LECTURE 2

TRANSFUSION OF BLOOD AND BLOOD SUBSTITUTES
I. Actuality of theme.

Clinical use of blood or blood components, and the related products (so-called haemotherapy or transfusion therapy) is a well-known and an effective therapeutic method for the treatment of various disorders. In view of that, biomedical and clinical qualification of blood replacement, as well as a variety of alternatives to "traditional" haemotherapy (autologous transfusions, pharmacological methods, blood substitutes, cytokines), make the specific elements of transfusion therapy.

The primary aim of transfusion therapy is the reconstitution of blood homeostasis and its maintenance through: (a) the improvement of red blood cell (RBC), white blood cell (WBC), platelet or coagulation factor deficiencies by replacement, or (b) the stimulation of synthesis of lacking blood constituent(s). The factors affecting the features of transfusion therapy are:

- the category and severity of patient's haematological deficit;
- the type of specific blood product(s) needed (such as leucodepleted RBCs, platelet concentrates (PCs), plasma products and occasionally granulocytes);
- the quantity of blood component(s) required, and
- the degree or level of the treatment urgency.

The above-mentioned factors ought to be determined before the initiation of blood replacement in all situations, and this is the highest priority in a well-working and high-quality transfusion therapy.

Despite the better donor selection, novel screening tests and procedures with the improved product quality, transfusion therapy is not administered without risks, and it occasionally results in a spectrum of adverse effects. Iatrogenic (transfusion-transmitted) infections and incompletely understood immunological adverse effects remain major concerns. Thus, clinicians and other practitioners should consider potential risks versus possible (expected) benefits of each transfusion. Only when the benefits clearly outweigh the risks should a blood transfusion be administered.

A large part of risks represents the potential of human error, and the subsequent transfusion of the incorrect blood component. The failure of correct identification and insufficient monitoring of patients receiving transfusions continue to be appreciable and avoidable causes of morbidity and mortality. Blood administration safety is the result of accurate identification of patients to blood products before transfusions, appropriate care and administration of blood products, and rapid recognition and intervention, when the adverse reactions occur. Transfusion specialists have to carefully evaluate every blood transfusion before its application as well as to observe signs and symptoms of transfusion reactions in order to minimize
the possible complications. Thus, the major purpose of transfusion policies and clinical practice guidelines as well as biomedical researches is to look for a "zero degree" or a "close to zero degree" risk.

In this lecture blood group serology, donation, preparation, and storage of blood, pretransfusion testing, indications (transfusion triggers) for blood replacement in patients with circulating blood volume deficiency, or depleted oxygen-carrying capacity (such as oxyform blood function), as well as with haemostatic or immune disorders, rational use of therapeutic fluids other than blood, anesthesiological and surgical technique and pharmacological agents to reduce surgical bleeding, autotransfusion and normovolemic hemodilution will be briefly described. In addition, this lecture discusses certain recent data about the manifestations of associated adverse effects, complications of transfusion therapy and haemovigilance. Thus, the objective of this lecture is to introduce the essential elements for establishing the most effective and safe transfusion programme in both clinical and non-traditional out-of-hospital settings.

II. Aims of lecture:

Educational:
- To describe the history of blood transfusion (β =I);
- To expound the blood group serology, donation, preparation, and storage of blood (β =II);
- To elucidate pretransfusion testing (tests for blood compatibility) (β =II);
- To characterize the indications and contraindications for transfusion of blood and blood components (β =II);
- To substantiate the adverse effects and complications of transfusion therapy (β =II);
- To elucidate rational use of therapeutic fluids other than blood (β =II);
- To describe anesthesiological and surgical technique and pharmacological agents to reduce surgical bleeding (β =II);
- To expound the methods of autotransfusion and normovolemic hemodilution (β =II);
- To describe the role of clinicians in promoting voluntary blood donation (β =I);
- To characterize the importance of a national policy for the clinical use of blood (β =I).
- To study the students the main principles of evidence-based medicine according the subject of lecture (β = IV).

Educative:
1. To study the students the main principles of system of non-paid blood donation.
2. To study the students to establish the psychological contact with patients who refuse blood transfusion (for example Jehovah’s Witnesses).
3. To characterize haemovigilance as a tool for progress in transfusion medicine.
### III. Plan and organization of structure of lecture

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<td>History of transfusiology; Blood Group Serology; Donation, Preparation, and Storage; Pretransfusion Testing; Transfusion Therapy; Indications (transfusion triggers) for blood replacement in patients with circulating blood volume deficiency, or depleted oxygen-carrying capacity (such as oxyform blood function), as well as with haemostatic or immune disorders; Adverse effects, complications of transfusion therapy; Rational use of therapeutic fluids other than blood; Anesthesiological and surgical technique and pharmacological agents to reduce surgical bleeding; Autotransfusion and normovolemic hemodilution; The role of clinicians in promoting voluntary blood donation; Haemovigilance as a tool for progress in transfusion medicine; The importance of a national policy for the clinical use of blood.</td>
<td>Facilities of activation of students are a questions, controversial situations, illustrative material</td>
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IV. Subject of a lecture
Blood replacement remains a highly effective and often life-saving benefit for many patients, and its use in clinical practice is common. The clinical use of blood components is particularly helpful in the treatment of life-threatening disorders caused by disorganized and/or disordered haemodynamic, oxygen-carrying, haemostatic, and immune and other blood functions. Thus, apart from the frequently life-saving properties of blood transfusion following acute (massive) blood loss, current surgery could not be considered without the availability of compatible and safe blood products for transfusion. Similarly, there are many conditions in internal medicine in which the therapy more or less depends on the application of the appropriate blood products in optimal quantity. By way of illustration, patients with cancer receive intensive anti-neoplastic therapy that affects their ability to produce and maintain circulating levels of blood cells. As a result, a variety of transfusions are needed for their treatment. These transfusion requirements necessitate a highly developed and specialized performance of transfusion therapy in cancer care.

History of blood transfusion. The first reported blood transfusion occurred in 1492. The transfusion was done on Pope Innocent VII in Rome. His doctors advised to transfuse blood from three healthy individuals as a therapeutic measure for his illness. However, the outcome of this blood transfusion was not successful and the Pope died soon after. In 1628, William Harvey, an English physician discovered how blood circulated around the body, with the heart pumping blood into the body through the arteries, and the blood returning back to the heart through the veins. In the year 1665 the first successful blood transfusion was recorded. Experiments were done by an English physician, Richard Lower, who transfused blood from one dog to another. Most of the dogs survived the transfusion. By the year 1667 Richard Lower and Jean-Baptiste Denis reported successful transfusions from animals to humans. Animals used included sheep and lamb. However, due to the deaths that were reported, within ten years this practice became illegal and for the next 150 years no studies in blood transfusion were recorded. Syng Physick did the first successful blood transfusion from one human to another human in America. The year was 1795; however he never published his findings. In 1818 the first successful transfusion was recorded. It was performed by James Blundell, a British obstetrician, on a mother that suffered post-partum haemorrhage (sever bleeding after delivery). Blood from her husband was
taken with a syringe and successfully injected into the patient. Between 1825 and 1830 he performed ten of these transfusions, with five of the patients showing clinical benefits. During the transfusions that were performed in the following years, two problems were being encountered. The first was that frequently, blood clotted during the procedure, as no anticoagulants (a solution that inhibits blood to clot) were being used until the year 1914. The second problem was that about half of the transfused patients had severe reactions, some of which led to death. The breakthrough came in 1900 when Karl Landsteiner, an Austrian scientist, discovered three human blood groups. These were the A, B and O blood groups. For this discovery he was awarded the Nobel Prize for medicine in 1930. In 1902, two students who worked with Karl Landsteiner discovered the fourth human blood group, the AB. These two were A. van Decastello and A. Sturli. These four blood groups together are what we today know as the ABO blood group system. In 1925, while Karl Landsteiner was working in New York he discovered two more blood group systems, the MN and the P blood group systems. In 1912, Roger Lee defined the terms ‘Universal donor’ and ‘Universal recipient’. He demonstrated that group O blood could be transfused in patients having anyone of the four blood groups, while group AB patients could receive blood having anyone of the four blood groups. As mentioned earlier in 1914 several anticoagulants were being introduced. In 1916, Francis Rous and J.R. Turner introduced a citrate-glucose solution, which was added to the collected blood. This allowed blood to be stored in containers and refrigerated for several days before being transfused. In the years to follow establishments where blood was collected and stored were being introduced. Later, these were to be known as 'Blood Banks', the first being introduced in a Leningrad Hospital in 1932. However the term 'Blood Bank' originated in 1937, by Bernard Fantus, who established the first Blood Bank at the Cook County Hospital in Chicago. In the following years Blood Banks spread throughout the United States. Although the discovery of the ABO blood group system reduced dramatically the number of deaths following blood transfusion, several other transfusion reactions (such as fever) were being observed. These were caused by other blood group systems, which yet had to be discovered. The most important of these systems was the Rhesus (RH) system. This discovery was made by Philip Levine and R.E. Stetson in 1939. They observed that after a mother gave birth to a stillborn child and subsequently transfused with her husband's blood, she suffered a severe reaction to the blood. Both the mother and the husband were group O. The two scientists explained the presence of a new factor as being the cause; however no name was given to it. The name was given by Karl Landsteiner and Alex Weiner in 1940. They conducted a study in which they injected blood from the monkey ‘Maccacus rhesus’ into rabbits and guinea pigs. The blood from the rabbits and the guinea pigs was then collected, and the serum (the liquid in which red blood cells flow), which contained the anti-Rh factor (a protein that binds to the rhesus antigen), was mixed with red blood cells from a number of samples from individuals of a population of New York City. Red blood cells from 85% of this population agglutinated (clumped together) with this serum. This population was called Rhesus
Positive (Rh Positive). The remaining 15% that did not have any agglutination were called Rhesus Negative (Rh Negative).

Other important blood group systems were discovered during the following years. The introduction of the anti-human globulin reagent (Coombs reagent) as part of the pre-transfusion testing was an important step for the detection and identification of incomplete antibodies that were causing transfusion reactions. The reagent was described in 1908 by Moreschi, and was rediscovered in 1945 by Dr. R. Coombs, Mourant and Race. The use of this reagent and the principles behind it are still an important tool today. By the year 1950, the number of blood banks was increasing around the world. In the United States alone the number of hospital blood banks reached 1500. During this year one of the most important technical developments in blood banking was introduced by Carl Walter and W.P. Murphy Jr. They introduced the plastic bags for the collection of blood, which replaced the breakable glass bottles that were in use. The scientific research that was done in the next fifty years revolutionized blood banking. New concepts and important techniques were developing, all of which moved blood banks towards a system that took into consideration the safety of both blood donors and patients receiving their blood. In 1970 blood banks started collecting blood from volunteers. Testing of blood for transfusion-transmitted diseases was being introduced. The first testing started in 1971 when blood collected for transfusion was being tested for Hepatitis B. In 1985 the first test to detect HIV was quickly implemented by all blood banks to protect the patients from infections of this virus. Testing for Hepatitis C was introduced in 1987. In the years to follow other testing was implemented and the techniques by which the testing was done improved, minimizing the risks of diseases transmitted through blood transfusions. Other important developments that occurred in these last thirty years were related to the storage of blood. Due to the fact that blood banks were collecting blood from volunteers and to the increasing demand of blood, several blood banks were starting to suffer shortage in their blood supply and were not coping with the demand. Although this problem still exists today the discovery that was made in 1979 reduced the problem. This was the development of a new anticoagulant preservative, CPDA-1, that extended the preservation of blood to 35 days. Subsequently the shelf life of red blood cells increased to 42 days in 1983 when a new additive, SAG-M was introduced.

Today, the experiments and methods that were used in the past may seem to be crude; however it is thanks to these researchers, that we have reached the safe standards that we have today. The only thing that never changed throughout the years is the importance of blood.

**The system ABO.** Differentiation of blood on groups by system ABO is based on four various combinations two agglutinogens (antigenes) A and B and two agglutinins (antibodies) a and b in red corpuscles and blood serum of people. The ABO antigens are carbohydrate (CHO) chains on large, membrane-bound glycosphingolipid molecules that differ only in their terminal sugar. The genes
responsible for these differences are alleles at the ABO locus on chromosome 9 that encode specific transferases. The A gene transferase appends an N-acetylgalactosamine onto the common precursor chain whereas the B gene transferase appends a galactose instead: these effects are genetically codominant. The 0 gene does not produce a functional transferase, and its effect is genetically recessive. ABO antigens are expressed early in gestation and at high density on almost all human cells. ABO antibodies do not arise spontaneously but are normally induced during the first year of life by exposure to ABO-like CHO common to flora that colonize the normal gut. Infants then develop antibodies to the A antigen or B antigen, or both, absent from their own cells. CHO antigens induce strong and persistent IgM antibody responses, and this class of immunoglobulin is an effective hemolysin and activator of serum complement. At faces of the first group red corpuscles do not contain agglutinogens A and B, therefore first group of blood designate 0(I) in blood serum are available agglutinins a and b. The second group of blood A(II) is described by presence in RBC agglutinogen A, and the blood serum contains agglutinin b. In red corpuscles of faces with the third group of blood B(III) is contained agglutinogen B and agglutinin a in blood serum. The fourth group of blood AB(IV) is contrast to first - in red corpuscles are available both agglutinogens A and B, agglutinins a and b are absent. a calls agglutination of red corpuscles with A, agglutinin b calls agglutination of red corpuscles with agglutinogen B. Agglutinin and agglutinogen of the same name cannot be present in blood of the person simultaneously.

Nomenclature. On Ukraine and in the countries of UIS, as well as in number of other countries, alphanumeric label of groups of blood is accepted: 0(I), A(II), B(III), AB(IV). Group isohemagglutinins are designated by letters a and b.

Subgroups of blood. Agglutinogen A, contained in red corpuscles of the people of group A(II) and AB(IV) is inhomogeneous and can be shown as two subgroups - A1 and A2. Agglutinogen B has not such differences, in this connection the concept "subgroups of blood " characterizes only intragroupe differences of agglutinogen A. Red corpuscles, which has agglutinogens A1, differs from red corpuscles with agglutinogens A2 by more expressed ability to agglutination and absorbtion in relation to agglutinins. Among people with the second and fourth groups of blood the subgroup A2 can be in 12 %, is more often at fourth group of blood (each 5-6 persons with the fourth group of blood is the launcher of weak A2-antigen).

Technique of determination of group of blood

With the help of standard serums.

Research is carryed out with the help of of two series of standard serums of groups - O(I), A(II), B(III) on special plate having an inscriptions at the left 0(I), in middle is A(II), on the right is B (III). On the upper boundarouse of a plate is written surname and initials of the patient, whose group of blood is determined. Under appropriate
labels put on one large drop (about 0.05 ml) of standard serum of two series in appropriate order: at the left 0(I), in middle is A(II), on the right is B(III). Thus on a plate six drops are received which will derivate two numbers (series) till 3 drops in each. With the help of pipette or small glass wand transfer on a plate (near to serum) one small drop (about 0.005 ml) of researched blood, then each drop of blood and the serum are mixed. The ratio of volume of researched blood and serum should be 1:10. Plate is cautious shaked during 5 minutes, then take into account an outcome. In accordance with approach of agglutination, but not earlier, than after 3 minutes, in drops is added one drop of isotonic solution of sodium chloride (about 0.05 ml). At an evaluation of outcomes of determination of groups of blood the following versions can be obtained:

1. The standard serums of all three groups do not call agglutination of red corpuscles. It indicates that red corpuscles do not contain agglutinogens A and B, i.e. the researched blood concerns to group 0(I).
2. Standard serums of group O(I) and B(III) call agglutination of red corpuscles, and with serum A(II) agglutination has not set in. The researched blood contains agglutinogen A, i.e. belongs to group A(II).
3. The standard serums 0(I) and A(II) have rendered a positive response, and serum of B(III) negative. Red corpuscles of examined sample of blood contains agglutinogen B, i.e. concern to group B(III).
4. Standard serums of all three groups calls agglutination of red corpuscles. The researched blood contains agglutinogens A and B. It attributes to group AB(IV). However for final inference it is necessary to conduct monitoring research on specificity and response with standard serum AB(IV). Only absence of agglutination of researched blood with serum of group AB(IV) can attribute blood to the fourth group.

**With the help of monoclonal anti-A and анти-B antibodies (colyclones anti-A and анти-B)**

They represent divorced ascitic fluid of mice-carriers of hybridoma, which contains IG M against antigen A and B. Colyclones gives more fast and more expressed response of agglutination, than standard ABO-serums.

Determination of group of blood is carried out at temperature from 15 up to 25 C. Put on one large drop of colyclones anti-A and анти-B, where introduce a drop of researched blood (red corpuscles) in 10 times smaller size and mix them. A plate is slightly shaked and response is observed in 2,5 min. The response usually comes in first 3-5 min. and is exhibited by formation of small-sized aggregates, flakes. The following versions of response are possible:

1. Agglutination is absent with colyclones anti-A and анти-B. Thus, the blood does not contain agglutinogens A and B, researched blood is 0(I) groupe.
2. Agglutination is observed with colyclone anti-A, is absent with colyclone anti-B. The blood is contained agglutinogen A is blood of A(II) groupe.
3. Agglutination is observed with colyclone anti-B, is absent with colyclone anti-A. Signifies, red corpuscles contains agglutinogen A researched blood is B(III) groupe.
4. Agglutination is observed with colyclone anti-A and colyclone anti-B. Accordingly, the blood is contained agglutinogens A and B, researched blood is AB(IV) groupe.

Colyclones anti-A and anti-B are made in liquid kind in small bottles also represent fluid of red (anti-A) or dark blue (anti-B) colour. They are kept in refrigerator at temperature 2-8 C. Term of storage is 2 years.

Standard isohemagglutinating serums for determination of groups of blood are prepared from donor blood being for this purpose most suitable, than cadaverous blood, transsudates and other fluids of an organism.

For marking standard serum of group A(II) is coloured in light-blue colour, serum B(III) in red colour, serum AB(IV) in yellow colour. The serum of group 0(I) has natural white-yellow colouring. Caption standard of isohemagglutinating serums should be below 1:32 for red corpuscles of group A(II), B(III), not beneath 1:16 for red corpuscles for AB(IV).

Serums are stored in temperature 4-8 C in sealed in glass small bottles by capacity 5-30 ml, with rubber fuses filled by Lassar's paste. On label indicates group of serum, it's caption, title of establishment, date of preparation and working life. Working life of standard serums is 2-3 month.

The Rh-factor, it's biological and clinical value, methods of determination
System rhesus (Rh-Hr)

The first antigene of this system Rh (D) called by the rhesus-factor, was opened in red corpuscles of the person in 1939 by American scientific Wiener with the help of serum of rabbits, immunized by red corpuscles of monkeys: macaques-rhesus. The Rh-factor is present in blood of 85 % people; in 15 % of people this factor does not contain. Analysis of antibodies exhibited in blood of the people, transferred posttransfusion complications or immunological incompatible of pregnancy, has resulted in discovery of other antigenes of this system called as varieties of the Rh-factor.

Rh antigens are proteins that appear early in gestation, are found only on the membranes of red blood cells and their precursors, and are expressed at relatively low density. Encoded by a multilocus complex on chromosome 1, the Rh system functionally behaves as three tightly linked genes, each having two major alleles: D and d; C and c; E and e. The D antigen is by far the most immunogenic. Its allele, d, is immunologically silent, apparently as a result of the deletion of several codons. Common usage of the term Rh typing refers to D antigen testing; ie, D(+) people are called Rh-positive, and D(-) are called Rh-negative, regardless of the other (C,c, E,e) antigenes of the Rh system. Rh-like antigenes are not encountered naturally in the environment, therefore, anti-Rh antibodies are produced only by patients exposed to these proteins through transfusions or pregnancies.
As expected for an immune response to protein antigen, anti-Rh antibodies are initially IgM but rapidly switch to IgG with persistence and immune memory (i.e., rapid recall with increased liters following rechallenge). IgG are less effective than IgM antibodies in activating complement, particularly with a low density of antigen on the target RBC. Therefore, immune destruction of RBC due to Rh alloimmunity is primarily by IgG-mediated extravascular phagocytosis rather than intravascular lysis. Of special note is the problem of Rh alloimmunity in pregnancy because maternal IgG crosses the placenta and can cause clinically significant hemolytic destruction of Rh-incompatible fetal RBC. This problem of hemolytic disease of the newborn primarily affects D(-) women carrying a D(+) fetus.

The clinical impact of the Rh system also is explained by its immunologic characteristics. The strong immuno-genicity of the D antigen dictates that donors and recipients should be typed for D compatibility in order to avoid anti-D sensitization. D(-) recipients should (except for emergencies) receive only D(-) RBC, but D(+) recipients can accept either D(+) or D(-) RBC. In the event that a nonsensitized D(-) patient is inadvertently exposed to D(+) RBC prompt administration of hyperimmune anti-D immunoglobulin preparation should be considered to block the induction of an active anti-D immune response. This is particularly important in women of childbearing age in order to prevent hemolytic disease of the newborn in future pregnancies. The anti-D immunoglobulin will cause removal of the D(+) cells. If more than 1 unit of D(+) cells has been transfused, consideration should first be given to performing an exchange transfusion to minimize the volume of RBC to be destroyed.

Rh transfusion reactions are less severe than ABO for several reasons. Patients without prior transfusions or pregnancies will not have pre-formed anti-Rh antibodies that could cause acute transfusion reactions. The antibodies induced by Rh sensitization will be primarily IgG, therefore, RBC destruction will be extravascular and relatively slow occurring over hours or days (i.e., a delayed hemolytic transfusion reaction), usually without overt clinical symptoms. Further, if incompatible RBC are given to an already Rh-sensitized patient (e.g., D(+) RBC to a patient with anti-D), the RBC destruction will still be primarily extravascular and, thus, more a delayed than an acute reaction. Because Rh expression is limited to RBC and because anti-Rh antibodies do not occur naturally, platelet and plasma transfusions and solid organ transplants are not affected by Rh incompatibility.

Typing for the other, less immunogenic, Rh antigens is not routinely performed unless there has been a positive antibody screen or crossmatch or the patient has a history of sensitization against one of those antigens.

Other RBC alloantigen systems produce relatively infrequent transfusion complications in the surgical setting. The most common (e.g., Kell, Kidd and Duffy systems) mechanistically resemble Rh, particularly in their absence of naturally occurring antibody and induction of mostly IgG immune response. Other plasma and
cellular allosen-sitization. such as anti-IgA, anti-platelet, anti-granulocyte and anti-drug antibodies, are of a range and complexity beyond the scope of this text and warrant consultation with a specialist trained in transfusion medicine.

**Nomenclature.** The system of antigenes rhesus is shown by 6 antigens, which, as well as other group indications of blood of the person, are transmitted by succession and during life do not vary. In the domestic and foreign literature uniformly widely use two nomenclatures of indicated antigenes: the Wiener and Fisher-Race), last is chosen by brackets Rho (D), rh' (C), rh " (E)", Hro (d), hr' (c), hr " (e)"

**HLA system**
Here the most relevant features are the protein nature of these antigens; their very low to absent expression on RBC but strong expression on white blood cells, platelets and almost all other tissue: and the absence of naturally occurring antibodies. Patients may become sensitized to HLA proteins through pregnancies, transfusions, or transplants. HLA antibodies do not diminish the efficacy of RBC transfusions, but may cause febrile transfusion reactions due to reactivity with donor leukocytes in the blood product. Because HLA antigens are expressed on platelets, sensitized patients may require platelets selected for HLA compatibility to attain a therapeutic effect.

**Determination of Rh-antigen in blood**
For determination of Rh-fittings is used standard anti-Rh serum and monocline antibodies anti-D.

At use of anti-Rh serum it is necessary to apply two samples of serums of two various series and simultaneously to use for the control standard red corpuscles, obtained from blood of Rh-positive and Rh-negative people.

**Technique of determination of the Rh-factor with application of 10 % gelatin solution.** Test is performed in centryfuged test-tubes. In test-tube by pipette is introduced one drop of researched red corpuscles, two drops of anti-Rh serum and two drops of 10 % gelatin solution, warmed-up up to temperature 46-48C. For the control in parallel investigate standard Rh-positive and Rh-negative red corpuscles, of the same with researched blood groupe. Test-tubes contents mixed by shaking; test-tubes placed on water bath-house with temperature 46-48C on 5 min. After incubation in test-tube is added 5-10 ml of isotinic solution of sodium chloride, mixes contents and estimates an outcome. Test-tubes looks through on light by unaided eye or through magnifier with repeated increase.
At presence of agglutination as red grains or flakes on transparent of almost decoloured hum of fluid treated as a positive outcome, i.e. the blood contains the Rh-factor. At negative outcome in test-tube is visible uniform painted in pink colour, slightly opalescent fluid.
The express method of determination of the Rh-factor.
The method is based on use of anti-Rh serum, divorced by 20-30% albumin solution, at the presence of which the serum gain ability to agglutinate red corpuscles on a plate at room temperature. Research is carried out on a plate for determination of groups of blood. Put a drop of anti-Rh serum AB (IV) group, where is added a drop of researched blood in 2-3 times smaller volume, and contents is mixed by glass wand. Near is carry out a response with control serum, which is serum of group AB (IV), divorced by albumin solution and dos'st contain rhesus-antibodies. On expiration of 5 min. is added one drop of isotonic solution of sodium chloride and take into account a response. At presence of agglutination of red corpuscles with anti-Rh serum blood is Rh-positive, at absence of agglutination blood is Rh-negative.

Determination of the Rh-factor with the help of monoclonal antibodies-D-super, which are intended for detection of D-antigen of system rhesus in red corpuscles.
The operational beginning of monoclonal antibodies-D-super are monoclonal human antibodies, which are produced by heterohybridoma, obtained in an outcome of confluence of human lymphoblast line with myeloma cell-like line of mouse. Anti-D-antibodies, which are Ig of class I, calls direct agglutination of red corpuscles, containing D-antigen.

Technique of determination. On a plate put a large drop (about 0,1 ml) of reactant, to which introduce a small drop (0,01-0,02 ml) of researched blood and mix blood with a reactant. The reaction of agglutination begins through 10s, precisely expressed agglutination comes in 30-60 seconds. The outcomes of reaction should be taken into account through 3 min. If is agglutination observed, the researched blood contains the Rh-factor, if agglutination is not present blood is Rh-negative.

Compatibility testing.
In transfusion medicine, cross-matching refers to the testing that is performed to determine the compatibility of a donated unit of blood for its intended recipient. It should not be confused with tests to determine a blood type or an antibody screen (indirect Coombs test) which are two entirely different tests that should always be completed prior to cross-match testing. Cross-match testing is done by a qualified laboratory technologist in a blood bank. Cross-matching can be done electronically with a computer database or serologically.

An electronic cross-match is essentially a computer assisted analysis of the data entered from testing done on the donor unit and blood samples drawn from intended recipient. This includes ABO/Rh typing of the unit and the recipient and an antibody screen of the recipient. Electronic cross-matching can only be used if a patient has a negative antibody screen. This means that they do not have any active red blood cell atypical antibodies or they are below the detectable level of current testing methods. If all of the data entered is compatible the computer will print a compatibility label stating that the unit is safe to transfuse.
In a **serological cross-match** red blood cells from the donor unit are tested against the plasma/serum of the patient who is in need of the blood transfusion. If the patient’s serum contains antibodies against the antigens present on the donor red blood cells agglutination will occur. Agglutination is considered a positive reaction indicating that the donor unit is incompatible for that specific patient. If no agglutination occurs the unit is deemed compatible and is safe to transfuse.

In the case of an emergency a physician can request **uncross-matched blood**, it is thought that the lifesaving measure is of more benefit than any risk of an antibody mediated transfusion reaction; this blood must be ABO compatible and preferably Rhesus (Rh) compatible as well to minimize the risk of a serious transfusion reaction. Type O Rh negative blood can be given if the recipients blood group is not known, as may happen in an emergency. In an emergency, blood grouping can be done easily and quickly in 2 or 3 minutes in the laboratory on glass slides with appropriate reagents, by trained technical staff. These slide methods depend on the presence or absence of agglutination that can usually be visualized directly, but occasionally a light microscope may be needed. If laboratory services are not available, another system of deciding which type of blood to use in an emergency is the bedside card method of blood grouping, where a drop of the intended recipients' blood is added to dried reagents on a prepared card. The card method may not be as reliable as laboratory methods, which are preferable.

### The Collection of Blood for Grouping and Compatibility Testing (Cross Matching)

1. Verify the identity of the patient. Ask the patient to state his/her own identification details and, if necessary, check them against the patient's identity wrist band.
2. With the exception of an emergency transfusion, the collection of a blood sample for blood grouping and red cell antibody screening and the collection of a second sample for blood group checking and compatibility testing should be taken at different times by two different people.
3. The transfusion of fresh frozen plasma or platelets does not require compatibility testing, but the blood group of the patient should be confirmed.
4. The blood samples should be stored in a refrigerator; the samples will remain suitable for compatibility testing for up to five days from the time of collection.

### Checking Procedure Prior to Transfusion

1. Verify the identity of the patient.
2. Ensure that the product is suitable and intended for the patient.
3. The patient's identification details must match with the identification details of the laboratory form.
4. The blood group of the product to be transfused must correspond with the patient's blood group.
5. Before a transfusion of red blood cells, check the result of the compatibility testing and verify that the correct product and patient was used for the test (i.e. the numbers
on the red blood cell unit and its compatibility test tubing correspond to the numbers on the compatibility form issued by the laboratory).

6. If the patient has red cell antibodies, ensure that the label on the red blood cell unit states the absence of the antigens corresponding to the antibodies detected in the patient.

7. Examine the blood product carefully.
   A. The integrity and cleanliness of the container.
   B. If you suspect haemolysis, check whether the plasma in the compatibility test tubing is red.
   C. The presence of clots, gas or a violet colour of a red blood cell product are suggestive of bacterial contamination, as is the cessation of platelet swirling when inspecting platelet products against light ("angel curls").

8. Confirm that the checks have been carried out by signing the transfusion form.

**Administration of a Blood Transfusion**

1. A transfusion of red blood cells should commence within six hours of removal of the unit from a refrigerator.

2. If a red blood cell product has been at room temperature for two hours, it must not be returned to a refrigerator for storage but it must either be transfused or discarded.

3. Before starting a transfusion check the patient's vital signs (i.e. blood pressure, pulse and temperature).

4. Blood products should be at room temperature before transfusion.

5. If the patient has significant cold agglutinins, red blood cell products should be warmed during transfusion with an approved commercial blood warmer. The temperature must not exceed +37 degree C due to the risk of haemolysis.

6. A blood administration set with a 200 µm filter should be used to transfuse all blood products (red blood cells, platelets, fresh frozen plasma).

7. A biological pre-check is recommended at the beginning of a red blood cell transfusion; the red cells are transfused slowly (10 to 15 drops/min) during the first 10 minutes whilst carefully observing the patient. The patient must then be monitored throughout the entire transfusion.

8. A transfusion of a unit of red blood cells should not last longer than six hours.

9. The same administration set may be used to transfuse several units of red blood cells without interruption (according to the capacity of the administration set filter), but it is recommended that the administration set is changed after six hours in order to reduce the risk of bacterial contamination.

10. It is recommended that platelets are administered via a special platelet administration set.

11. Record the start and end time of the blood product transfusion in the patient's notes and confirm the completion of transfusion with your signature. This will ensure the blood product may be traced back from the patient to the donor and vice versa.
Pretransfusion checks
When administering any fresh blood product the following checks must be made:
1. Prior to collecting the blood from blood bank ensure the patient has patent IV access and is ready to receive the transfusion.
2. Check medical orders. Are the product type, special requirements and administration requirements correct?
3. To ensure the right patient receives the right blood product the following checks must be made at the bedside prior to administration of the blood product:
   A. Patient identification. Check the name, DOB and UR on the blood transfusion record, blood pack and tag and on the patient's wristband. Are they identical?
   NB: If parent or guardian are present or child is of appropriate developmental age include them in the patient identification checking process.
   B. Blood product identification. Check the pack number on the blood transfusion record, pack label and the pack. Are they identical?
   C. Blood Group. Check the blood group (ABO and RhD) of the product on the blood transfusion record, pack tag and pack. Do they match?
   D. Check expiry date. Is the product in date?
   E. Check blood product for any signs of leakage, clots or abnormal colour
   F. Complete documentation: sign, date, start and finish time the Blood Transfusion Record and file in the patient's medical record.

Care of transfused patients
The following must be undertaken for each unit of fresh blood product that the patient receives
1. Monitor your patient for adverse effects of transfusion; observe closely during the first 15 minutes.
2. As a minimum take and record vital signs (Temperature, Pulse, Respiration and Blood Pressure) on the observation chart
   A. Before starting the transfusion
   B. 15 minutes after commencement of the transfusion
   C. On completion of each pack of fresh blood
This is a minimum requirement. Some clinical areas may require more frequent observations particularly in unstable/unconscious patients. Patients should be observed closely during the first 15 minutes of transfusions some life-threatening reactions may occur after the infusion of only a small amount of blood. Where possible, patients should be informed of possible symptoms of a transfusion reaction and should inform staff immediately if they feel unwell during transfusion.

Indications for blood transfusion
As mentioned earlier, transfusion therapy includes the clinical use of specific blood products and/or appropriate alternative to transfusions (e.g. the application of
recombinant haematopoietic growth factors) in the management of patients. Although, in the clinical area, it still commonly designates a supportive therapy based on replacing a blood component that is deficient in patient's circulation. The basic postulates for a satisfactory and safe transfusion therapy are both the determination of the deficient blood constituent type and the degree of its depletion.

Clinical use of packed RBCs and related products

Currently, when speaking in terms of blood transfusion, one mostly thinks of therapeutic application of allogenic packed RBCs, or related products. Packed RBCs are the most frequently transfused blood products worldwide. In view of transfusion therapy, oligaemia has to be characterized as a pathological condition with the decreased total RBC volume in which packed RBC transfusions are advantageous, or necessary. Therefore, in clinically recognized oligaemia, the need for the increased oxygen delivery is the major indication for the transfusion of packed RBCs. This requirement typically arises from acute blood loss or chronic oligaemia. The final volume, residual WBC contents and shelf-life of the various RBC products depend on the method of the whole blood processing, and on the type of anticoagulant-preservasive or additive solution added.

Depending on the actual medical requests (e.g. allosensitisation, chronic transfusions, organ and tissue transplantation, etc), different packed RBC products are used in the therapy of oligaemic patients. As mentioned earlier, the application of each one has numerous potential advantages, but also possible adverse effects. Determination an appropriate threshold for initiating blood component replacement (transfusion trigger) could help avoiding the unjustified blood transfusion, as well as "under-transfusion" situations, i.e. the inadequate support with packed RBCs when they are helpful or necessary.

Packed RBC products should be administered only for the purpose of increasing oxygen-carrying capacity in oligaemic patients who are at risk of ischaemic events. In clinical use, the decision whether to transfuse packed RBCs or not is usually based on the patient's haematocrit (Htc) and haemoglobin (Hb) values. However, exact and rigid Htc and Hb thresholds (as universal transfusion triggers) have been considered inappropriate in several guidelines. Evaluation of Htc and Hb concentration do not always give a true indication of RBC deficiency, because they might yield to the incorrect estimation of RBC volume and/or total blood volume. Namely, the majority of patients requiring transfusion are at the same time hypovolemic and oligaemic, and thus they could exhibit false increases in Htc and Hb values. Consequently, these measurements should not be the only consideration in defining the transfusion trigger for packed RBC transfusion.
Over the past several years, conventional "Hct < 0.30 and Hb < 100 g/L" has been used as generally acceptable trigger for RBC transfusion in normovolemic oligoaemia. After the discovery of transfusion-transmitted infections, the number of preferred and fulfilled blood transfusions was clearly decreased in most countries, because the criteria for their performance became far more rigorous.

Currently, the qualified decision to transfuse should be made based on individual patient's vital symptoms of the decreased tissue oxygenation, cardiopulmonary reserve, rate and magnitude of blood loss, oxygen consumption rate, and the presence of considerable atherosclerotic disorder. The use of rigid "transfusion triggers", precisely the preference of exclusively Hct and Hb values as triggers for RBC administration (i.e. the concept of transfusing packed RBCs solely based on a Htc < 0.30 or Hb < 100 g/L), has little scientific support and is outdated. As a general concept only, transfusion of PRBCs is rarely indicated when the Hb concentration is greater than 100 g/L, and is almost always indicated when its concentration is less than 60 g/L. Finally, it is important to say that each RBC unit administered to adult patients with normovolaemic oligoaemia results in the increase of Hct of approximately 0.03 (i.e. 3%), along with the simultaneous increase of Hb concentration for around 10 g/L upon transfusion.

There are two major hazards of allogenic RBC transfusions: a) the transmission of viral infections, and b) potentially lethal acute immune HTR. The immunological risk associated to RBC transfusions is bound to the blood groups polymorphism and to the respect of blood transfusion regulations. The majority of HTRs results from administration of ABO-incompatible RBCs. Sometimes RBC transfusion errors in practice lead to life-threatening and even fatal consequences. Many factors contribute to these errors, resulting from the misidentification of either the patient, or the blood product. Even a small amount of incompatible RBCs might initiate a reaction and cause harmful consequences (haemoglobinaemia, disseminated intravascular coagulation, and renal failure, leading to death. Careful monitoring of the anaesthetized patient is important in recognizing symptoms of a HTR, so that the reaction could be promptly detected and treatment quickly initiated.

Transfusible fluids that may be used as alternatives to red blood cell transfusion offer the promise of preserving tissue perfusion and minimizing hypoxic cellular damage, and this promise may soon be fulfilled. Two classes of blood substitutes have undergone clinical study: (1) cell-free hemoglobin solutions and (2) perfluorocarbon solutions. The clinical applicability of perfluorocarbon solution is limited by the requirement of ventilation with oxygen concentrations of 100% and the complicated process of preparing the solutions for intravenous administration. Thus, current research is limited to hemoglobin solutions.
Cell-free hemoglobin solutions utilize free hemoglobin molecules to bind oxygen at the concentration present in alveolar capillaries and unload it at the levels found in tissues. Four different sources of hemoglobin have been used—human, bovine, recombinant, and transgenic.

The toxicity of these solutions has been secondary primarily to the tendency of the native tetrameric hemoglobin to dissociate to a dimeric form, which is toxic to the kidney and gastrointestinal tract and causes vasoconstriction. Subsequent modifications of the hemoglobin molecule have reduced this dissociation; however, clinical studies continue to demonstrate clinically significant vasoconstriction, which limits potential oxygen delivery to tissues.

Another limitation is the short circulatory half-life of these hemoglobin solutions. Polymerized bovine hemoglobin has an 8-hour half-life, and its effectiveness is limited to approximately 24 hours. That hemoglobin solutions will replace allogeneic blood transfusion totally is difficult to believe, but they may play a role in the resuscitation of patients after acute life-threatening hemorrhage and during acute normovolemic hemodilution.

**Current platelet transfusion practices**

Platelet transfusions are indicated in prevention or therapy of haemorrhage caused by thrombocytopenia or platelet dysfunction. Therapeutic platelet transfusions are established as helpful treatment in bleeding patients, but the issue of the benefit of prophylactic platelet transfusions in the prevention of haemorrhage remains controversial. Generally, in platelet replacement substantial improvements were achieved in the last several years, but platelet transfusions remain a critical part of supportive therapy in many patients. Moreover, a number of these patients require specialized components, including leucodepleted (filtered), gamma-irradiated and cross-matched or HLA-matched platelets. Platelet transfusions are also useful in the therapy of some other thrombocytopenic conditions such as dilution thrombocytopenia during surgery, including cardiopulmonary bypass, etc. However, the cause of thrombocytopenia should be determined before PC transfusions are used, because platelet support is not always advantageous. Thus, PC transfusions are contraindicated in some conditions, for example in thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome (TTP/HUS) and heparin-induced thrombocytopenia. Numerous reports, related to the determination of "platelet transfusion trigger", i.e. a threshold for their clinical use based on platelet count, have occurred recently. Typically, severe haemorrhage (haematuria, haematemesia and melaena) is present at platelet counts < 5 x 10⁹/L. However, there are reports on cases of rare and moderate bleedings in patients with around 5 x 10⁹/L platelets. Correspondingly, due to the possible existence of a critical "individual
thrombocytopenic bleeding threshold", a decision related to the introduction of PC transfusion should never be made exclusively upon the patient's platelet count.

The British Committee for Standards in Haematology provides guidelines for platelet transfusions in thrombocytopenic patients subjected to invasive procedures, such as bone marrow or liver biopsy, lumbar puncture, insertion of catheters in which platelet count should be raised to at least 50 x 10^9/L. For surgery in critical sites such as brain, platelet count should be raised to at least 100 x 10^9/L.

Two types of platelet components are available in most hospital settings: platelet concentrates ("random-donor platelets") and apheresis-derived platelets ("single-donor platelets" or "apheresed platelets"). The haemostatic activity of platelets is similar in each component. However, random-donor PC and apheresis-derived PC differ in methods of preparation, platelet contents, and potential for adverse effects in the transfusion recipient. Anyhow, daily dose of platelets is approximately 0,7 x 10^10 cells per Kg of patient's body mass.

Each random-donor PC unit is prepared from a single donated unit of whole blood by sequential low and high "g-force" centrifugation. These PCs contain around 5,5-7 x 10^10 platelets in approximately 50 mL of plasma. Prior to transfusion, the transfusion staff pools 5-8 PCs (each from a different donor) into a single product containing approximately 3-6 x 10^10 platelets. The resulting pooled platelet product is designated as a single therapeutic dose. Therefore, each pooled platelet product exposes the transfusion recipient to the risk of infectious disease transmission from 5-8 donors. In addition, each pooled platelet product also contains 10^8 or more WBC, which may induce adverse transfusion reactions.

Each apheresis-derived PC is prepared from a single volunteer platelet donor by platelet-apheresis. The process takes about 1-2 hours and results in an apheresis-derived PC unit with approximately 3-8 x 10^11 or more platelets in about 150 mL of plasma. Apheresis-derived PC usually contains 10^4 to 10^6 leukocytes (i.e. = 1% of leukocytes in a random-donor PC pool) and less than 0,5 mL of contaminating RBCs, demonstrating the selectivity of the apheresis process. As compared with the random-donor PC, the low leukocyte content of apheresis-derived PC reduces the risk of leukocyte-mediated adverse transfusion effects. In addition, each apheresis-derived PC exposes the recipient to only the infectious disease risk of a single donor. Finally, apheresis-derived platelets can be efficiently cross-matched and HLA-matched for transfusion to refractory recipients.

PCs should be administered immediately after preparation, and, if necessary, stored in liquid state up to 5-7 days on 20 ± 2°C, with permanent agitation of units using shakers. During storage in liquid state, platelet count in PCs is progressively decreased. Morphological distribution is also altered, resulting likewise in the change
of platelet morphological score (PMS) value. Long-term storage of platelets in frozen state requires the application of safe and effective cryopreservation procedures. Reduction of leukocyte count in stored blood products not only decreases the frequency of various transfusion reactions, but also prevents \textit{in vitro} production of inflammatory cytokines. Leucoreduction could be accomplished by filtration, or by using separators with integral leucoreduction system.

**Plasma and coagulation factor replacement**

Since the clinical use of fresh-frozen plasma (FFP) is not free of adverse effects, knowledge concerning plasma constituents and assessment of plasma replacement efficiency in specific clinical situations are essential. The application of FFP should be reserved only for clinical situations in which plasma products have been proven evidently beneficial, or in conditions in which more specific replacement is not available.

**Albumin** is quantitatively the mostly represented plasma protein and it has an important role in maintaining oncotic pressure in blood. Other plasma proteins include immunoglobulins, complement components, proteins involved in haemostasis, transport, enzymatic processes and hormones. One unit of plasma product is the quantity of plasma that has been obtained from 1 unit of the whole blood, and usually contains 180-300 mL of anticoagulated plasma. Plasmapheresis of a single donor can yield as much as 500-800 mL of plasma per unit, known as "jumbo-plasma units". Plasma products contain minimum numbers of RBCs and platelets, but may contain a small number of viable WBCs.

The **cryoprecipitate** is the blood product containing the highest concentrations of factor VIII (F VIII:C), von Willenbrand's factor (vWF), factor XIII (F XIII), fibrinogen, and fibronectin. It is an insoluble cold precipitate formed when FFP is thawed between 1 and 6°C. The recovered precipitate is then refrozen in 10-15 mL of plasma within 1 hour of separation and stored at -30°C or less for a maximum period of 1 year. Each bag of cryoprecipitate contains between 150 and 250 mg of fibrinogen, 30-60 mg of fibronectin, and 80-120 units of F VIII. In addition, cryoprecipitate also contains approximately 40-70% (i.e. 80 units) of original vWF and approximately 30% (i.e. 40-60 units) of the initial concentration of F XIII.

With the development of methods of extracting factor concentrates from plasma, improved procedures for viral inactivation of these factor concentrates, and recombinant protein manufacture, the role of cryoprecipitate in the management of these entities has largely become secondary. Cryoprecipitate is currently used for the treatment of: a) congenital and acquired states of hypofibrinogenemia; b) F XIII
deficiency; c) certain types of vWD; d) rarely for mild haemophilia A; and e) for the production of fibrin glue.

Prior to 1960s, the only treatment available for bleeding episodes in coagulation factor deficiency patients was the infusion of FFP. In 1964, Pool discovered that plasma cryoprecipitate contained F VIII in high concentrations. Investigators then started purifying F VIII (named as "intermediate-purity F VIII concentrates") from cryoprecipitate, using chromatography. Further purification steps (immunoaffinity chromatography) led to the production of "high-purity F VIII concentrates". Therefore, the main expectation during the preparation of coagulation factors was to obtain: a) the highest possible yield; b) optimal therapeutic and minimum side-effects, and c) maximum reduction of risks of transfusion-transmitted infections.

The production of recombinant coagulation factor products was made possible through the cloning of the desired factor gene, optimisation of the expression system, and characterization of a unique cell line through recombinant DNA technology. These products permit rapid reversal of bleeding episodes through self-infusion, and therefore limit irreversible joint damage and other bleeding complications. It is now possible to perform long-term out-patient prophylaxis in order to prevent bleeding episodes, minimizing costly hospitalisations and the effects of chronic joint bleeding and its attendant morbidity.

**Application of prothrombin complex concentrate (PCC)**

The risk of virus transmission with PCC is clearly lower than with quarantine-FFP. FFP is still preferable if it is possible to improve the haemostasis quickly enough, and the patient is not exposed to too high a risk of citrate intoxication and hypervolemia by FFP. If the haemostasis has to be normalized very quickly, plasma therapy alone often is insufficient and has to be supplemented by PCC. This particularly is the case in cerebral bleeding of patients having taken coumarin agents or sometimes after trauma with large blood loss or in peracute bleeding of esophageal varices when the coagulation is already initially below the critical levels.

The **indications of PCC** are surgery and/or haemorrhage in acquired coagulation disorders, as in peracute massive transfusion, acute esophageal varices bleeding, acute coumarin bleeding, and factor VIII inhibitors (in case FEIBA® or rFVIIa are not available). Furthermore, PCC may be indicated in inherited coagulation factor deficiencies where no concentrates are commercially available (factor II, VII, X deficiencies).

There is some increased risk of thrombo-embolism and disseminated intravascular coagulation (DIC) in patients treated with PCC. This is explained by the content of activated clotting factors, by an imbalance of the levels of the containing factors, and by the more or less low concentration of the inhibitors protein C and S. The
commercially available PCCs in Europe have improved during the last 8-10 years with regard to activated clotting factors. However, as a precaution, following rules should be observed:

- use of PCC with low contents of activated coagulation factors;
- preference for PCC containing protein C ands S for coumarin patients;
- restrictive use especially in patients with increased risk of thrombo-embolism;
- slow infusion (< 1 mL/min);
- no large single doses, dosage according to body weight (< 30 E/Kg BW);
- effective anticoagulation (heparin/low molecular heparin, antithrombin in case of significantly reduced AT levels);
- no use in combination with antifibrinolytics:
- determination of prothrombin time, PTT and antithrombin.

Application of factor concentrates

Fibrinogen concentrate

For many years fibrinogen concentrates have been refused by many haemostaseologists because of thrombo-embolic complications. The fibrinogen concentrates obviously have improved. Fibrinogen concentrates are indicated for preparation of surgery or in bleeding complications when the fibrinogen concentration is below the critical levels. For brain surgery or cerebral bleeding even higher levels may be critical (< 1.5 g/L). The necessary dose of fibrinogen concentrate can be calculated as:

\[
\text{Dose [g]} = \text{aimed increase [g/L]} \times \text{plasma volume [L]}
\]

In adults single doses of 2-3 g are mostly sufficient.

Other factor concentrates

Factor VIII and IX concentrates are only indicated in inherited deficiencies of these factors and the rather rare cases of acquired spontaneous inhibitors against these factors. For replacement therapy see under general recommendations.

Factor XIII often is underestimated in its importance. Replacement often is been done as restrictive as in haemophilia. However, factor XIII deficiency is already critical at higher concentrations when systemic or local hyperfibrinolysis occurs or wound healing is delayed. Therefore, we recommend factor XIII therapy in ulcerative colitis, Crohn's disease, and urologic surgery when bleeding persists and factor XIII is below 50%. Patients with dehiscence of sutures receive factor XIII for the same reason. However, bleeding because of thrombocytopenia is no indication for factor XIII replacement.
**Recombinant factor VIIa concentrates**

Recombinant factor VIIa (rFVIIa, NovoSeven®) has proven to be very efficacious in haemophilic patients with inhibitors as well as lrracquired inhibitor haemophilia. Meanwhile haemorrhage due to inhibitors against FVIII and FIX and possibly also to other coagulation factors is a generally accepted indication for rFVIIa. In surgery, a bolus of about 90 ug/Kg BW every 2 hours or a continuous application of 14-16 ng/Kg/h after an initial bolus have been used successfully. In muscle bleeds and haemarthroses the recommended doses are twice as high. Additionally, rFVIIa has been shown to be also efficacious in Glanzmann's thrombasthenia. This is of particular importance as platelet transfusions may become inefficient due to platelet-specific antibodies against glycoprotein IIb/IIIa. Furthermore, quite a lot of reports document the efficacy of rFVIIa in various other bleeding complications due to deficient coagulation, e.g. during surgery or after trauma (dosage 60-240 < mg/Kg BW). Surprisingly, no increase of thrombo-embolic complications has been reported. This might be explained by the platelet-based coagulation which only takes place at the injured vessels after adherence and activation of platelets. However, treatment with rFVIIa is extremely expensive. Therefore, besides for treatment of haemorrhage due to inhibitors or Glanzmann thrombasthenia, high dose rFVIIa is only indicated in case that all the other therapeutic means inclusive of antifibrinolytics and desmopressin have failed.

**Inhibitor concentrates**

Of the commercially available protease inhibitors anti thrombin, recombinant human activated protein C and Cl -esterase-inhibitor, only antithrombin (AT) has been used in bleeding disorders as a therapeutic agent. Recombinant human activated protein C has been used in severe sepsis and Cl-esterase-inhibitor in hereditary neurotic angioedema which are beyond the scope of this article. Therefore, only AT is discussed here with respect to its effectiveness in haemostatic disorders and the differentiation of its use in this field from its purported indications in other diseases.

The main indication of AT is inefficacy of heparin because of low AT level. This especially applies to DIC. Effectiveness of heparin cannot easily be documented given the alterations of the coagulation tests in DIC, and low platelet counts do not allow administering higher heparin doses. Therefore, AT therapy is generally recommended in DIC. AT levels should be normalized (at least to > 70%).

For many years there was a controversy whether increasing of AT to highly normal levels (> 120%) in septicaemia may decrease mortality. Animal experiments support the hypothesis that AT has an antiinflammatory and anti-proteolytic effect, reduces release of lysosomal proteinases, cytokines, etc, and has vascular protective properties. In a recent study with a relatively small number of patients early application of high-dose AT in septic shock significantly reduced outcome. In a large sepsis study, a positive effect could only be found in a small subgroup not receiving...
heparin. Therefore, this expensive therapy cannot be recommended in septicaemia as long as no DIC has developed.

AT application in case of PCC infusion is only indicated if no adequate anticoagulation with heparin can be performed and/or AT level is low.

Low AT levels per se are no indication for AT application at least as long as the haemostatic balance between procoagulant factors and inhibitors is maintained, e.g. in liver failure or dilutional coagulopathy. If there is a particular thrombo-embolic risk, a higher heparin dosage mostly ensures sufficient anticoagulation. Despite selective loss of AT in nephrotic syndrome and sometimes in inflammation of the bowel there is no need of AT therapy but adequate anticoagulation with heparin.

AT should not be given as a bolus to patients getting already heparin and having an increased bleeding risk. This especially applies to patients with liver and/or kidney failure who may have heparin accumulation. The dosage of AT is calculated as for the coagulation factors.

**Alternative drugs in haemostatic disorders**

Considering the very small but still existing risk of virus transmission even by virus-inactivated blood products, alternative drugs should be preferred whenever possible.

**Vitamin K** application to patients with liver failure, after large blood loss, during parenteral feeding and high-dose antibiotic therapy enhances production of vitamin K-dependent coagulation factors. Particularly patients on intensive care need regular vitamin K application (twice a week 10-20 mg) in order to prevent post-operative bleeding and/or the requirement of plasma product application.

In moderate and mild haemophilia as well as in vWD often factor concentrates are not required if **desmopressin** is used. At least the amount of necessary concentrates can significantly be reduced. Moreover, desmopressin is very effective in improving primary haemostasis in many circumstances with platelet function abnormalities. It is the measure of choice when patients with ASA have to undergo surgery. The effect is due to a 2-3 fold increase of von Willebrand factor by endothelial release. The maximal effect can be seen 1-2 hours after i.v. application (0,3 ug/Kg BW). Therefore, the operation has to be scheduled with regard to the onset and decrease of the desmopressin effect. If required, desmopressin application can be repeated every 12 to 24 hours for a few days with decreasing efficacy. Desmopressin slightly activates fibrinolysis. Therefore, combination with antifibrinolytics is recommended, at least in patients with increased risk of hyperfibrinolysis (cardiac, urological and oral surgery, neurosurgery).
**Antifibrinolytics** have their accepted indication whenever systemic or local hyperfibrinolysis can be assumed. In addition, there are a lot of reports showing positive effects in other coagulation disorders inclusive of impairment of platelet function. Significant reduction of blood loss can even be achieved in general surgery without detectable coagulation disorders. Therefore, antifibrinolytics should be tried when unclear haemorrhage cannot be stopped by other means. However, adequate anticoagulation has to be provided with regard to the risk of thrombo-embolic complications and re-occlusion of bypasses. In addition, antifibrinolytics should not be used in DIC.

**Granulocyte transfusions**

Granulocyte transfusions have been utilized in the setting of severe neutropenia with progressive infections or neutrophil dysfunction, with varying results and controversies concerning their therapeutic efficiency. Using potent antibiotics, recombinant haematopoietic growth factors (i.e. rHuG-CSF), and immunoglobulins in neutropenic patients, the risk of life-threatening infections has been reduced, but not completely eliminated. Despite rapid reconstitution of haematopoiesis (promoted by the use of rHuG-CSF and other growth factors), severe neutropenia might limit the intensity of radio-chemotherapy. However, more recent advances in granulocyte collection, with the administration of rHuG-CSF to enhance granulocyte yield, have produced re-established concern for this therapy (so-called "modern granulocyte transfusion therapy"). Granulocyte collections are apheresis-derived WBC components, usually about 300 mL in volume, composed of a mixture of granulocytes and other elements that are considered as contaminants, such as RBCs (60-70 g/L of Hb per granulocyte product), platelets and citrated plasma. The use of rHuG-CSF has made possible markedly increased blood granulocyte count of normal donors. Both higher granulocyte count in donor's circulation, and processing of larger blood volume provide collection and transfusion of evidently greater granulocyte doses (6-8x10^10/unit), compared with previously acceptable but marginal granulocyte doses (1-2 x 10^10/unit).

Consequently, renewed interest has extended the use of granulocyte transfusions in the treatment of transplanted adults and other oncological patients in whom critical neutropenia (transfusion trigger is 200 to 500 granulocytes per mL of blood) was complicated by severe infections.

However, the clinical use of biological products is limited not only by the availability, but also by the public concern for safety. Blood is a living tissue, and its transfusion from one individual to another is not free of hazards. Transfusion adverse effects (reactions or complications) might be immunologically or non-immunologically mediated, immediate or delayed, and may vary from mild to fatal.
A variety of transfusion adverse effects are still a major concern in transfusion therapy.

**Transfusion therapy: the common adverse effects**

- Febrile non-haemolytic transfusion reaction (FNHTR);
- Transfusion-induced immunomodulation (immunosuppression);
- Haemolytic transfusion reaction (HTR);
- Hypotensive reactions;
- Transfusion-related acute lung injury (TRALI);
- Platelet transfusion refractoriness;
- Post-transfusion purpura;
- Allergic and anaphylactic reactions;
- Vasovagal syncopal reactions;
- Circulatory overload;
- Transmission of parasites and bacteria (transfusion-associated sepsis);
- Complications of massive transfusion and apheresis;
- Transfusion-induced haemosiderosis;
- Transfusion-associated graft-versus-host disease (TA-GvHD);
- Transfusion-transmitted (blood-borne) infections.

The most important hazards of transfusion are transfusion-transmitted infections and acute haemolytic transfusion reactions (HTR) caused by the use of ABO-incompatible RBCs. The advent of AIDS has raised concern regarding blood-borne diseases. Blood transfusion is safer than ever before through continual improvements in safe donor recruitment and donor screening (e.g. removal of high-risk donors from the donor pool), testing of each donation with a panel of viral markers, and appropriate clinical use of blood. The risk of residual infections is further reduced through inactivation of pathogens in blood components. However, despite the improved peri-donation evaluation of each donor and donated blood, transfusion therapy still carries a considerable risk of transmission of blood-borne diseases. The most important immune-mediated risk of transfusion is associated with the polymorphism of blood group systems. Pre-transfusion compatibility testing, if properly performed, helps to maximize the effectiveness of transfusion therapy and to prevent incompatible RBC replacement that could lead to HTR. Each laboratory should select the procedures and protocols best suited to meet their patient's care and institutional needs. Quality assessment monitoring of the elements of compatibility testing helps to ensure a high level of safety for patients receiving transfusion therapy.

In each of the general issues mentioned above the hazards of transfusion therapy can be radically reduced when blood replacement is performed according to the accepted standards of Transfusion Medicine. Under these circumstances three major steps are very important for the increase of transfusion safety: (a) epidemiological step, i.e.
donor selection; (b) biological step, i.e. qualification of each blood donation and (c) technological step, i.e. obtaining an agreeable final product. The final blood products have to meet the requirements concerning their quality and safety. These requirements could be ensured only if ethical, clinical, epidemiological and biological parameters are clearly defined. For efficiency and safety it is necessary to improve clinical transfusion practice through alternatives to the traditional blood replacement (such as autologous transfusion), and audit blood request by hospital Transfusion Committees.

Instituted by law in 1994, **haemovigilance** is a national system of surveillance and alarm, from blood collection to the follow up of the recipients, gathering and analysing all untoward effects of blood transfusion in order to correct their cause and prevent recurrence. Haemovigilance contributes to transfusion safety. It enables recognition of already known risks, alerts for emergent risks, preventive actions on critical steps of the transfusion chain, and warrants follow up of safety measures efficiency.

The complications can be broadly classified into two categories:

- Immune Complications
- Non-immune Complications

**Immune Complications**

**Immune complications can further be classified into two categories:**

- Hemolytic (acute and delayed)
- Non-Hemolytic (includes febrile, urticarial, anaphylactic, purpura, etc.)

**Hemolytic reactions** usually involve the destruction of transfused blood cells by the recipient's antibodies. Less commonly, the transfused antibodies can cause hemolysis of the recipient's blood cells. There are acute (also known as intravascular) hemolytic reactions and delayed (also known as extravascular) hemolytic reactions.

**Acute hemolytic reactions** are usually due to ABO blood type incompatibility - in other words, human error plays a large part in these reactions. Blood given to the wrong patient has been attributed to physician error approximately 20% of the time, the operating room is the most common site of this error, and the anesthesiologist is the most commonly implicated physician. This type of reaction has been reported to occur approximately 1 in 25,000 transfusions - but it is often very severe and accounts for over 50% of reported deaths related to transfusion. The severity of the reaction often depends in the amount of blood given.

Symptoms of acute hemolytic reactions include chills, fever, nausea, chest pain and flank pain in awake patients. In anesthetized patients, you should look for rise in temperature, unexplained tachycardia, hypotension, hemoglobinuria, oozing in the surgical field, DIC, shock and renal shutdown.
Management of acute hemolytic reactions mandates that the transfusion be stopped immediately. The unit should be re-checked. Blood from the recipient patient should be drawn to test for hemoglobin in plasma, repeat compatibility testing and coagulation tests. A foley catheter should be placed to check for hemoglobin in the urine. Osmotic diuresis with mannitol and fluids should be utilized (low-dose dopamine may help renal function and support blood pressure). With rapid blood loss, platelets and fresh frozen plasma may be indicated.

**Delayed hemolytic reactions** are generally mild in comparison. These are caused by antibodies to non-D antigens of the Rh system or to foreign alleles in other systems such as the Kell, Duffy or Kidd antigens. Following a normal, compatible transfusion there is a 1-1.6% chance of developing antibodies to these foreign antibodies. This takes weeks or months to happen - and by that time, the original transfused cells have already been cleared. Re-exposure to the same foreign antigen can then cause an immune response. Thus the reaction is typically delayed from two to twenty-one days after transfusion. Symptoms are generally mild and include malaise, jaundice, fever, a fall in hematocrit despite transfusion, and an increase in unconjugated bilirubin. Diagnosis may be facilitated by the direct Coombs test which can detect the presence of antibodies on the membranes of red cells. Treatment is generally supportive. These reactions occur in approximately 1 in 2,500 transfusions and most often in females with previous exposure secondary to pregnancy.

**Non-Hemolytic reactions** are due to sensitization of the recipient to donor white cells, platelets or plasma proteins. These reactions include:

- Febrile
- Urticarial
- Anaphylactic
- Pulmonary Edema (non-cardiogenic)
- Graft vs. Host
- Purpura
- Immune Suppression

**Febrile reactions** are typically due to white cell or platelet sensitization. This reaction is relatively common occurring in 1-3% of all transfusions. The presenting symptom is a rise in body temperature without evidence of hemolysis. Patients with a history of this reaction that require additional transfusions should receive leukocyte poor transfusions. Use of a filter traps most contaminants.

**Urticarial reactions** are characterized by erythema, hives and itching without fever. Again, this is a relatively common reaction and occurs in about 1% of all transfusions. It is thought to be due to sensitization against plasma proteins. The use of packed red blood cells rather than whole blood has decreased the likelihood of this problem. Treatment is with antihistamines for symptomatic relief.
**Anaphylactic reactions** are rare and occur in about 1 of 150,000 transfusions. These are severe reactions that can occur with very small amounts of blood (a few milliliters). Typically, these reactions occur in IgA-deficient patients with anti-IgA antibodies. These antibodies react to transfusions containing IgA. Treatment is with epinephrine, fluids, corticosteroids and supportive measures. IgA deficiency occurs in 1 of 600-800 patients in the general population. Patients with known IgA deficiency should receive thoroughly washed packed red blood cells, deglycerolized frozen red cells or IgA free blood units.

Some patients can develop **pulmonary edema** and present with a picture that looks like adult respiratory distress syndrome (ARDS). This is a rare but serious complication that is thought to be due to the transfusion of antileukocyte antibodies that interact with the patient's white blood cells causing them to aggregate in the pulmonary circulation. Subsequent damage to the alveolocapillary membrane triggers the syndrome. Treatment involves respiratory support as needed.

**Graft versus Host disease** is seen exclusively in immunocompromised patients where cellular blood products containing lymphocytes are given. These lymphocytes can mount an immune response against the compromised recipient. Irradiation of transfusions can be utilized to inactivate the lymphocytes prior to transfusion.

**Post-transfusion purpura** is common with the development of platelet antibodies. The external purpura signal a reaction that may lead to profound thrombocytopenia which usually occurs about one week post transfusion. Plasmapheresis is the recommended treatment.

**Immune suppression** is a debatable complication. The transfusion of leukocyte-containing blood products appears to be immunosuppressive causing a decrease in Natural Killer cell function, decreased phagocytosis and decreased helper to suppressor cell ratios. The reason for this is unclear. The effect was first seen in renal transplant patients in whom preoperative blood transfusions appeared to improve graft survival. The clinical significance of this effect on such things as cancer recurrence and post-operative infection is still unclear.

**Non-Immune Complications**

The non-immune complications can also be classified into two broad categories:

- Complications associated with massive blood transfusion
- Infectious complications

**Massive Transfusion** is usually defined as the need to transfuse from one to two times the patient's normal blood volume. In a "normal" adult, this is the equivalent of 10-20 units. Potential complications from this include coagulopathy, citrate toxicity, hypothermia, acid-base disturbances and changes in serum potassium concentration.
Coagulopathy is common with massive transfusion. The most common cause of bleeding following a large volume transfusion is dilutional thrombocytopenia. This should be suspected and treated first before moving on to factor deficiencies as the cause of coagulopathy.

Citrate toxicity results when the citrate in the transfused blood begins to bind calcium in the patient's body. Clinically significant hypocalcaemia does not usually occur unless the rate of transfusion exceeds one unit every five minutes or so. Citrate metabolism is primarily hepatic - so hepatic disease or dysfunction can cause this effect to be more pronounced. Treatment is with intravenous calcium administration - but identification of the problem requires a high index of suspicion.

Hypothermia should not occur on a regular basis. Massive transfusion is an absolute indication for the warming of all blood and fluid to body temperature as it is being given.

Acid-Base balance can be seen after massive transfusion. The most common abnormality is a metabolic alkalosis. Patients may initially be acidotic because the blood load itself is acidic and there may be a prevailing lactic acidosis from hypoperfusion. However, once normal perfusion is restored, any metabolic acidosis resolves and the citrate and lactate are then converted to bicarbonate in the liver.

Serum potassium can rise as blood is given. The potassium concentration in stored blood increases steadily with time. The amount of potassium is typically less than 4 milliequivalents per unit - so you can see that large amounts of blood at a high rate of delivery is required to raise serum levels of potassium.

Infectious Agents can be passed along with blood transfusion as well.

- Hepatitis
- AIDS
- Other viral agents (CMV, EBV, HTLV)
- Parasites and bacteria

Hepatitis has been an ongoing problem. Until recently, the incidence of hepatitis following transfusion was as high as 10% with the overwhelming majority of these infections caused by hepatitis C. Now that this virus is identified and tested for, the risk is decreased. Currently the risk from transfusion is estimated to be in the range of 1:1,000,000 for hepatitis A, 1:30,000 - 250,000 for hepatitis B and 1:30,000 - 150,000 for hepatitis C.

AIDS is a feared disease but the actual risk is quite low. All blood is tested for the anti-HIV-1 antibody which is a marker for infectivity. Unfortunately, there is a 6-
8 week period required for a person to develop the antibody after they are infected with HIV and therefore infectious units can go undetected. The current risk for HIV infection due to transfusion is estimated to be 1:200,000 to 2,000,000.

**CMV and EBV** are usually the cause of only asymptomatic infection or mild systemic illness. Unfortunately, some of these people become asymptomatic carriers of the viruses and the white blood cells in blood units are capable of transmitting either virus. Immunocompromised and immunosuppressed patients are particularly susceptible to CMV and should receive CMV negative units only.

**Human T cell Lymphocytic Viruses (HTLV-1 responsible for tropical spastic paresis and HTLV-II)** are leukemia and lymphoma retro-viruses that have been reported to be transmitted via transfusion. Screening is done for these, but again those in the window before antibodies are made can be missed. Current risk is estimated at 1:250,000 - 2,000,000.

**Parvovirus B19** has been reported to be transmitted by factor concentrates and occurs in approximately 1:10,000 transfusions. It is associated with aplastic anemia and liver failure, especially in immunologically compromised children.

**Parasitic diseases** reported to be transmitted via blood transfusion include malaria, toxoplasmosis, and Chagas' disease. Fortunately, such cases are rare and the prevalence of these diseases is very low in the United States. Therefore, these are of very little concern in this country.

**Both gram-positive and gram-negative bacteria** can rarely contaminate blood transfusions. To avoid the possibility of significant bacterial contamination, blood should be administered over a period of **shorter than four hours**.

Recognition of the potential risks of allogeneic blood transfusions has resulted in a search for alternative therapies to reduce the frequency of exposure to community-acquired blood products. Meticulous surgical techniques, surgical protocols to reduce blood loss, and the acceptance of lower hemoglobin levels as triggers for transfusion have reduced banked blood use. Acute normovolemic hemodilution, preoperative autologous donation, and intraoperative autotransfusion of shed blood all have been utilized to reduce exposure to allogeneic blood. More recently, the recognition that preoperative anemia is a risk factor for later transfusion has led to research into the role of erythropoietin before both surgery and autologous donation.

**Acute normovolemic hemodilution**

Acute normovolemic hemodilution involves the preoperative exchange of the patient's whole blood with appropriate volumes of crystalloid or colloid solutions so that normovolemia is maintained. The collected blood is stored in bags, with the use of standard anticoagulation. The blood is stored temporarily in the operating room and is delivered as needed during the procedure.
Advantages of acute normovolemic hemodilution include the immediate availability of fresh blood with normal levels of clotting factors, avoidance of the infectious risks of allogeneic stored blood, reduced cost in the processing of the collected units, and avoidance of administrative errors that can lead to hemolytic reactions, even with the use of preoperatively collected autologous units of blood. Unexpected blood loss during the operative procedure may result in hypovolemia, a fall in cardiac output, and physiologic decompensation. This condition is exacerbated with extreme hemodilution. Because acute normovolemic hemodilution is most useful in complex cases with the potential of sudden unexpected blood loss and decompensation, the technique remains confined to a few centers with anesthesiologists experienced in it use.

**Preoperative autologous donation**

Preoperative autologous donation limits exposure to the infectious, immunologic, and hemolytic complications of allogeneic blood transfusion. Because of concern about transfusion-transmitted HIV infections, autologous donation increased until, by 1992, 1 per every 12 units of blood collected in the United States was obtained in this manner. Because autologous donation often involves patients who eventually require no blood and the use of excess blood for patients other than the donor is not recommended, approximately one third of the collected blood is discarded.

Advantages of autologous donation include prevention of transfusion-transmitted viral infections, avoidance of immunosensitization, reduction in the risk of delayed hemolytic transfusion reactions, and augmentation of the blood supply. Autologous donation also is useful in patients with known blood compatibility problems due to alloantibodies. Disadvantages include the risk of administrative errors resulting in ABO incompatibility, risk of bacterial contamination, increased administrative cost, waste of unused blood, and the creation of preoperative anemia that actually may increase the requirement for transfusion. The compensatory erythropoiesis depends on the patient's iron status, but not on age or sex. Increasing the time between the last donation and the date of surgery and supplementing with iron decrease the degree of anemia.

Approximately 10-20% of patients donating autologous blood still require allogeneic blood transfusion. The development of anemia at the time of the first donation has been identified as the most important indicator of the requirement for perioperative transfusion of allogeneic blood. This finding has led to clinical trials of erythropoietin during the period of autologous blood donation.

**Intraoperative autotransfusion of blood**

The intraoperative collection and autotransfusion of shed blood frequently is used in cardiovascular, orthopedic, and trauma surgery. Relative contraindications include
the presence of infection, gross contamination with enteric contents or amniotic fluid, and the potential presence of malignant cells.

Blood may be collected and transfused directly or after cell washing. Washing reduces contamination and cellular debris but also eliminates plasma clotting factors and platelets. Washing does not remove bacteria completely from the recovered blood, and blood should not be autotransfused in the presence of gross bacterial contamination.

Coagulopathy has been described following intraoperative autotransfusion. Several factors may play a role in the clotting dysfunction. As noted above, washed cells are deficient in clotting factors and platelets. Furthermore, the washing process may not eliminate soluble tissue procoagulants. Finally, cell washing may not completely remove residual heparin used in the collection of the shed blood. While the incidence of clinically significant coagulopathy increases as the number of autotransfused units increases, so does the incidence of hypothermia, hypotension, acidosis, and other contributors to a coagulation defect. Thus, defining the exact impact of the autotransfused blood on the resultant coagulopathy becomes difficult to estimate.

Multiple studies of intraoperative autotransfusion have failed to reveal a significant reduction in patient exposure to allogeneic blood transfusion. Nevertheless, intraoperative autotransfusion remains beneficial in selected cases due to the decreased cost of the autotransfused units and the immediate availability of the blood in the event of rapid blood loss. The latter fact makes intraoperative autotransfusion especially attractive in trauma and vascular surgery, where catastrophic blood loss may occur without the warning necessary for proper crossmatching.

V. Materials of activation of students
(questions, tasks, controversial situations, illustrative materials and other).

VI. Materials of selftraining of students on the topic of lecture: literature, questions, tasks.

Literature
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28. Materials of Residential Courses of ESTM.

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