Haemostasis and coagulation exploration

Bleeding is the loss of blood from the circulatory tree. The consequences of bleeding are: decrease of red blood cells that leads to insufficient oxygenation of tissues; decrease of nutrients in plasma volume affecting the tissues; decrease of blood pressure. A loss by 10% does not lead to obvious changes while a loss by $35 \div 45\%$ is followed by death.

Haemostasis includes all processes through which bleeding is stopped. When a blood vessel is broken hemostasis is achieved through several mechanisms in two phases: primary and secondary haemostasis. Primary haemostasis consists of: vasoconstriction and platelet plug (its forming is enough for small defects). Vasoconstriction occurs immediately after cutting the vessel thus reduces blood flow through the damaged vessel. The contraction of the smooth muscle present in the vessel structure is produced by: local muscle contraction, action of local factors released from injured tissue and platelet as thromboxane A2, nerve reflexes. Tests for primary haemostasis involve:

⦿ the evaluation of the platelet number,
⦿ the test of capillary fragility or by the Rumpel- Leede,
⦿ the bleeding time test and
⦿ assessment of the von Willebrand factor.

Blood clotting (secondary hemostasis) is a series of enzymatic reactions leading to soluble fibrinogen transformation into insoluble fibrin. Enzymes involved in the process of coagulation are factors I-XIII (secondary haemostasis). Coagulation occurs by:

-Intrinsic mechanism - no blood contact with tissues, takes place with the participation of plasma and platelet factors only;
-Extrinsic mechanism-after contact with external factors.

Secondary haemostasis can be explored by global tests (used as screening tests) and tests that explore the intrinsic pathway or the extrinsic pathway.

ASSESSMENT OF PRIMARY HAEMOSTASIS

1. Platelets count

Platelets are fragments of cytoplasm without nucleus with very important roles in haemostasis. It has a biconvex disc shape and a surface layer of glycoproteins on which different substances are absorbed: ADP, coagulation factors V, VIII, and IX. Inside there are numerous ducts which communicate with the plasma. In the cytoplasm there are grains of type alpha, lysosomal and dense containing: fibrinogen, fV, ATP, Ca^{2+}, serotonin, enzymes, etc. Average life: $8 \div 10$ days. They are produced in bone marrow from megakaryocytes.

⦿ Normal number: $150\ 000 \div 400\ 000/mm^3$.

The decrease below $100\ 000\ /\ mm^3$ is called thrombocytopenia; to below $50\ 000\ mm^3$ spontaneous bleeding occur.

The increase over $700\ 000\ /\ mm^3$ is called thrombocytosis and may be associated with blood clotting in the vessels (thrombosis).

Functional role:
-maintaining the integrity of vascular endothelium;
-carrying coagulation factors, adrenaline, and serotonin;
-role in haemostasis and coagulation;
-retraction clot;
- phagocytosis of antigen-antibody complexes;
-viscous metamorphosis is the property of joining rough surfaces and changes their shape (it becomes a viscous mass).

Thrombocyte count is an exploration of current use being made with the help of microscopic or electronic method.

Microscopic method

Principle: the counting number of platelets in a blood sample diluted with a solution that produces hemolysis and prevents platelet aggregation in a Thoma type counting chamber.
Materials required: Winthrobe solution, Thoma type counting chamber (or Burker-Turk), Potain pipette, cotton, alcohol, needle.
Interpretation
 Normal values are between: 150 000 ÷ 400 000/mm ³
        The decrease is called thrombocytopenia seen in thrombotic purpura, in the first phase of the disseminated intravascular coagulation syndrome, acute leukemia, etc.
        The increasing in the number of platelets, thrombocytosis is seen in polycythemia Vera, thrombocythemia bleeding, chronic myelogenous leukemia, anemia posthaemorrhagic, etc.

Capillary fragility test (RUMPEL- LEED test)
Principle: capillary resistance is evaluated on a circular area 2,5 cm^2 in diameter at the elbow by counting petechiae occurred after compression achieved with a sphygmomanometer cuff.
Materials required: Tensiometer
Technique: applies compression using a tensiometer cuff for five minutes at a pressure equal to the arithmetic mean between the maximum and minimum blood pressure. The number of petechiae that appear on the 2,5cm^2 surface is counted. The test should not be repeated on the same arm within some time (1 week).
Interpretation
Normal values: 0-10 petechiae point (or negative test)
                10 ÷ 20 petechiae - test positive +
                20 to 40 petechiae - test positive + +
                40 to 60 petechiae - test positive + + +

        An abnormal number of petechiae sometimes appears in some healthy persons, especially in women, including pregnancy, women over the age of 40, in children; the elderly capillary resistance is lower than in men, in the newborn is increased. Pathological positive test is found in: some thrombocytosis, thrombocytopenia, arterial hypertension, diabetes, avitaminosis C, etc.

Bleeding time (Duke Method)
Principle is to measure elapsed time up to stop bleeding injury produced by a standard capillary made from a stinging or a small incision in the skin; is the time elapsed until the platelet plug formation.
Materials required: needle or disposable lancet (Frank), cotton, alcohol, filter paper, stopwatch.
Technique: A prick is made at the level of the ear lobe or on the fingertip in order to cause bleeding, after skin disinfection. The timers start and from 30 to 30 seconds absorb drops of blood with filter paper without a pressure maneuver. When the paper no longer stains, stop the timer and record the elapsed time.
Interpretation
    Normal values: 2 ÷ 5 minutes

⊙	A prolonged time (pathological than 5 minutes) is found in quantitative or qualitative abnormalities of platelets, von Willebrand disease, angiopathy, administration of drugs such as acetyl salicylic acid derivatives, indomethacin, treatment with anticoagulants.

⊙	Causes of errors: failure to wound size may lead to prolonged time (incision too deep) or shortening (incision too shallow).

ASSESSMENT OF SECONDARY HEMOSTASIS

Coagulation time
The coagulation time represents the time from the collection of blood until its complete coagulation. It is a global test that explores the overall coagulation. It has relevance when it is prolonged, but one cannot establish where the deficiency is (on the intrinsic, extrinsic or common pathway). It can be performed by two methods: the Lee and White method (in the test tube) and the Millian method (on the slide).

1. **Lee and White method**
Materials: Alcohol, cotton, strand, syringe, needles, slides, test tubes, stopwatch, water bath at 37°C, filter paper, Petri plate.
Procedure: 2 ml of blood are drawn by venous puncture and discarded onto a test tube. The stopwatch is started. The test tube is introduced into the bath at 37°C and the test tube is verified every minute for the appearance of coagulation. The stopwatch is stopped when the coagulation occurs.
Normal values: 8 - 10 minutes

2. **Millian method**
Procedure: After the finger is disinfected with cotton soaked in alcohol it is pricked. The first drop is swept away and the second and the third drops are placed on a slide. The diameter of the drop must be 0,5 cm. The slide is introduced into a Petri plate lined with wet filter paper. At every minute the coagulation is checked by tilting the slide.
Normal values: 6-8 minutes
Interpretation
⊙	The coagulation time is prolonged in hemophilia, disseminated intravascular coagulation, hypofibrinogenemia, vitamin K deficiency, hepatic diseases and anticoagulant therapy. It signifies a state of hypocoagulability of the blood and a bleeding diathesis.
⊙	Its value is shortened in thrombocytosis, hypothyroidism and bleeding. When it is reduced it reflects a hypercoagulability of the blood and the risk of thrombosis.

Activated Partial Thromboplastin Time (aPTT)

This test assesses the intrinsic and common pathways of coagulation. The term "partial thromboplastin" is derived from the fact that this assay differs from the prothrombin time (which uses "complete thromboplastin") in that no tissue factor is used to initiate clotting. The coagulation of blood is initiated by the contact of plasma with the negatively charged glass surface of the test tube. This process may be enhanced by adding kaolin, silica or ellagic acid.
Principle: a surface activator (such as kaolin or ellagic acid), phospholipids and calcium are added to citrated plasma at 37°C and the time that takes to initiate clot formation is recorded.
Materials: anticoagulant (sodium citrate), test tubes, pipettes, syringe, needles, garrotte, cotton, stopwatch, water bath at 37°C, cephaline, kaolin, calcium chloride solution M/40.

Procedure: plasma is obtained in the same manner as for the prothrombine time. 0.1 mL of plasma (at 37°C) is mixed with 0.1 mL of a kaolin suspension, 0.1 mL cephalin and 0.1 mL of calcium chloride. At every 5 seconds the appearance of fibrin is checked. The time until fibrin formation can be seen is recorded. The test is repeated also with the control sample.

Normal values: 35 - 55 seconds

Interpretation

◉ Prolongation of aPTT shows a global deficiency of plasma factors of coagulation through the intrinsic pathway (XII, XI, IX, and VIII).

◉ Higher values are noted in hemophilia, treatment with heparin, circulating anticoagulants, hepatic diseases, hypofibrinogenemia and von Willebrand disease.

Quick or prothrombin time (PT or T.Q)

Principle: a test witch use a platelet poor-plasma performed in the presence of excess tissuellar thromboplastin; explores the extrinsic coagulation path, that activity factors II, VII, IX and X.

Materials required: 3.8% sodium citrate, needle, syringe or vacutainer for blood clotting, thromboplastin solution, calcium chloride M / 40, water bath at 37 ° C (or coagulometer), test tubes, pipettes, stopwatch.

Technique: reagents and plasma are kept separate in the water bath; a tube is placed in a water bath with 0.1 ml plasma, 0.1 ml calcium chloride and 0.1 ml thromboplastin. When calcium chloride is added start the stopwatch. Lightly tilt the tube and watch the appearance of coagulation. Perform at least two determinations of the plasma investigated and a witness.

Interpretation   Normal values are between:

♣13-15 seconds for coagulation;

♣percentage of normal activity of prothrombin prothrombin activity between 63 ÷ 100%;

♣ the prothrombin ratio between normal plasma PT and PT patient- INR (International Normalized Ratio)

$$INR = \left(\frac{PT\ patient}{PT\ normal}\right)^{ISI}$$

ISI is the international sensitivity index of the thromboplastin used, calculated in report with the reference thromboplastin for which it is equal to 1. ISI values of tromboplastins vary between 1 and 3 for each batch of reagents. The more the ISI value is closer to 1, the higher the sensitivity is.

◉ The prothrombine time is prolonged in: hepatic diseases, vitamin K deficiency, hypofibrinogenemia, hypoprothrombinemia, under the treatment with anti vitamin K drugs, circulating anticoagulants, deficiency of one of the factors of the prothrombinic complex (II, VII, IX, X).

◉ It is normal in hemophilia.

◉ The test is used in medical practice for monitoring treatment with anti vitamin K drugs. It is intended to maintain prothrombin activity levels between 20 to 30% for prevention of thromboembolic diseases.