HAEMATOLOGY NOTES

BLOOD FILM

Target cells are red cells with central staining with precipitated haemoglobin seen in conditions with abnormal haemoglobin as well as cell membrane.

Causes of target cells are:
Sickle cell disease
thalassaemia
iron deficiency anaemia
liver disease

Howell Jolly bodies contain nuclear remnants. Causes are**:**
Post splenectomy
Leukaemia
Megaloblastic anaemia
Iron deficiency anaemia

Heinz bodies are precipitated, denatured Hb within red cells. They are also present in G6PD deficiency. (Fava beans cause haemolysis in G6PD - 'Beans means Heinz' mnemonic).

Leuco erythroblastic picture is due to extensive infiltration of bone and may be seen in malignancies like lung cancer metastases, myeloproliferative disorders, severe vitamin deficiency and severe infections. White cell and red cell precursors are found in the bloodstream in leucoerythroblastic picture.

Reactive lymphocytes are caused by
Ebstein Barr virus infection / infectious mononucleosis
CMV infection
toxoplasmosis
HIV

The **direct antiglobulin test (Coomb's)** is positive if there is autoimmune haemolytic anaemia. It is used to detect IgG or C3 bound to the surface of the red cell. Immune causes of hemolysis including autoimmune hemolytic anemias, drug induced hemolysis, and delayed or acute hemolytic transfusion reactions are characterized by a positive DAT.

The **red blood cell enzyme assay** is a device used to measure the activity in red blood cells of clinically important enzymatic reactions and their products, such as pyruvate kinase or 2,3-diphosphoglycerate. A red blood cell enzyme assay is used to determine the enzyme defects responsible for a patient`s hereditary hemolytic anemia.

SICKLE CELL DISEASE

Sickle cell disease is due to substitution of valine for glutamic acid (position 6 of the beta chain). All forms of sickle cell include HbS.
Aseptic necrosis of the hip, cholecystitis, renal papillary necrosis and proliferative retinopathy are clinical features of sickle cell disease.
Hb SS patients sickle at 6 kPA hypoxia but SC patients at a lower pO2 of 4 kPa.

When there is neurological damage or visceral sequestration crisis in sickle cell crisis, *exchange transfusion* is indicated. Exchange transfusion involves drawing out the patient's blood while exchanging it for donor red blood cells. It can be done manually or automatically with erythrocytapheresis.

The **acute chest syndrome** in *sickle cell disease* can be defined as:

1. a new infiltrate on chest x-ray
2. associated with one or more NEW symptoms: fever, cough, sputum production, dyspnea, or hypoxia.

The most common cause of **aplastic crisis**, is parvovirus infection.
Patients with sickle cell anemia also exhibit increased susceptibility to other common infectious agents, including Mycoplasma pneumoniae, Staphylococcus aureus, and Escherichia coli.

THALASSEMIA

Mutations in globin genes cause **thalassemias**. **Alpha thalassemia** affects the alpha-globin gene(s). **Beta thalassemia** affects one or both of the beta-globin genes. The genetic defect usually is a missense or nonsense mutation in the beta-globin gene (not deletion).

β thalassemia is caused by mutations of the β globin gene on chromosome 11. In beta thalassemia major (ie, homozygous beta thalassemia), the production of beta-globin chains is severely impaired, because both beta-globin genes are mutated. In beta thalassemia major, one of the beta-globin chains is impaired.

The severe anemia resulting from this disease, if untreated, can result in high-output cardiac failure, which causes the highest mortality.

Iron overload which occurs relatively quickly due to recurrent transfusions, can be reduced with INTRAVENOUS desferrioxamine. The side effects are deafness and retinal damage.

HAEMOLYTIC ANAEMIAS

Hereditary spherocytosis gene for ankyrin (cell membrane protein) has been mapped to chromosome 8 and is autosomal dominant. The condition is commoner among Northern Europeans. It presents in childhood with jaundice and splenomegaly. Spherocytes on blood film suggests **hereditary spherocytosis** (HS). In HS the red cells are smaller, rounder, and more fragile than normal. The *osmotic fragility test* is

the best diagnostic test, although AGLT (Acidified glycerol lysis test) may be used as a screening tool for relatives. Treatment is with splenectomy.

Glucose 6 phosphate dehydrogenase deficiency (X linked recessive) is seen in African, Mediterranean, Iraqi, Jew and South East Asian Chinese people. It predisposes to a haemolytic anaemia reaction to drugs and infection. The blood film in G6PD deficiency shows characteristic **blister cells**, where the membrane protrudes out like a blister.

Drugs causing **haemolysis in G6PD deficiency** are:
Sulphonamides
Antimalarials (chloroquine, quinine, primaquine)
Antipyretics (aspirin + paracetamol)
Chloramphenicol
nitrofurantoin
Dapsone
Probenecid
Vit K

OTHER ANAEMIAS

A high MCV with normal folate and B12 levels, normal iron and a blood film showing anisocytosis and poikilocytosis suggests **sideroblastic anaemia**.

Sideroblasts are abnormal red cell precursors with iron loaded mitochondria, forming a ring around the nucleus. In **sideroblastic anaemia**, there is increased bone marrow iron. This is reflected in the increased iron stores in ferritin and also haemosiderin and ringed premature red blood cells (sideroblasts) due to excess iron.

Sideroblastic anaemia is associated with:
δALA synthase 2 deficiency
Alcohol
Lead
Myelodysplasia
drugs (paracetamol, phenacetin, pyrazinamide and chloramphenicol).

In sideroblastic anaemia, there is a defect in haem synthesis (failure to incorporate iron into the haemoglobin molecule). This leads to excess loading of iron to compensate in red cell precursors and into iron stores, sometimes causing haemosiderosis in the liver. Desferrioxamine therapy may help.

Aplastic anaemia can be congenital (Fanconi's anaemia) or acquired due to drugs (benzene compounds, insecticides, gold or penicillamine).

Pernicious anaemia (PA) is a disease of the stomach that is characterised by megaloblastic anaemia due to vitamin B12 deficiency. It is secondary to intrinsic factor deficiency and gastric atrophy. It usually has an autoimmune basis. Pernicious Anaemia primarily affects the elderly - most patients are over 60 years of age. Women are affected more often than men, in a ratio of 3:2. It may be associated with

autoimmune diseases, such as Addison's disease, hypothyroidism and also an increased risk of gastric carcinoma.

CLOTTING DISORDERS

Haemophilia A (factor VIII deficiency) or **B** (factor IX deficiency) both produce a prolonged APTT. They are both X-linked recessive.

An isolated prolonged APTT will be caused by deficiencies in factors VIII, IX, XI and XII and by von Willebrand's disease. Factor XII deficiency is not associated with increased bleeding.

Von Willebrand's disease is autosomal dominant. Bleeding following trauma, epistaxis and menorrhagia are described.
The tests to diagnose vWD include:
bleeding time (prolonged)
factor VIII level test (measures the level of factor VIII and its ability to function)
von Willebrand factor antigen test (the disorder is considered mild if a person has 20% to 40% of the normal amount, severe if the amount is less than 10% of normal)
ristocetin cofactor activity test (measures how well the von Willebrand factor is working)
Therapy includes DDAVP , Factor VIII concentrates and plasma products, and Tranexemic acid (fibrinolytic inhibitor).

Type I **heparin induced thrombocytopenia** (HIT) occurs within a few days of heparin and is usually mild.

In this case, type II HIT is more likely, and this occurs slightly later (5-15 days). It is associated with thrombosis and a low platelet count. Alternative anticoagulation should be used (hirudin, danaparoid sodium).

Side effects of heparin:
Alopecia
thrombocytopenia
osteoprosis

The main inherited **thrombophilic** defects are:
Protein C deficiency
Protein S deficiency
Antithrombin deficiency
Factor V Leiden
Prothrombin 20210 gene mutation

PLATELET DISORDERS

Disseminated intravascular coagulation is caused by inappropriate and excessive activation of the haemostatic systems. 60% are caused by Gram negative sepsis. Other causes include viral infections, metastatic carcinoma, leukaemia, obstetric causes, extensive trauma and burns.

APTT, PT and TT are all prolonged, platelets and fibrinogen are low, D-dimers/FDPs are high. Treatment is of underlying causes and by control of the haemorrhagic state. Platelets, blood, cryoprecipitate and fresh frozen plasma may all be required.

Features consistent with a diagnosis of **immune thrombocytopenic purpura** (ITP) are thrombocytopenia with platelets being normal in size or may appear larger than normal, but uniformly giant platelets (approaching the size of red cells) should be absent. The morphology of red blood cells and white blood cells should be normal. In younger patients with **ITP**, the disease usually remits spontaneously within several weeks and no treatment is usually required unless there is significant bleeding. However, after adolescence, the disease tends to run a chronic relapsing course and therefore requires therapy. First line therapy is oral steroids. Patients who are refractory to, or are intolerant of steroids may respond to intravenous immunoglobulins (IVIg) or anti-D.

Thrombotic thrombocytopenic purpura (TTP) is characterised by microangiopathic haemolysis and thrombocytopenia. There is a spectrum of presentations with TTP-HUS. Neurological features are present in 60% of patients of TTP and renal failure is often associated in HUS (haemolytic uraemic syndrome). With the introduction of *plasma exchange* (recommended treatment), the survival rate has improved from approximately 3% prior to the 1960s to 82%.

MYELOPROLIFERATIVE DISORDERS

In **myelofibrosis**, splenomegaly occurs with a fibrotic process.
Leucoerythroblastic anaemia (red cell and white cell precursors) are seen on the blood film. Fibrous tissue infiltration of the bone makes it difficult to aspirate bone marrow. Bone pain, bleeding (platelet dysfunction) may occur.

The criteria for **Polycythaemia Rubra Vera** are:
1) increased red cell mass
2) splenomegaly
3) increased platelets, leucocytes, INCREASED NAP score and B12 (increased B12 binding protein release).
Increased serum viscosity may arise from hyperglobulinaemia or from an increased red cell mass, polycythaemia. Treatment of hyperviscosity syndrome should be with fluid replacement and venesection.

The **NAP** score is a semiquantitative cytochemical assessment of alkaline phosphatase in neutrophils. The NAP score is based on staining intensity, with a possible score of 0-400.
It differentiates **chronic myeloid leukaemia** (low) from reactive leucocytosis (high), eg bacterial infection.
It may assist in the differentiation of **polycythaemia rubra vera** (high) from other causes of erythrocytosis (normal).

LEUKAEMIAS AND LYMPHOMAS

Acute leukaemia is defined as blast cells comprising 30%

Chronic lymphocytic leukaemia : high white cell count with predominant lymphocytosis and anaemia . Immunophenotyping can be used for classification of undifferentiated leukemia as lymphoid or myeloid and subclassification of leukemias. Chronic lymphatic leukaemia is characterised by a lymphocytosis. The blood film shows mature lymphocytes with **smear** or **smudge** cells (they are squashed cells).

Gleevec (imatinib mesylate, Novartis), is an oral drug which interferes with the action of the abnormal Bcr-Abl tyrosine kinase in **CML** white blood cells.

Before Gleevec, the most common drugs used to treat CML were the oral treatments hydroxyurea and busulphan.

Acute Lymphoblastic Leukaemia : The bcr gene is found on chromosome 22 and abl gene on 9. This is known as the Philadelphia chromosome (named because it was discovered at hospital in Philadelphia). The Philadelphia chromosome is found in 25% of ALL patients (not AML). The philadelphia chromosome is associated with bad prognosis.

Prognostic factors are more important in ALL than the other leukaemias. These are the prognostic factors:
Age at diagnosis: Children younger than 1 year and children older than 10 years are considered high-risk patients.
White blood cell (WBC) count: Children with ALL who have especially high WBC counts are classified as high risk
Sex: Girls with ALL have a slightly higher chance of being cured than do boys.
Organ Spread: Spread of the leukemia into the spinal fluid, or the testicles increases the chance of a poor outcome.
Immunophenotype of the leukemia cells: Children with pre-B or early pre-B-cell ALL do better than those with T-cell or mature B-cell (Burkitt) leukemia.
Number of chromosomes: Patients are more likely to be cured if their leukemia cells have an increased number of chromosomes (called hyperdiploidy). Children whose leukemia cells have fewer chromosomes than normal (hypodiploidy) have a less favorable outlook.
Chromosome translocations: Children with a translocation between chromosomes 9 and 22 (the "Philadelphia" chromosome), 1 and 19, or 4 and 11 have a less favorable prognosis.
Response to therapy: Children whose leukemia responds completely to therapy within 7 or 14 days of chemotherapy have a better outlook.

The Retinoic acid receptor- **RAR alpha** gene maps to chromosome 17q21, close to the t(15;17), (q21-q11-22) translocation. A fusion of the promyelocytic gene (PML) with the RAR gene is specifically associated with **acute promyelocytic leukaemia**.

In **Acute Myeloid Leukaemia**, 8:21 and 15:17 translocations are associated with better prognosis compared to 9:22 and deletions of chromosome 5 and 7.
Auer rods are due to stacking of granules in myeloblasts (granulocytes). Promyelocytes and Auer rods are found in M3 (morphological classification of M1-M7).

M6 and M7 which are the most differentiated have the worst prognosis.
In **AML**, the most common abnormality is DIC, which results in an elevated prothrombin time, a decreasing fibrinogen level, and the presence of fibrin split products. Acute promyelocytic leukemia (APL), also known as M3, is the most common subtype of AML associated with DIC.

Hairy cell leukemia is a B cell lymphoproliferative disorder in which monocytopenia occurs.

Burkitt's lymphoma is a high grade B-cell NHL which is very sensitive to chemotherapy and radiotherapy (95% response rate). It is associated with Ebstein Barr virus infection. Abnormalities of c-myc are almost commonly seen in Burkitt's lymphoma with about 75% of cases carrying t(8;14) and 20% carrying t(2;8) translocations.

Hodgkin's lymphoma is rare in children aged less than 6. Common presenting features for **Hodgkin's disease** are Pel Ebstein fever, weight loss, alcohol induced pain and lymphadenopathy. *Cold agglutinins* can occur, leading to possible haemolytic anaemia.

Histology demonstrates Reed-Sternberg cells, which are pathognomonic. Reed-Sternberg cells are characteristic bi-nucleate or multinucleate cells found in Hodgkin's disease (owls eye nuclei or church plate nuclei).
Nodular sclerosing is the commonest and **lymphocyte depleted** is the rarest form. The lymphocyte predominant form has the best prognosis, whilst the lymphocyte depleted form has the worst.

Staging of **Hodgkin's lymphoma** is via the Modified Ann Arbor classification:
I - Involvement of a single lymph node region or a single extralymphatic site or organ.
II - Involvement of two or more lymph node regions on the same side of the diaphragm (II) or one or more lymph node regions plus an extralymphatic site (IIE).
III - Involvement of lymph nodes on both sides of the diaphragm.
IV - Involvement of one or more extralymphatic organs (Lung, liver, bone marrow, with or without lymph node involvement).

MISCELLANEOUS DISORDERS

Cold Agglutinins: IgM which agglutinate red cells between 0 and 4ºC. Causes are: coxsackie virus, EBV, CMV, Mumps, HIV, syphilis, malaria, legionella, mycoplasma, listeria, E coli, lymphoma, leukaemia, myeloma, Waldenstrom's macroglobulinaemia, .

Folate deficiency is treated by giving folic acid orally at 1 to 5 mg daily. **B12 deficiency** is usually treated by parenteral administration of B12. Therapeutic doses of folate will correct the hematologic abnormalities due to cobalamin deficiency also but the neurologic abnormalities can worsen, it is best to give B12 first or both B12 and folate but never folate alone.

Methaemoglobinaemia a cause of cyanosis because it causes the formation of reduced Hb >1.5 g/dl. It is due to oxidised iron from Fe2+ to Fe3+ in Hb and may cause precipitation as Heinz bodies.

About forty substances have been implicated in causing this condition, the most prominent being dapsone, nitrates, prilocaine, antimalarials, sulphonamides and dyes.

Standard pulse oximeters give spuriously low readings in the presence of excess methaemoglobin. *Methylene blue* is indicated in any patient with symptoms and/or signs of hypoxia (mental changes, tachycardia, dyspnoea, chest pain).

Hypoxia, acidosis, high CO2, raised 2,3 DPG, raised temperature, high altitude and anaemia all shift the oxygen dissociation curve to the *right*, reducing haemoglobin's affinity for oxygen.
Raised levels of **HbF** (fetal hemoglobin) shifts the curve to the *left*.

Myelodysplasia can be classified into five subtypes -
Refractory anaemia
Refractory anaemia with ring sideroblasts
Refractory anaemia with excess blasts
Refractory anaemia with excess blasts in transformation (near AML)
CML.

Myelodysplastic syndromes are associated with pancytopenias along with dyserythropoietic ringed sideroblasts and blast cells in the peripheral circulation.

Few patients require aggressive therapy such as chemotherapy, it is reserved for younger patients to prevent progression to AML. Supportive therapy includes blood transfusions, platelet transfusions or **G-CSF** to improve blood counts. However median survival is only 2 years.

In **multiple myeloma**, the bone marrow shows increased amounts of plasma cells (>30%).

In **multiple myeloma** there is raised protein (60-80 normal range). This paraproteinaemia is due to **IgG** proliferation. There is also low white cell count due to bone marrow infiltration, hypercalcaemia and renal failure.

Waldenstrom's macroglobulinaemia is a type of non-Hodgkin's lymphoma. It is a condition which typically presents in the seventh and eighth decade of life.
Hepatosplenomegaly occurs.
Increased serum proteins leads to a variety of symptoms:
Neuropathy
Headache and focal nervous system impairment
congestive cardiac failure.

It is characterized by the presence of a high level of a macroglobulin immunoglobulin M [**IgM** – note that myeloma is IgG] and elevated serum viscosity in the presence of a

lymphoplasmacytic infiltrate in the bone marrow. The treatment is chemotherapy (Chlorambucil or Fludarabine)

Marble bone disease is due to a defect in osteoclast function, causing extensive mineralisation of bone and reduction in bone marrow space.

Primary **thrombocytosis** can be caused by:
Essential thrombocytosis
Chronic myeloid leukaemia
Polycythaemia vera
Myelofibrosis
Myelodysplastic syndromes

Following **splenectomy**, patients should receive lifelong penicillin prophylaxis. The major complication of splenectomy is overwhelming sepsis with encapsulated bacteria (eg, S pneumoniae, H influenzae, N meningitidis). The overall risk of sepsis in asplenic patients is approximately 2% but varies depending on the age and underlying diseases.

Paroxysmal nocturnal haemoglobinuria (PNH) is an aplastic anaemia like syndrome which red cells are predisposed to complement lysis and resultant haemolytic anaemia. There is a pancytopenia as well as a tendency towards Budd Chiari thrombosis.

The diagnostic test is the **HAM** test. Serum (which contains complements) is acidified (activates the complement pathway) and mixed with red cells which undergo lysis. **Haemosiderin** is a by product of haem breakdown containing iron. Excess amounts leads to renal damage, and is also lost in the urine.

In PNH, there is a loss of anchor protein (GPI glycosylphosphatidyl inositol) which hold different antigens e.g. CD59, CD14. These are regulatory proteins for the complement pathway.

Tumours and virus associations:
Burkitt's lymphoma - Ebstein Barr virus
Kaposi's sarcoma - Herpes virus and HIV
adult T cell leukaemia - HTLV
cervical carcinoma – HPV

In the UK every **blood donation** is tested for evidence of *hepatitis B, hepatitis C, HIV-1, HIV-2* and *syphilis*. However, although there are recent concerns regarding tranmission of new variant CJD, there are no reliable screening methods yet.