: Minkowski-Chauffard’s disease, hereditary microspherocytic hemolytic anemia, congenital spherocytic anemia,
**congenital hemolytic jaundice** is a common type of hemolytic anemia with a genetic defect of the RBC membrane contributing to hemolysis. It is inherited in an autosomal dominant manner, but in most cases neither parent is affected and it is presumed that this disease has occurred by spontaneous mutation.

**Pathogenesis**

Impairment of protein structure (spectrin) leads to alteration of cell membrane and structure. There is an increased permeability of the cell membrane, through which a great number of sodium ions and water enter the cells. As a result, the red cells change their forms and are not capable of squeezing through splenic microcirculation, for example, in the splenic sinuses. They are entrapped and later engulfed by macrophages in the spleen. This is so-called extravascular (intracorpuscular) hemolysis.

**Clinical features**

1. Anemia is the first clinical sign in children.
2. Jaundice, splenomegaly, pain in the left hypochondrium, sometimes hepatomegaly, pigment gallstones (due to chronic hemolysis) and symmetric ulcers on the leg.
4. Periodically hemolytic crises (provoked by infections): pain in the liver, spleen, hepatosplenomegaly, chills, fever up to 39-40°C, vomiting, leucocytosis with shift-to-left, normocytes or normoblasts in blood and dark urine.
5. Aplastic crisis provoked by parvovirus - aregenerative anemia develops rapidly and may lead to lethal outcome. If survival occurs, immunity of the patients is usually compromised.
6. Megaloblastic anemia occurs as a result of folic acid depletion owing to the bone marrow hyperactivity.
**Investigations**

1. **CBC** – normochromic, microspherocytic, hyperregenerative anemia, possible sign of hypersplenism. For example, RBC - 2.1 x $10^{12}$/L, Hb - 68 g/L, CI-1.0, reticulocytes - 5.6 %, thrombocytes-190 x $10^{9}$/L, WBC - 6.1 x $10^{9}$/L: eosinophils - 1, stab cells - 4, segmented cells - 60, lymphocytes - 30, monocytes - 5, ESR - 12mm/Hr, anisocytosis ++++, Ht - 30 %. **microspherocytes.**

2. Osmotic fragility is increased or osmotic resistance is decreased (N: minimal hemolysis 0.46-0.48% NaCl, maximal hemolysis 0.32-0.34% NaCl). For example, min-0.64%, max-0.42% NaCl.

3. Bone marrow: stimulation of the erythroid cell line (accumulation of erythroblasts and normocytes).

4. Rise in serum bilirubin and plasma LDH.

5. Raised urine urobilinogen and fecal stercobilinogen (pleochromia).

6. Test for shortened red cell life span with the aid of radioactive Cr (life span is shortened to 22-30 days in moderate hemolysis and to 5-6 days in severe hemolysis).

7. Skull X-ray shows the characteristic “hair on end” appearance of bony trabeculation as a result of expansion of the bone marrow into cortical bone.

**Differential diagnosis**

It is made with AIHA and aplastic anemia in aplastic crisis.

**Management**

1. Splenectomy is encouraged in the following cases:
   - In those aged 7-8 years and more;
   - Severe anemia with hemolytic crises, hypersplenism and splenomegaly;
   - Secondary gallstone disease (together with cholecystectomy);
   - Persistent jaundice.

In the past, splenectomy, which has a low operative mortality, was considered routine in patients with hereditary microspherocytosis. However, the risk of
overwhelming post-splenectomy infection (OPSI) and the recent emergence of penicillin-resistant pneumococci have led to a reevaluation of the role of splenectomy in the treatment of this disease. Antipneumococcal vaccination is carried out before this procedure.

2. Transfusion of RBC mass is carried out in extremely severe anemia, severe hemolytic crises and severe anemia (when splenectomy is contraindicated).

3. In aplastic crisis, human Ig is injected intravenously 1-2g/day for five days.

4. Antiplatelet agents and anticoagulants for prevention of thrombosis (Flebodia 600®) (Diovenor).

**SOUTHEAST ASIAN OVALOCYTOSIS**

Southeast Asian ovalocytosis (syn: Melanesian elliptocytosis, stomatocytic elliptocytosis) is a dominantly inherited disease, characterized by the presence of oval RBC. This disease is widespread in parts of Southeast Asia, predominantly Malaysia, Papua New Guinea, the Philippines, and Indonesia. Numerous abnormalities of Southeast Asian ovalocytosis erythrocytes have been reported, including increased red cell rigidity, decreased osmotic fragility, increased thermal stability, resistance to shape change by echinocytic agents, and a reduced expression of many red cell antigens. All these conditions are believed to be caused by the underlying defect in band 3 protein, the major transmembrane protein, which has abnormal structure and function. Of note, Southeast Asian ovalocytosis confers resistance against Plasmodium falciparum and Plasmodium knowlesi infection likely because of alterations in band 3, which is one of the malaria receptors.

**Clinical features**

Most patients are asymptomatic and do not have any obvious physical signs. Patients with clinically significant hemolysis have splenomegaly, pallor, scleral icterus, and (in rare cases) leg ulcers. Southeast Asian ovalocytosis is often associated with renal tubular acidosis.
Investigations
1. Microscopic examination of peripheral smears reveals ovalocytes. Fragmented red cells may also be found;
2. Signs of hemolysis: low haptoglobin levels, high reticulocyte count, raised LDH, indirect bilirubin and urobilinogen;
3. Decreased osmotic fragility.

Differential diagnosis
It is made with the following diseases:
• G6PD deficiency;
• Iron deficiency anemia;
• Megaloblastic anemia;
• MDS;
• Myeloproliferative diseases;
• Pyruvate kinase deficiency;
• Sickle cell anemia;
• Hereditary spherocytosis.

Management
Splenectomy is the best approach to fight against anemia in patients with clinically significant hemolysis. The pneumococcal, meningococcal, and Haemophilus influenzae vaccines should be administered before this operation.

HEREDITARY HEMOLYTIC ANEMIA WITH RED CELL ENZYME DEFECTS (ENZYMOPATHY) GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY ANEMIA

G6PD is the first enzyme in the hexose monophosphate shunt, which oxidizes glucose-6-phosphate to 6-phosphoglycerate with the reduction of NADP to NADPH. This reaction is very important in RBC because it is the only source of NADPH, which is used via glutathione to protect them from any oxidative damage.
In G6PD deficiency anemia, this protective mechanism is deranged, leading to hemolysis. G6PD deficiency anemia is a sex-linked disease, which affects Africans (type A), Caucasians (type B) and eastern countries (Favism). This disease is widespread in the Mediterranean, Iran, Iraq, Azerbaijan, Africa, Latin America and Dagestan.

**Etiology**

1. Drugs: antimalarials (quinine, primaquine, chloroquine), antibacterials (sulfonamides, dapsone, nitrofurantoin, furazolidone, chloramphenicol), NSAIDs (aspirin, amidopyrine), vikasol, probenecid, dimercaprol (syn: BAL), nalidixic acid.
2. Ingestion of fava beans.
3. Infections, acidosis in diabetes mellitus or renal failure.

**Pathogenesis**

G6PD deficiency leads to reduced levels of glutathione in RBC. This results in oxidation and precipitation of Hb within the red cells forming Heinz bodies.

**Clinical features**

Manifestations usually appear 2-3 days after intake of above-mentioned drugs:

- Dark urine due to hemoglobinuria;
- Fever;
- Agonizing headache, pain in extremities, vomiting;
- Breathlessness, hypotension;
- Splenomegaly and sometimes hepatomegaly;
- Rarely acute renal failure with anuria.

**Investigations**

1. CBC – normochromic, hyperregenerative anemia, Heinz body, leucocytosis with shift-to-left, myelocytes, free hemoglobin.
2. Methemoglobinemia.
3. Haptoglobinemia.
5. Elevated serum bilirubin and plasma LDH.
6. Hemosiderin in urine, hemoglobinuria.
In favism, clinical signs develop in few hours (till 2 days). Symptoms of acute hemolysis include dyspepsia and renal failure.

G6PD deficiency anemia is differentiated with AIHA in negative direct Coombs’ test.

**Management**
1. Offending drugs and toxic agents should be ceased.
2. Drugs restoring glutathione: riboflavin 0.015g 2-3times/day intramuscularly, ksilit 5-10mg 3times/day.
3. Antioxidant drugs (Erevit®).
4. NaHCO3 4-5% - 200-400 ml intravenously in drops.
5. Mannitol 1g/kg in 10% of solution.
6. Lasix® 40-60mg intravenously.
7. Hemodialysis, peritoneal dialysis.
8. Blood transfusion is life saving.
9. Splenectomy is not effective.

**SICKLE CELL ANEMIA**

Sickle cell anemia is a severe type of hereditary anemia with production of sickle-shaped RBC. It is a homozygous state of sickle cell Hb (Hb S), in which an abnormal gene is inherited from each parent. HbS occurs due to a single-base mutation of adenine to thymine producing a substitution of valine for glutamine at the 6th position of the β-globin chain.

**Pathogenesis**

Deoxygenated HbS molecules are insoluble and polymerized. The flexibility of RBC is reduced and they become more rigid and take out characteristic sickle appearance. Sickling of RBC increases blood viscosity, produces a shortened red cell survival and impairs passage of cells through the microcirculation contributing to tissue hypoxia and infarction.

This disease is mainly found in the central Africa, India, Turkey, Iran, Iraq, Azerbaijan and Algeria.
**Clinical features**

Patients suffering from sickle cell anemia may have the following features: infantilism, hypogonadism, “turreted skull” and distorted spine. Patients may also complain of pain and swelling of the joints. Osteoporosis of the skull and spine, thrombo-necrotic injury to the bones and pathological fractures, fat embolism, dactylitis, chronic ulcers on the extremities, ischemia of the myocardium and kidneys, priapism and CNS disorders are also important signs.

In homozygous form, the disease commences several months after birth. In heterozygous form, the disease occurs after provocations such as pregnancy, delivery, physical stress, infections and hypoxia.

**Four types of crises are distinguished:**

1. Hemolytic crisis due to intravascular hemolysis (dark urine, sharp reduction of Hb level and fever);
2. Thrombocytic crisis: thrombosis in the vessels leading to tenderness and swelling of the joints of the hands, feet, legs, aseptic necrosis of the heads of the humerus and femur (so called “African rheumatism”); infarcts involving spleen, lungs, kidneys, abdomen and skin;
3. Aplastic crisis occurs after severe infections (bone marrow disorder of erythrokaryocytes);
4. Acute sequestration in which a significant number of erythrocytes are destroyed in the internal organs, contributing to development of shock.

**Investigations**

1. CBC – normochromic and hyperregenerative anemia. Sickling of red cells can be better revealed in the presence of sodium metabisulphite. In hemolytic crisis – severe anemia, leucocytosis with neutrophilic shift and thrombocytosis.
2. Rise in serum bilirubin.
3. Urobilinuria; in macroinfarcts involving the kidneys – hematuria.
5. Ultrasound imaging: hepatosplenomegaly, gallstones.
6. X-ray of the bones: “hair on end” appearance of the skull and osteoporosis of the long bones.
Management

1. Prevention of hypoxia and dehydration.
2. Prophylaxis of infectious complications.
3. Antiplatelet therapy.
4. Treatment of hemolytic crisis:
   • Hemodilution: rheopolyglucin 400-800ml, isotonic solution 1000ml;
   • Stimulation of diuresis: Lasix® 80mg, repeat every 2-4 hours in 40-80mg;
   • NaHCO3 4-5% - 200-400 ml intravenously in drops (to fight against metabolic acidosis).
   • Transfusion of red cell mass (in sharp reduction of Hb level and reticulocyte count, infarction, relapses of attacks and trophic ulcers on the legs).
5. Oral folic acid 1mg 1 time daily.

**THALASSEMIA**

Thalassemia (Greek thalassa, sea) is a hereditary homozygous or heterozygous hemolytic hemoglobinopathy, characterized by microcytic, hypochromic, and a shortened life span of RBC caused by defective synthesis of globin chains (α or β) and decreased synthesis of hemoglobin A.

It is an autosomal dominant hemolytic anemia. The thalassemias are the commonest monogenic diseases in Man. They occur at a high gene frequency throughout the Mediterranean populations, the Middle East, the Indian subcontinent, and Burma and in a line stretching from southern China through Thailand and the Malay peninsula into the island populations of the Pacific. In Thailand there are approximately 600 000 affected individuals and more than 20 million carriers in the population of 60 million.

A normal adult Hb consists of a heme and two polypeptide globin chains, i.e. Hb A (95%) – α and β, Hb A2 (3.5%) – α and δ, Hb F (1-1.5%) – α and γ. In patients with thalassemia, there is defective synthesis of one or few globin chains. This causes excessive production of unpaired globin chains and leads to formation of an insoluble tetramer, i.e. destruction of RBC membrane.
Classification

According to globin chain deficiency, thalassemia is classified into α-thalassemia and β-thalassemia. Severity of this disease depends on the type of inheritance – homozygous or heterozygous. Table 5 shows the types of thalassemia, subtypes and their clinical features.

Clinical features

In homozygous form, clinical manifestations are observed at the end of the first or second year of life:

- Anemia;
- Jaundice, pallor, marked hepatosplenomegaly, gallstones, hemochromatosis (liver cirrhosis, diabetes mellitus, hypofunction of the reproductive organs, cardiосclerosis, skin pigmentation) due to persistent hemolysis;
- Skeletal changes due to bone marrow hyperplasia: quadrangular skull, thickened nasal bridge, protruding zygomatic bone, narrow eye slit, physical growth retardation;
- Synovitis of the large joints without increased local temperature and deformation;

Investigations

1. CBC – hypochromic, hyperregenerative anemia, basophilic stippling, microcytosis, target cells, Howell-Jolly bodies. For example, RBC-3.0 x 10¹²/L, Hb-90g/L, CI-0.9, reticulocytes-14%, thrombocytes-180 x 10⁹/L, WBC-2.8 x 10⁹/L: eosinophils-2, stab cells-3, segmented cells-67, lymphocytes-26, monocytes-2, ESR-22mm/Hr, Ht-29%, target cells, Heinz body, Howell-Jolly bodies.
2. Hemoglobin electrophoresis reviews reduced amounts of Hb A and elevated amounts of Hb F.
3. Decreased osmotic fragility or increased osmotic resistance of RBC. For example, minimal hemolysis is less than 0.46% and maximal hemolysis is less than 0.32% in NaCl.
4. Bone marrow aspirate examination shows normoblastic erythroid hyperplasia.
5. Raised serum bilirubin (unconjugated).
6. Desferal test shows an increased level of iron storage (inject intramuscularly 500mg of Desferal, N:0.6-1.3mg iron is excreted in urine in 24 hours; in thalassemia, this level is increased)

7. In β-thalassemia – marked increased of Hb F and Hb A2. In α-thalassemia, their level is not altered. It is necessary to confirm the diagnosis by a study of globin biosynthesis in vitro in reticulocytes.

8. Ultrasound imaging and X-ray examination: hepatosplenomegaly, gallstones, osteoporosis of the long bones, “hair on end” appearance of the skull (see photo № 6 on the last page of the cover).

Differential diagnosis

It is necessary to differentiate heterozygous thalassemia with:

- **Iron deficiency anemia**, in which low serum iron, unchanged serum bilirubin level and marked stimulation of the erythroid cell lines in myelogram with an elevated reticulocyte count are usually found.
- **AIHA**, in which there is no hemochromatosis but with positive direct Coombs’ test.
- **Hereditary microspherocytosis** – normochromic anemia and microspherocytosis.

Management

1. Transfusion of red cell mass since childhood. At the beginning, bolus therapy is administered (8-10 transfusions in 2-3 weeks), and then (20 ml/kg in every 3-4 weeks).

2. Desferal 0.5-1.0 g/day + ascorbic acid 250-500 mg intravenously in drops with the speed 15 mg/kg/hour.

3. Splenectomy is indicated in marked splenomegaly, signs of hypersplenism and hemoglobin H disease. Antipneumococcal vaccination is carried out before this procedure.

4. Bone marrow transplantation – in first few days of life in homozygous form of thalassemia.

In majority cases, prognosis is unfavorable. Patients of thalassemia major rarely live up to 20 years. Prognosis is more favorable in thalassemia minor.
HEMOGLOBIN E DISEASE

Hemoglobin E (E is embryonic or epsilon) is a variant hemoglobin with a mutation in the β globin gene (α2β226glu→lys) causing substitution of glutamic acid for lysine at the position 26 of the β globin chain. HbE is the second commonest abnormal hemoglobin after sickle cell hemoglobin (HbS). HbE is common in Thailand, Cambodia, Laos, Sri Lanka, Bangladesh, Pakistan, Nepal, Vietnam and Malaysia.

Homozygous Hb E disease causes a mild hemolytic anemia, usually without splenomegaly. Heterozygotes (Hb AE) are asymptomatic. Patients with combined heterozygous Hb E and β-thalassemia are more severe than those with S-thalassemia or homozygous Hb E disease and usually have splenomegaly.

Clinical features

HbE trait is an asymptomatic condition with no clinical relevance, except for the risk of compound heterozygous states with β thalassemia in the offspring.

Investigations

1. CBC: Mild anemia, reduced MCV and MCH. Blood film may be normal or may show hypochromic, microcytosis, target cells, basophilic stippling or any combination of these features.

2. Electrophoresis: Hemoglobin electrophoresis at alkaline pH on cellulose acetate shows that the variant HbE has the same mobility than that of the variant HbC and the adult hemoglobin HbA2.

Management

1. During childhood, regular follow-up of growth and facial deformities, hemoglobin level, prophylaxis of infections with vaccines, treatment of potential infectious sites are essential. Daily oral penicillin is recommended.

2. Transfusions are indicated in case of bad tolerance of anemia and facial deformities. If iron overload is developed after prolonged transfusions, chelation therapy (Desferal®) is administered.

3. Splenectomy can diminish or suppress transfusion requirement. It is performed only after five years old.
4. Recombinant erythropoietin or associated with hydroxyurea may be useful in reducing transfusions requirements, improving quality of life and diminishing hematopoietic ectopic extramedullary masses.

**Quired immune hemolytic anemias**

These anemias are characterized by presence of antibodies against RBC or erythrokaryocytes causing their destruction. The following forms are distinguished:

- **Isoimmune hemolytic anemia** – isoantibodies or isoantigens enter the organism from the external environment (hemolytic disease of newborn, transfusion of incompatible blood groups);

- **Transimmune hemolytic anemia** – formation of antibodies in the newborn, whose mother suffers from AIHA;

- **Heteroimmune hemolytic anemia** – formation of antibodies against haptens. These haptens may be drugs (penicillin, sulfonamides and etc.) or viruses;

- **Autoimmune hemolytic anemia** – formation of autoantibodies against patients’ own RBC antigens.

**AUTOIMMUNE HEMOLYTIC ANEMIA**

Autoimmune hemolytic anemia (AIHA) is characterized by hemolysis due to production of antibodies against RBC antigens. It is an acquired disease.

**Etiology**

- Idiopathic: Etiology is unknown.

- Symptomatic: There is an underlying disease causing AIHA, e.g. autoimmune hepatitis, SLE, autoimmune thyroiditis (syn: Hashimoto’s thyroiditis), CLL, AIDS and others.

**Types of antibodies** may be the following:

1. AIHA with incomplete warm agglutinins;

2. AIHA with warm hemolysins;

3. AIHA with complete cold agglutinins;

4. AIHA with biphasic cold hemolysins;
Warm antibodies are activated at body temperature (37°C); whereas cold antibodies – at 4°C).

**Clinical features**

They include symptoms of anemia (pallor, weakness, dyspnea, signs of myocardial dystrophy), syndrome of hemolytic jaundice (jaundice, hepatomegaly, dark urine and feces) and signs of intravascular hemolysis (dark urine, hemoglobinuria, complications of thrombosis). Very often, patients may complain of asthenic vegetative syndrome.

In some cases, the disease develops rapidly with manifestations of hemolytic crisis (weakness, pain in the lumbar and heart regions, breathlessness, fever, and rapidly-developed jaundice). In other cases, the disease progresses with a chronic duration with periods of exacerbations and remissions.

**Clinical manifestations** depend on the character of antibodies. Complete cold antibodies cause agglutination of RBC in the peripheral vessels at cold temperature (characteristic of Raynaud’s phenomenon and cold intolerance). Incomplete antibodies derange functions of RBC enzymes and alter permeability of cell membranes to sodium chloride (may cause microspherocytosis). Hemolysins cause RBC destruction immediately in the vessels (signs of intravascular hemolysis – dark urine, hemoglobinuria and complications of thrombosis).

**Investigations**

1. CBC – Normochromic, hyperregenerative anemia, increased ESR, anisocytosis, poikilocytosis; in crisis – leucocytosis with shift-to-left. Positive indirect Coombs’ test.

2. Increased unconjugated bilirubin. In intravascular hemolysis – elevated (free) hemoglobin and LDH with reduced haptoglobin.

3. Protein and urobilin are detected in urine. Free hemoglobin, sometimes hemosiderin are detected in urine in intravascular hemolysis.


5. Positive Coombs’ test. In direct Coombs’ test, agglutination of RBC is obtained after mixing patient’s RBC (with fixed autoantibodies) with immunized
antiglobulin of the sheep. In indirect Coombs’ test, patient’s serum (containing autoantibodies) is incubated with donor’s RBC and mixed with antiglobulin serum. In short, direct Coombs’ test detects antibodies fixed to RBC; indirect Coombs’ test detects antibodies in serum.


**Differential diagnosis**

**Differential diagnosis** of different anemias is listed in table.

### Differential diagnosis of different anemias

<table>
<thead>
<tr>
<th>Signs</th>
<th>Thalassemia</th>
<th>Acquired hemolytic anemia</th>
<th>Iron deficiency anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographical distribution</td>
<td>Mediterranean and Southeast Asia</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pathogenesis</td>
<td>Impaired globin synthesis</td>
<td>Formation of antibodies or toxic metabolites</td>
<td>Iron deficiency</td>
</tr>
<tr>
<td>Onset of disease</td>
<td>Childhood</td>
<td>Any age</td>
<td>Any age</td>
</tr>
<tr>
<td>Hereditary predisposition</td>
<td>Usually</td>
<td>Sometimes</td>
<td>–</td>
</tr>
<tr>
<td>Skeletal changes</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Jaundice</td>
<td>Usually</td>
<td>In marked hemolysis</td>
<td>–</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Osmotic fragility / osmotic resistance</td>
<td>Decreased / increased</td>
<td>Normal or increased / Normal or decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Elevated indirect</td>
<td>Elevated indirect</td>
<td>Normal</td>
</tr>
<tr>
<td>CI</td>
<td>Hypochromic</td>
<td>Normochromic</td>
<td>Hypochromic</td>
</tr>
<tr>
<td>Serum iron</td>
<td>Raised</td>
<td>Normal or raised</td>
<td>Reduced</td>
</tr>
<tr>
<td>Desferal test</td>
<td>+</td>
<td>- or weakly +</td>
<td>-</td>
</tr>
<tr>
<td>Hemosiderosis</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Management**

1. Patients should be admitted to hospital. Drugs causing anemia should be changed.

2. **Glucocorticoids**: in hemolytic crisis – prednisolone 1-2mg/kg/day, middle dose 60mg/day. To prevent ulcer, antacids are administered 20-30 minutes before prednisolone intake. To prevent osteoporosis, active metabolites e.g. vitamin D3 and calcium preparations are administered. Ideos (containing 400IU vitamin D3 and 500mg calcium carbonate) – 1 tablet 2 times daily. Patients with diabetes mellitus
and arterial hypertension can take this drug because it does not contain sugar and sodium. Ideos® reduces risk of fractures and pathologic compression of the spine; increases bone mass and decreases bone resorption.

3. **Splenectomy** is indicated when glucocorticoid therapy is ineffective. Effectiveness attains 66%.

4. Immunosuppressive agents (used in ineffectiveness of glucocorticoids and splenectomy, systemic diseases): azathioprine 100-150 mg/day, cyclophosphamide 400 mg every other day, vincristine 2 mg 1 time/week, Cyclosporin A 5 mg/kg/day or other cytostatics.

5. Transfusion of red cell mass – in sharp reduction of Hb until 30-40 g/L with signs of hypoxia of the brain and heart.

6. Plasmapheresis.

**Prognosis**

Prognosis is favorable in most cases. Factors worsening prognosis include age more than 60 years, shock, thromboembolism, hemorrhagic syndrome (syn: **Evans’s syndrome** and resistance to therapy.

**PAROXYSMAL NOCTURNAL HEMOGLOBINURIA** (SYN: **MARCHIAFAVA-MICHELI DISEASE**)

Paroxysmal nocturnal hemoglobinuria (syn: Marchiafava-Micheli disease) is an acquired hemolytic anemia, characterized by increased sensitivity of RBC to complement system due to defective synthesis of RBC membrane.

**Pathogenesis**

It is connected with pathologic clones of erythrocytes (owing to spontaneous mutation) resulting in membrane defect. This defect causes red cell destruction in acidosis. Hemoglobinuria is a typical sign, which predominantly occurs at night. It is because acidosis predominates at night.

**Clinical features**

1. Anemia;

2. Pallor with yellow coloration, hepatomegaly, splenomegaly;
3. In the period of crisis (usually after infections, at night) – pain in the lumbar region, chills, fever, dark urine;

**Investigations**

1. CBC – normochromic, then hypochromic and hyperregenerative anemia, in crisis – leucocytosis with shift-to-left;
2. Rise in serum iron, raised Hb level;
3. Undue sensitivity of the red cell membrane to complement demonstrated in vitro by Ham’s test (hemolysis of RBC in 0.1 % HCl solution) and sugar test (in 5 % sugar solution); 4. Raised serum unconjugated bilirubin;
5. Myelogram: stimulation of the erythroid cell line;
6. Urobilinuria, in crisis – hemoglobinuria and hemosiderinuria;
7. Raised amount of stercobilin in feces.

**Differential diagnosis**

It is made with hypoplastic anemia. In PNH, reticulocytosis, stimulation of the erythroid cell line in the bone marrow and intravascular hemolysis predominate.

**Management**

1. Transfusion of red cell mass in patients with severe condition or low Hb level; 2. Anabolic hormones (possessing antioxidant action) – nerobol 0.005 g 4 times/day for several months with control of functional condition of the liver; 3. Antioxidants – Aevit, Erevit (vitamin E preparations) 3-4 ml/day, tocopherol acetate 2 capsules daily or 30% solution 1-2 ml intramuscularly; 4. Anticoagulants, antiplatelets, Flebodia 600 (Diovenor) in thrombosis.

**APPROACH TO PATIENTS WITH ANEMIA**

Every doctor should have a meticulous plan of investigations and tactics in management of anemia. Very often, the diagnosis is made based on complaints, anamnesis and different methods of investigations. Besides anemic syndrome, doctors should find out other specific signs: sideropenic syndrome in iron deficiency anemia; paresthesia, glossalgia (syn: glossodynia), GIT
disorder in B12 deficiency anemia; changes of urine color in hemolytic anemias and porphyria; bleeding and tendency to infections in aplastic anemia; dysphagia is often met in iron deficiency anemia and PNH.

**Anamnesis:** it is necessary to question patients closely about bleeding (e.g. dark stool in intestinal bleeding), nutrition, chronic diseases, drug intakes, operations, alcoholism, narcomania, intoxications and ionizing radiations. For women, it is necessary to ask about the character of menstruation (duration, amount, regularity and interval), pregnancy, labor, abortion and others. Family history may reveal hereditary anemias.

**Physical examinations:**
- Condition of the patient – satisfactory, light anemia, anemic, cachectic;
- Position of the patient – horizontal, paralysis of lower extremities in severe B12 deficiency anemia; joint deformations and tetraparesis in porphyria; skull deformation in microspherocytosis; shortened little finger and arachnodactyly in other anemias;
- Gait of the patient – unsteadiness in funicular myelosis;
- Skin and its appendages, mucus membranes – color (bluish sclera in iron deficiency anemia), erosion, ulcer, scar, hyperpigmentation in porphyria; brittle hair and nails in iron deficiency anemia; condition of lymph nodes in secondary anemias;
- Respiratory system – degree of breathlessness;
- Cardiovascular system – tachycardia, cardiac arrhythmias, murmurs;
- GIT – angular stomatitis in iron deficiency anemia; high position of the palate in microspherocytosis; erythrodontia (red coloration of the teeth) in porphyria; glossitis, atrophy of the papillae of the tongue, dysphagia, gastritis ulcer, liver disease, gallstone, hepatomegaly, splenomegaly, hemorrhoids, stool incontinence;
- Genitourinary system – urinary incontinence;
- Nervous system – polynueiritis, paresthesia, sensitivity disorder, funicular myelosis, psychiatric disorder, mental retardation;
- Endocrine system – hypertrophy of the thyroid gland, endocrinopathies;
**Hematological values:**

- Erythrocyte count and Hb level reveal the degree of severity;
- CI – hypochromic in iron deficiency anemia; hyperchromic in megaloblastic anemia;
- MCV is decreased in iron deficiency anemia and microspherocytosis; increased in megaloblastic anemia; remains unchanged in autoimmune and aplastic anemias;
- Reticulocyte count shows regenerative function of the bone marrow. Reticulocyte count is elevated in hemolysis, after vitamin B$_{12}$ therapy; decreased in aplastic anemia;
- Platelet count may be reduced in aplastic anemia, B$_{12}$ deficiency anemia and Evans’s syndrome; raised in iron deficiency anemia;
- Leukocyte count may be reduced in B$_{12}$ deficiency anemia and aplastic anemia;
- Morphological changes of the blood cells – hypersegmentation of the neutrophils, Howell-Jolly bodies and Cabot’s ring in B$_{12}$ deficiency anemia;

**In anemia with unknown etiology,** X-ray examination of the chest, EGD, ultrasound imaging of the abdominal organs, barium enema, rectosigmoidoscopy, CT and scintigraphy may be conducted. For women, consultation with the gynecologist is compulsory. These investigations are to reveal the underlying cause (e.g. malignant neoplasm, ulcer, erosion, varicose vein, diaphragmatic hernia etc.). In women, iron deficiency anemia is often met. Megaloblastic and secondary anemias are often seen in middle-aged patients. Aplastic anemia may occur in any age.
BIBLIOGRAPHY

