

MINISTRY OF HEALTH PROTECTION OF UKRAINE ODESSA NATIONAL MEDICAL UNIVERSITY DEPARTMENT OF GENERAL SURGERY

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Methodical recommendations for classes of 3rd year students

Academic discipline: «General surgery » Topic: «Transfusion of components and drugs of blood in surgery. Use of modern blood substitutes» Level of higher education: second (master's) Field of knowledge: 22 "Health care" Specialty: 222 "Medicine" Educational and professional program: Medicine

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The program was discussed at the meeting of the department of general and military surgery

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Content module 1-3:

Blood physiology. Transfusion of blood components and drugs. Use of modern blood substitutes.

Plan:				
Blood as a tissue.				
Antigenic systems of blood.				
Determination of blood group.				
Components and drugs of blood.				
Blood Transfusion.				
Complications of blood transfusion.				
Reinfusion of blood. Autohemotransfusion. Donation				
Blood substitutes.				

Topic content:

Blood as a tissue.

Blood (haema, sanguis) - it is a liquid tissue consisting of plasma and blood cells suspended in it. Blood is contained in the vascular system and is in a state of continuous movement. Blood, lymph, and interstitial fluid are the body's internal environments, which wash all cells, delivering them the substances necessary for vital activity, and taking away the end products of metabolism. The internal environment of the body is constant in its composition and physicochemical properties. The stability of the body's

internal environment is called homeostasis and is a necessary condition for life. Homeostasis is regulated by the nervous and endocrine systems. Cessation of blood flow during cardiac arrest leads to the death of the body.

Blood functions:

- I. Transport (respiratory, nutritional, excretory)
- II. Protective (immune, protection against blood loss)
- III. Thermoregulating
- IV. Humoral regulation of functions in the body.

Amount of blood

Blood makes up 6-8% of body weight. Newborns have up to 15%. On average, a person has 4.5-5 liters. The blood circulating in the vessels is peripheral, part of the blood contained in the depot (liver, spleen, skin) is deposited. Loss of 1/3 of blood leads to the death of the body.

Environmental reaction (pH) - normally 7.36 - 7.42. Life is possible if the pH is

between 7 and 7.8.

Blood osmotic pressure = 7,6-8,1 atm.

Composition of blood

Formed elements of blood - blood cells, make up 40 - 45% of blood.

Blood plasma is a liquid intercellular substance of blood, which makes up 55-60% of blood. The ratio of plasma and formed blood elements is called the hematocrit index, because it is determined using hematocrit. When the blood is standing in the test tube, the formed elements settle to the bottom, and the plasma remains on top.

Formed elements of blood

Erythrocytes (red blood cells), leukocytes (white blood cells), thrombocytes.

Erythrocytes are red blood cells without a nucleus, having the shape of a biconcave disc, 7-8 microns in size.

They are formed in the red bone marrow, live for 120 days, are destroyed in the spleen ("graveyard of erythrocytes"), liver, macrophages.

Hemoglobin (Hb) - red respiratory pigment found in erythrocytes. It is synthesized in the red bone marrow, destroyed in the spleen, liver, and macrophages.

Hemoglobin consists of a protein - globin and 4 heme molecules. Heme is a non-protein part of Hb, contains iron, which combines with O2 and CO2. One hemoglobin molecule can attach 4 O2 molecules.

Leukocytes - these are colorless (white) blood cells, containing a nucleus and protoplasm. They are formed in the red bone marrow, live for 7-12 days, are destroyed in the spleen, liver, and macrophages.

Клітини крові



Types of leukocytes, their number

Leukocytes are divided into two large groups: granular leukocytes, or granulocytes, and non-granular - agranulocytes.

Granular leukocytes got their name due to the presence of characteristic granularity in their cytoplasm. Depending on the ability to perceive certain dyes, granulocytes are divided into neutrophils, eosinophils and basophils. Neutrophils make up 60-70% of all white blood cells, eosinophils – 1–4%, basophils 0–0.5%. Agranulocytes are represented by lymphocytes and monocytes. Lymphocytes make up 25-30% of all leukocytes, monocytes - 6-8%. Only 1 mm3 of blood contains 6,000–8,000 leukocytes. An increase in their number in the blood is called leukocytosis. It is noted in acute infectious diseases, inflammatory processes, in various intoxications, after eating. A decrease in the number of leukocytes is called leukopenia. It can be observed when bone marrow function is suppressed.

The structure and functions of various types of leukocytes

Neutrophils have a rounded shape, their diameter is 12 μ m. Cytoplasm in the stained preparation is pink, its granules are stained bluish-pink. The composition of the granularity includes the most various enzymes that ensure the synthesis and splitting of substances, amino acids, glycogen, lipids, RNA. The nucleus, as a rule, consists of 3-4 segments. Nuclei have processes - nuclear appendages.

Neutrophils have a pronounced ability to phagocytosis.

Phagocytosis is the ability of a cell to capture and digest all kinds of substances (microbes, paint, cell debris, etc.).

Neutrophils are short-lived: their lifespan is 8–12 days. In addition to phagocytic neutrophils, they also perform a transport function. They carry antibodies that adsorb them on their surface. Neutrophils also enhance mitotic activity, contributing to the restoration - *regeneration* - of damaged tissues.

Eosinophils have a diameter of $12-15 \mu m$. Their cytoplasm contains spherical or oval granules that are colored yellow-pink. Other cytoplasm is stained blue. The granules contain enzymes, but they lack glycogen.

The core consists of two segments. Eosinophils have weak phagocytic activity. Their main function is to inactivate histamine, which is formed especially in large quantities in diseases associated with increased sensitivity to foreign elements. Eosinophils contain an enzyme that breaks down histamine. In addition, adsorbing the latter, they transfer it to the lungs and intestines, where it is excreted.

It is clear that in the case of increased histamine formation in the body, the number of eosinophils increases.

Basophils – cells with a diameter of 10 μ m. Granules of their cytoplasm are stained dark purple. They contain RNA, glycogen, enzymes, heparins, histamine. Cytoplasm is stained pink. The core is paw-shaped. The main function of basophils is the synthesis of histamine and heparin. Half of blood histamine is in basophils.

Lymphocytes depending on their size, they are divided into three groups: large (15–18 μ m), medium (10–14 μ m), and small (6–9 μ m). Most of all in the blood of small lymphocytes. The shape of lymphocytes is round or oval. Their core is colored dark blue. It occupies almost the entire cell.

Cytoplasm is stained with basic dyes. It contains enzymes, nucleic acids, ATP. Not all lymphocytes have glycogen. The function of lymphocytes is related to the production of γ -globulins. The more cytoplasm contains RNA, the stronger its ability to produce antibodies. Just like neutrophils, lymphocytes can adsorb antibodies and transport them to the focus of inflammation. Lymphocytes neutralize various toxins.

Monocytes – the largest blood cells. Their diameter reaches $13-25 \mu m$. The nucleus is irregular, oval or bean-shaped, with depressions and protrusions. Cytoplasm is stained blue-gray or gray-blue. The cytoplasm contains RNA, polysaccharides and enzymes. Monocytes have a greater capacity for amoeba-like movement than lymphocytes, which is why they have a characteristic phagocytic function. Unlike neutrophils, it is carried out in an acidic environment. Therefore, monocytes actively participate in the fight against infection in foci of inflammation.

Platelets - platelets, colorless anucleate bodies 2-5 μ m in size. They are formed in the red bone marrow, live for 2-5 days, are destroyed in the spleen, liver, and macrophages.

Functions:

- Participation in blood clotting.
- Enzymatic.
- Transport.
- Protective capable of phagocytosis and binding of toxins.

Properties of platelets:

- Adhesiveness the ability to stick to a foreign surface
- Aggregation the ability to stick to each other
- Agglutination sticking together
- Ability to phagocytosis.
- Amoebic movement.

Bernestrum	Діти			Дорослі	
Показник	1 - 6	7 - 12	13 - 15	Чоловіки	Жінки
гемоглобін (Hb), г/л	110 - 140	110 - 145	110 - 150	130 - 160	120 - 140
еритроцити (Er), г/л	3,5 - 4,5	3,5 - 4,7	3,8 - 5,1	4 - 5,1	3,7 - 4,7
лейкоцити (WBC), %	5 - 12	4,5 - 10	4,3 - 9,5	4 - 9	4 - 9
нейтрофіли (NEUT), %					
паличкоядерні	0,5 - 5	0,5 - 5	0,5 - 6	1 - 6	1 - 6
сегментноядерні	25 - 60	35 - 65	40 - 65	47 - 72	47 - 72
еозинофіли (ЕО), %	0,5 - 7	0,5 - 7	0,5 - 6	0 - 5	0 - 5
базофіли (ВА), %	0 - 1	0 - 1	0 - 1	0 - 1	0 - 1
лімфоцити (LY), %	26 - 60	24 - 54	25 - 50	18 - 40	18 - 40
моноцити (MON), %	2 - 10	2 - 10	2 - 10	3 - 11	3 - 11
ШОЕ, мм/г	4 - 12	4 - 12	4 - 15	1 - 10	2 - 15
тромбоцити	160 - 390	160 - 380	160 - 360	180 - 320	180 - 320

Norm of general blood analysis

Antigenic systems of blood.

Isoserology studies the antigenic structures of blood. The AB0 antigen system was discovered in 1900 by the Austrian scientist K. Landsteiner. The presence of group-specific antigens-agglutinogens in erythrocytes and antibodies-agglutinins in blood plasma determines a person's blood group. When the erythrocyte agglutinogens of one person and

plasma agglutinins of another person interact, erythrocyte agglutination occurs, i.e. isohemagglutination reaction. Currently, more than 500 different agglutinogens have been identified in humans, which, like a mosaic, cover blood cells. The most important are those that can cause post-transfusion complications. These agglutinogens are divided into 9 systems: AB0, Rh-Hr, MNSS, R. Kell-Chelano, Duffy, Kidd, Lewis, Lutheran. Several blood groups are distinguished for each of them, each person has about 30 combinations.

Antigens (agglutinogens) – substances of a protein nature, located on surface of formed elements of blood, mainly erythrocytes. The main properties of antigens: immunogenicity, that is, the ability to cause the formation of antibodies in the body and react with them, specificity - they interact only with antibodies of the same name. The temperature optimum of the reaction is +15 - +25 °C.

Antigens of leukocytes identical to antigens in human tissues, are important in transplantology for organ and tissue transplantation. About 200 leukocyte antigens are known, but the most studied are HLA antigens, which are called histocompatibility antigens. Platelet antigens are similar to antigens erythrocytes and leukocytes, but less active. Currently, 4 types are known: PI, ZVS, WAX, Co.

Plasma antigens – α , β , γ - globulins that cause homologous blood syndrome.

Antibodies (agglutinins) – plasma globulins that have the properties to combine with blood cell agglutinogens of the same name, to cause their gluing (agglutinins) and destruction (hemolysins). Agglutinins can be cold - active at temperatures from +4 to \pm +18 °C, thermal - active at \pm 37 °C. Regarding the environment in which agglutinins act, a distinction is made between complete agglutinins, which are active in a physiological solution, and incomplete agglutinins, which are active in a colloidal environment. The agglutination reaction is possible only in the presence of a high-molecular environment: human serum, albumin, gelatin, dextran, polyglukin. That is why, when determining the Rh factor with standard serums with incomplete agglutinins, it is necessary to take the studied erythrocytes in their own serum or add

gelatin. There are agglutinins in human plasma that combine either only with erythrocyte agglutinogens (anti-erythrocyte antibodies) or with leukocyte agglutinogens (anti-leukocyte antibodies), or with platelet antigens (anti-platelet antibodies). The sixth part of agglutinins is inherited and exists throughout life - these are natural agglutinins. Others appear at any period of life as a result of immunization with agglutinins, for example, when Rh-positive blood is transfused to a Rh-negative person - these are immune agglutinins.

Key provisions

- Human blood group is a set of antigens that are present in erythrocytes, leukocytes, platelets, and plasma proteins.
- Blood group is unchanged throughout a person's life.
- Group properties of blood are transmitted according to classical laws of genetics.

AB0 system

Depending on the content of antigens (agglutinogens) A and B in erythrocytes, 4 blood groups are distinguished. In our country, alphabetic and numerical designations of blood groups are accepted: $O\alpha\beta$ (I), $A\beta$ (II), $B\alpha$ (III), ABo (IV).

I group does not contain antigens, blood serum contains agglutinins α i β .

II group містить аглютиноген A на еритроцитах, у сироватці міститься аглютинін β.

III group contains agglutinogen B on erythrocytes, serum contains agglutinin α.IV group contains agglutinogens A and B, there are no agglutinins in blood serum.

During a deeper study of blood groups, it was established that agglutinogen A has three varieties: A1, A2, A3, which differ from each other in their ability to glue erythrocytes. Erythrocytes containing A1 agglutinogens give rapid (up to 1 min) coarse-grained agglutination; erythrocytes containing agglutinogen A2 give delayed (after 5 min) fine-grained agglutination; agglutinogen A3 has weak agglutinating properties. In humans, agglutinogen A1 occurs in 88% of cases, and agglutinogen A2 in 12%.

When determining blood groups, it is necessary to take into account special conditions of a congenital or acquired nature. These include:

- blood chimeras when erythrocytes of different groups are present in human blood, for example, if a patient with Aβ(II) group was transfused with a lot of blood of Oαβ(I) group, or congenital chimerism (more often in fraternal twins);
- 2) 2Bombay blood blood that does not contain agglutinogens A and B, H-antigen, but contains agglutinins; people with such blood are considered carriers of the Oαβ(I) group, but they can only be transfused with Bombay blood, since it contains anti-H antibodies, and the blood of Oαβ(I) people has H-antigen;
- 3) defective blood groups the blood lacks any sign, for example, O,

O1, Ao, Bo, Ooo.

Rhesus system

In 1937, Landsteiner and Wiener discovered the Rhesus factor (Rh factor). In the course of experiments on rabbit immunization with erythrocytes of rhesus macaques (Makakus rhesus), a serum was obtained that agglutinates 85% of human erythrocyte samples, regardless of group affiliation. Thus, it was established the presence in human erythrocytes of a substance of an antigenic nature, similar to that found in Macakirhesus. It was called the Rhesus factor. 6 agglutinogens of the Rhesus system

are of practical importance: three of them are a type of Rh agglutinogen, and three are a type of Hr agglutinogen. The antigen of the Rhesus system is found in erythrocytes in 81-85% of people, they are "Rhesus-positive". 16-19% of people lack Rhesus agglutinogen, they are "Rhesus-negative". Two nomenclatures are used to designate Rhesus antigens. One proposed by Wiener and denoted by the symbols RHQ, rh, rh", HrO, hr, hr". Another nomenclature proposed by Fisher and Reiss, the letters D, C, E, d, c, e are used. Rhesus antigens are inherited from parents and do not change during life. The Rh factor is the strongest antigen and is most often the cause of immunization during blood transfusion, Rh-conflict pregnancy, and can cause post-transfusion complications. When rhesus-positive blood is transfused to a rhesus-negative patient, he may develop immune rhesus antibodies. They can also appear in the blood of a Rhnegative pregnant woman in response to a Rh-positive fetus. Rhesus incompatibility (Rhesus conflict) occurs in case of repeated contact of a sensitized person with the Rhesus factor (blood transfusion, pregnancy). It is necessary to know that a donor who does not have RhO, rh, rh in his blood can be considered Rh-negative." All Rh-negative and most Rh-positive people are Hr-positive, that is, they have Hr agglutinogen. Immunization and complications due to Hr agglutinogens occur rarely. There are many other antigens in the blood. They are designated as MNSS, Kell, Duffy, Kidd, Lutheran, etc. systems, and extremely rarely cause severe post-transfusion complications and hemolytic disease. Currently, antigens are found in leukocytes, platelets, and other protein structures with a total number of about 300 species.

Key provisions

- The Rhesus system is one of the most complex isoserological systems.
- The antigen of the Rhesus system is contained in human erythrocytes, is inherited from parents and does not change throughout life.

- Natural antibodies to Rh-Hr agglutinogens are practically not found, therefore belonging to the Rh-Hr system depends on the combination of agglutinogens.
- The Rh factor is the strongest antigen that can cause immunization.

Previously, **Ottenberg's law** was observed, according to which erythrocytes of transfused donor blood stick together. According to this law, a recipient with the first group was allowed to transfuse donor blood of only group 1, a recipient with the second group donor blood of groups 2 and 1, a recipient with the third group - donor blood of groups 3 and 1, a recipient with group 4 - donor blood of all 4 groups. However, in recent years it has been proven that each group is strictly individual.

So, agglutinogen A has 2 subgroups: A1 and A2, thus, II blood group can be A1b or A2b, IV group A1B0 or A2B0.

In addition, Ottenberg's inverse law became known: with large volumes of blood transfusion, gluing of the recipient's erythrocytes is possible. Therefore, it is currently allowed to transfuse only blood of the same group.

Blood of any group can be either Rh positive or negative, depending on the presence of the Rh factor (Rh factor). About 85% of people have this factor, or are Rh-positive, 15% do not have it, or are Rh-negative. But in recent years it became known that there are 5 main (D, C, c, E, e) and many non-main subgroups of the Rh factor. Subgroup D is found in 85%, the rest - in descending order c>E>C>e. Therefore, the determination of blood groups and the Rhesus factor is of great importance in the clinic.

Determination of blood group

Blood group is a set of agglutinogens - isogenic antigens embedded in the erythrocyte membrane, and agglutinins - antibodies contained in blood plasma. Such a set of blood

agglutinogens and agglutinins is a specific individual biological feature that distinguishes one organism from others.

Agglutination (lat. agglutinatio — gluing) - gluing (sometimes sticking together) into lumps and sedimentation of suspended in liquid microorganisms or individual cells — erythrocytes or leukocytes. Agglutination is caused by special substances that are formed in the body during various diseases, during immunization. The phenomenon of agglutination is used for laboratory diagnostics: human blood group, some infectious diseases, determination of the type of microorganisms, etc.

Using monoclonal antibodies.



Determination of the blood group (erythrocytes) according to the AB0 system with monoclonal reagents (anti-A and anti-B monoclonals, anti-A and anti-B tsoliclones) is carried out using the usual isoserological methods of detecting erythrocyte antigens: in the case of mass determination in blood service institutions - on tablets or in automatic systems; in the case of an individual - on a white porcelain or any other plate with a wettable surface. In patients, determination of blood group can be carried out on identification cards. Although monoclonal reagents are characterized by high activity and avidity, in order to avoid unforeseen errors, two series of anti-A and anti-B reagents are used for each blood group determination. Monoclonal antibodies anti-A and anti-B are applied to a tablet or a plate in one large drop (0.1 ml) under the appropriate labels: "anti-A" or "anti-B". Along with drops of antibodies, one small drop (0.01 ml) of the tested blood is applied. After mixing the reagents and blood, observe the agglutination reaction for 2.5 minutes. In a saline environment, the agglutination reaction is carried out in test tubes by mixing equal volumes (0.1 ml) of antibodies and 2.0 ml of the suspension of the examined erythrocytes.

The results of the reaction are evaluated by the presence or absence of agglutination in the test tubes after urgent centrifugation and shaking. On the identification cards, the blood group is determined according to the instructions attached to them. The evaluation of the results of the agglutination reaction with anti-A and anti-B monoclonal antibodies is given in the table. 2, which also includes the results of determination of agglutinins in the serum (plasma) of donors using standard erythrocytes.

Реакция агглютинации с цоликлонами			Кровь принадлежит
Анти-А	Анти-В	Анти-АВ	к группе
нет	нет	нет	O(I)
есть	нет	есть	A(II)
нет	есть	есть	B(III)
есть	есть	есть	AB(IV)

T.

N⁰	erythro	on of studied ocytes with nal antibodies	The reaction of serum (plasma) research with standard erythrocytes of the group		The examined blood (erythrocytes) belongs to the group	
	анти-А	анти-В	A(II)	B(III)		
1	-	-	+	+	0(1)	
2	+	-	-	+	A(II)	
3	-	+	+	-	B(III)	
4	+	+	-	-	AB(IV)	

1. No agglutination (-) with either anti-A or anti-B monoclonal antibodies. Therefore, the examined red blood cells do not have antigens A and B, and the blood belongs to group O(I). This is confirmed by the presence of agglutinins α (alpha) and β (beta) in the serum (plasma), which are examined by the results of a positive agglutination reaction with standard red blood cells of groups A(II) and B(III).

2. Agglutination (+) is observed only with monoclonal antibodies anti-A. Thus, such red blood cells have only antigen A, and the blood belongs to group A(II). This is confirmed by the presence of agglutinins b (beta) in the serum (plasma) of the examined, based on the results of a positive agglutination reaction with standard red blood cells of group B (III).

3. Agglutination (+) is observed only with anti-B monoclonal antibodies. Thus, the examined red blood cells have only B antigen, and the blood belongs to group B(III). This is confirmed by the presence of agglutinins a(alpha) in the serum (plasma) of the examined erythrocytes, based on the results of a positive agglutination reaction with standard erythrocytes of group A(II).

4. Agglutination (+) is observed with both anti-A and anti-B monoclonal antibodies. Thus, the examined red blood cells have both antigens (A and B), and the blood belongs to group AB(IV). This is confirmed by the absence of agglutinins a(alpha) and b(beta) in the serum (plasma) of the examined erythrocytes, according to the results of a negative agglutination reaction with standard erythrocytes of group A(II) and B(III).

5. In order to exclude autoagglutination, which can be observed in some patients (myeloma, burn disease), as well as in the umbilical cord blood of newborns, in case of AB(IV) blood group, it is necessary to conduct a control study: mix one drop (0.1 ml) of isotonic sodium chloride solution with a small drop (0.01 ml) of the blood under study. Agglutination reaction should be absent.

2. direct method: using standard sera: serum of group 1 contains agglutinins a and b; serum of group 2 - agglutinin b, serum of group 3 - agglutinin a; serum of group 4 (control) does not contain agglutinins.

Blood group is determined in 2 series, sera are applied, which are mixed with the test blood.

	serums				
Blood groups	1	2	3	4	
	ab	b	a	0	
0(ab)	-	-	-	-	
A(b)	+	-	+	-	
B(a)	+	+	-	-	
AB(0)	+	+	+	-	

2. reverse method: using standard red blood cells: standard red blood cells of the 1st group do not contain agglutinogens (0),

2nd group - contain agglutinogen A,

group 3 - agglutinogen B, group 4

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- A and B.
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Blood groups	Standard red blood cells			
	1	2	3	4
	0	А	В	AB
0 (ab)	-	+	+	+
A (b)	-	-	+	+
B (a)	-	+	-	+
AB (0)	-	-	-	-

4 Cross method: a combination of 2 and 3 methods.

Determination of the Rh factor

1. in a water bath:

Standard anti-Rhesus serum is applied to a Petri dish, mixed with red blood cells of the blood under study.

The Petri dish is placed in a water bath for 7 - 10 minutes at a temperature of 46 degrees.

If agglutination occurs, the blood is Rh-positive.

2. express method:

At the bottom of the test tube apply 1 drop of anti-Rhesus serum and 1 drop of the test blood, mix them, the test tube is inverted so that the contents spread along the wall.

After 3 min the presence of agglutination is checked.

To exclude false aggregation of erythrocytes, it is necessary to add 2-3 ml of saline solution.

In the presence of agglutination, the blood is Rh-positive.

3. laboratory methods:

These are gelatin test, indirect Coombs test, determination of Rh factor using monoclonal antibodies, in the presence of polyglucin, albumin, etc.

Determination of Rh factor D (Rh0) by conglutination reaction using gelatin (in a test tube heated to 46 °C) Special equipment:

Standard anti-Rhesus sera with incomplete antibodies.

Standard red blood cells for control.

Centrifuge tubes or any other thin-walled tubes with a capacity of 10-15 ml. Water bath at a temperature of 46°C or dry air thermostat at a temperature of 46°C

10% gelatin solution. Gelatin can be stored with preservatives: sodium sulfacyl (albucid) at the rate of 100 mg of albucid per 10 ml of 10% gelatin solution or with sodium azide at the rate of 10 mg per 10 ml of 10% gelatin solution.

Blood for the study should be taken in the amount of 2-5 ml in a tube without stabilizer. Sign the name, initials and blood group of the person from whom the blood was taken on the tube. Of course, after blood clotting, a small amount of free red blood cells remains at the bottom of the tube, which should be used for testing. If these red blood cells are not enough, you should shake the clot to separate more red blood cells. If blood is taken with 3.8-5.0% sodium citrate solution (0.25 ml of sodium citrate per 1 ml of blood), heparin or other stabilizer, the red blood cells must be washed by adding 0.9% sodium chloride solution to the top of the tube, mixing and centrifuging at 1,500 rpm for 5 minutes at room temperature. For the study it is necessary to use washed red blood cells. To determine Rh blood can be taken from the puncture site of the finger with a glass rod and immediately injected into a test tube with antiretus serum mixed with gelatin in a ratio of 1:2.

To determine Rh blood can be stored in the refrigerator for three days at a temperature of $+(6 \pm 2)$ °C.

In the case of using two series of antiresus serum, three rows of centrifuge tubes or any other tubes (at least 10 ml in volume) in each row according to the number of erythrocyte samples to be tested and two tubes for standard Rh-positive and Rh-negative erythrocytes shall be placed in the rack. Each of the three tubes is marked with the name and initials of the person whose blood will be tested. In the identically marked tubes (three rows) inject one drop (0.05 ml) of the tested red blood cells, and in the control tubes - one drop (0.05 ml) of standard (Rh0+) and (Rh0-) red blood cells. In all tubes add two drops (0.1 ml) of 10% gelatin solution, preheated to dissolution in a water bath at a temperature of 46 °C. In all tubes of the first row add one drop (0.05 ml) of

antiretroviral serum of one series, in all tubes of the second row add 1 drop (0.05 ml) of antiretroviral serum of the second series. The third row is a control to exclude possible nonspecific adhesion of the studied erythrocytes, for example, due to auto-, heat or cold antibodies, and no antiresus serum is added there. The contents of the tubes are mixed by shaking, and the rack with the tubes is placed in a water bath at a temperature of 46 °C for 5-10 minutes or in a dry-air thermostat at the same temperature for 30 minutes. After removing the tubes from the water bath or from the thermostat, 5-8 ml of 0.9% sodium chloride solution is added to them and the contents are mixed by inverting the tubes 1-2 times.

The tubes are viewed in the light with the naked eye or through a magnifying glass with a twofold magnification. The result is evaluated by the presence or absence of erythrocyte agglutination. In case of a positive result, agglutinates look like red lumps on a transparent, almost discolored background of the liquid. In the case of a negative result, the test tube shows an evenly colored light red, slightly opalescent liquid. Samples of red blood cells that agglutinated with anti-D(Rh0) serum are Rh positive (Rh0+). Samples of red blood cells that did not agglutinate with anti-D(Rh0) serum are Rh negative (Rh0). However, the results are considered as reliable if both series of anti-Rhesus serum coincide and after checking the control samples confirming the specificity and activity of anti-Rhesus serum, that is, in the absence of agglutination with standard Rh-negative erythrocytes of the same group and the presence of agglutination with standard Rh-positive erythrocytes of the same group and group 0(I). There should be no agglutination in the tubes of the third (control) row. The presence of agglutination in any tube of the control series indicates the nonspecificity of the reaction. Under these conditions, a positive result with anti-Rhesus serum cannot be taken into account as probable. In such cases, antiresus serum with full antibodies should be used to determine the Rh status or erythrocytes should be pre-washed with warm 0.9% sodium chloride solution to wash out autoantibodies. In case of doubtful results, microscopic examination should be performed.

Detection of Rh antigens using monoclonal antibodies.

Monoclonal reagents (antibodies) are intended for the detection of individual Rh antigens on human red blood cells. They can be used instead of isoimmune sera or in parallel with them. Monoclonal testreagents are monoclonal antibodies produced by a heterohybridoma. Monoclonal antibodies are obtained from the culture fluid of hybridoma progenitor cells. The technology of manufacturing test reagents excludes the possibility of their contamination with pathogenic viruses for humans. Anti-D-super monoclonal antibodies are intended for detection of D(RhO) antigen on human erythrocytes and are used in serological tests instead of or in parallel with anti-D immune serum. Monoclonal anti-D antibodies are available as full (IgM) and partial (IgG) antibodies. Since IgM antibodies do not agglutinate some erythrocyte samples with a weak D variant (in particular, DU), it is necessary to further investigate such "negative" erythrocytes in the gelatin test or indirect Coombs test using anti-D reagents with IgG antibodies. Such reagents are polyclonal sera or monoclonal anti-D-IgG reagents. Anti-D-IgM antibodies induce direct agglutination of D-antigen red blood cells and can be used in any modification of direct agglutination: in tubes, on a plane, in microplates.

Anti-C-super monoclonal antibodies contain antibodies capable of inducing direct agglutination of red blood cells carrying the C(rh') antigen of the Rh system. This reagent has no antibodies of other specificity and is therefore suitable for the detection of C-antigen in erythrocytes of any blood group according to the AB0 system. Monoclonal anti-C antibodies can be used in direct agglutination reactions in tubes, on the plane, in microplates. In case of using the test on a plate, the glass should be preheated slightly. Monoclonal anti-CDE antibodies are made from monoclonal antibodies (IgM and IgG) for the detection of C-antigen (one IgM clone), D-antigen (one IgM clone and one IgG clone) and E-antigen (one IgM clone) in erythrocytes by direct agglutination in tubes, on the plane and in microplates. It should be emphasized that DU-positive erythrocytes are also agglutinated with this reagent. However, in case of a negative reaction, a weak DU phenotype can be detected in an indirect Coombs test using IgG components of anti-CDE monoclonal antibodies. Reaction with

monoclonal anti-Rhesus test reagents can be performed in tubes, on a plate and in microplates.

Types of errors in determining the blood group:

A) Technical errors.

- B) Inferiority of standard sera and standard red blood cells.
- C) Biological characteristics of the blood under study.

Technical errors in determining blood group A) Incorrect location of sera on the plate. B) Incorrect quantitative ratios of sera and erythrocytes.

C) Use of insufficiently clean plates and other objects that

in contact with blood:

a) there should be a separate pipette for each serum;

b) only 0.9% sodium chloride solution should be used to rinse pipettes

sodium chloride solution only.

d) Incorrect recording of the tested blood.

E) Failure to observe the time required for the agglutination reaction:

a) when the reaction is taken into account before the expiration of 5 minutes, it will not have time to occur,

if the test blood contains weak agglutinogens;

b) if the reaction is held for more than 5 minutes, the drops may dry out, simulating

agglutination, and this will also lead to a false conclusion. E) Lack of agglutination due to

high (above 25°C) ambient temperature.

Errors depending on the use of inferior sera A) Weak standard sera with a titer below 1:32 or expired sera may cause weak or late agglutination.

B) The use of standard sera that have been prepared non-sterilely and insufficiently preserved leads to nonspecific "bacterial" agglutination.

Errors that depend on the biological characteristics of the blood under investigation.

A) Errors that depend on the biological characteristics of the red blood cells under study:a) late and weak agglutination is due to "weak" forms of antigenserythrocytes, more often

- presence of weak agglutinogen A2 in groups A(II) and AB(I \lor);

- in the case of determining the blood group only by standard sera without testing the patient's serum for the presence of agglutinins, errors may be observed, as a result of which the blood of group A2B(I \lor) is determined as group B (III), and the blood of A2 (II) - as group 0 (1);

- for the identification of agglutinogen A2, it is recommended to repeat the study with other series of reagents, using other laboratory glassware, with an increase in the reaction registration time;

- in laboratory conditions, the determination is carried out using standard red blood cells, anti-A1 test reagent and anti-A colicion;

b) "panagglutination", "autoagglutination", that is, the ability of blood to give the same nonspecific agglutination with all sera and even with its own;

- the intensity of this reaction decreases after 5 minutes, while true agglutination increases;

- most often "autoagglutination" occurs in hematological, oncological patients, severely burned patients, etc;

- for control, it is recommended to assess whether the tested erythrocytes agglutinate in standard $AB(I \lor)$ serum and isotonic solution;

- blood group at "panagglutination" can be determined after three times washing of the tested erythrocytes;

- to eliminate nonspecific agglutination, the plate is placed in a thermostat at 37°C for 5 minutes, after which the nonspecific agglutination disappears and the true agglutination remains;

- it is advisable to repeat the determination using monoclonal antibodies

(anti-A and anti-B coliciones);

c) erythrocytes of the tested blood are folded into "coin columns", which

can be mistaken for agglutinates under macroscopy;

- adding 1-2 drops of isotonic sodium chloride solution followed by gentle rocking of the plate usually destroys the "coin columns";

d) mixed or incomplete agglutination: part of the red blood cells agglutinates,

and some remain free: is observed in patients of groups A(II), B(III) and AB(I \lor) after bone marrow transplantation, or during the first three months after blood transfusion of group 0(1);

- heterogeneity of peripheral blood erythrocytes is clearly verified in the Oia Mes gel test.

B) Errors that depend on the biological characteristics of the tested serum (when determining the blood group by the cross method):

a) detection of antibodies of other specificity in the serum during routine testing, which appeared as a result of previous sensitization:

- it is advisable to determine the specificity of the antibodies;

- individual selection of compatible donor blood is mandatory for the immunized recipient;

b) absence of anti-A and anti-B antibodies (possibly in infants and patients with suppressed humoral immunity);

c) agglutination of standard erythrocytes, including group 0(1) with

formation of "coin columns" or in the presence of cold antibodies.

Components and blood products. Classification of transfusion media Red blood cells: - erythrocytes;

- red blood cells in an additional solution (red blood cell suspension); - red blood cells with the platelet layer removed;

- red blood cells with the removed platelet layer in an additional solution (suspension of red blood cells with the removed platelet layer);

- red blood cells depleted in leukocytes;

- leukocyte-depleted red blood cells in an additional solution (suspension of leukocytedepleted red blood cells);

- washed red blood cells; - red blood cells, apheresis.

Platelets:

- platelets recovered from a dose of blood;

- platelets recovered from a dose of blood, depleted of leukocytes;

- platelets (platelet concentrate), apheresis;

- platelets recovered, combined into one dose;

- platelets recovered, combined into one dose, depleted of leukocytes.

Plasma:

- frozen plasma;
- fresh frozen plasma;
- plasma depleted of cryoprecipitate (cryosupernatant plasma);
- leukofiltered plasma;
- frozen cryoprecipitate.

Granulocytes:

- granulocytes, apheresis.
- Blood components are virus-activated:

- virucidally inactivated blood components (indicating the method of inactivation).

List of donor blood products:

- 1. complex action preparations: albumin 5, 10, 20%; polybiolin;
- haptoglobin; ceruloplasmin; glutamine.
- 2. immunologically active drugs:
- immunoglobulins (normal and specific);
- hydrolyzates of donor blood cells; leukocyte human interferon.
- 3. blood coagulation system preparations:
- cryoprecipitate;
- prothrombin complex;
- fibrinogen;
- concentrates of coagulation factors VIII, IX, XI.
- 4. protease inhibitors:
- alpha-1-antitrypsin and alpha-2-macroglobulin.
- 5. recombinant drugs that are biotechnological:
- blood coagulation factors VIII, IX, VII;

- recombinant activated protein C.

Red blood cells are a hemotransfusion medium containing red blood cells and having a hematocrit of up to 80%. Red blood cells are obtained from preserved blood by extracting plasma. In medical practice, several types of red blood cells can be used, depending on the method of procurement and indications for administration.

Indications:

Anemia requiring red blood cell transfusion:

1) in hemodynamically stable hospitalized patients, follow restrictive transfusion tactics - consider transfusion at Hb \leq 7 g/dL (in patients after orthopedic or cardiac surgery or with pre-existing cardiovascular disease, consider transfusion at Hb \leq 8 g/dL) or when clinical symptoms of anemia occur (chest pain orthostatic hypotension, tachycardia unresponsive to infusion of solutions, congestive heart failure);

2) in some patients in a life-threatening condition, more liberal transfusion criteria should be applied:

a) in patients with acute coronary syndrome - keep Hb >8 g/dl;

b) in the early stages of severe sepsis (first 6 hours), if insufficient oxygen delivery is confirmed, consider transfusion to maintain Hb

9-10 g/dl;

3) in patients with ischemic stroke, as well as in cases of traumatic brain injury with symptoms of brain ischemia - keep Hb level >9 g/dl, and in patients with subarachnoid bleeding - Hb >8 g/dl.

Contraindications:

Alloimmunization with leukocyte antigens (in this case - prescribe leukocyte-depleted red blood cell mass), hypersensitivity to plasma proteins (then - washed red blood cells), in case other methods are sufficient for the treatment of anemia.

Platelet concentrate (PC)

Platelet concentrate is a cellular component isolated from fresh whole blood, which contains platelet concentrate in a therapeutically effective form and suspended in a small amount (50-60 ml) of plasma. Freshly prepared donor blood intended for platelets is not cooled below the temperature of +20 - (+) 24 °C and platelet-rich plasma is separated by centrifugation (no later than 8 hours after exfusion).

The platelet content per unit volume obtained by centrifugation is from 45 to $85.0 \times 109 / 1$ platelets. In thrombocytapheresis, a larger number of platelets can be obtained - 500.0- $600.0 \times 109 / 1$. The widespread use of CT in medical practice is evidenced by the fact that in Western Europe, a significant amount of freshly prepared donor blood (from 25 to 50%) is used to obtain platelet concentrate.

Indications:

Thrombocytopenia or platelet dysfunction with symptoms of hemorrhagic diathesis. In each case, the decision to transfuse platelet mass should be based not only on the platelet count, but also on a holistic assessment of the patient's clinical condition.

The mechanism of action of donor blood components and calculations for their use.

The main mechanism of action of the transfused red blood cell components is the oxygen transport function (the ability of red blood cells to bind oxygen to hemoglobin in the lungs, transfer and give it to the tissues, as well as take carbon dioxide from the tissues). In addition to the basic respiratory function, red blood cells are involved in blood clotting and fibrinolysis due to an agent similar to factor PI in platelets, as well as erythrokinase, which activates and converts profibrinolysin to fibrinolysin. The large surface of red blood cells (4000 m2), rapid turnover of non-deposited red blood cells (45 seconds), pronounced sorption capacity determines the detoxification function. According to the modern concept of component hemotherapy, red blood cells are transfused to the patient mainly for replacement purposes. The advantages of transfusion of red blood cell components over the transfusion of preserved donor blood are

- increased oxygen transport activity in a small volume of transfusion medium;

- absence or minimal content of sensitizing factors (plasma proteins, leukocytes, platelets), especially in washed red blood cells;

- absence or minimal content of decay products and vasoactive substances

(K, Na, nitrogen, serotonin, histamine);

- reduction in the number of microaggregates;
- absence of plasma hemocoagulation factors and a significant number of platelets;
- reduced risk of infection with vector-borne infections.

Fresh frozen plasma (FFP) is native plasma frozen at -30-(-)40 °C, obtained from donor blood no later than 4-6 hours after blood donation. Plasma composition: water - 90%, protein - 7-8%, organic matter - 1.1%, inorganic matter - 0.9%.

Plasma contains a large number of biologically active components - more than 100 types of proteins, lipids, carbohydrates, lipoproteins, glycoproteins, metalloproteins, enzymes, vitamins and hormones.

Plasma is prepared by the following methods:

- centrifugation;
- manual (interrupted) plasmapheresis;
- automatic plasmapheresis with the use of blood separators.

SPP has high therapeutic properties due to the preservation of all protein coagulation factors in it, depending on the storage time and temperature.

Transfusions of fresh frozen plasma (FFP) are widely used in clinical practice. However, according to the results of scientific studies, a significant number of FFP transfusions are performed unreasonably due to the lack of unified recommendations for determining the indications for FFP transfusions. In medical practice, fresh frozen plasma (FFP), cryoprecipitate and plasma preparations are widely used: albumin, gamma globulins, concentrates of blood coagulation factors, physiological anticoagulants (antithrombin III, proteins C and S), components of the fibrinolytic system.

Indications:

1) symptomatic blood coagulation disorders, especially in patients with deficiency of several blood coagulation factors (the method of choice in DIC syndrome in case of active bleeding, or when it is necessary to perform invasive intervention, in massive transfusions), in cases of deficiency of one of the factors, when there are no appropriate preparations of

plasma coagulation factors, the technology of preparation of which includes inactivation of infectious agents;

2) thrombotic thrombocytopenic purpura and atypical form of hemolytic uremic syndrome

3) if necessary, immediate discontinuation of vitamin K antagonists, if there is no prothrombin factor concentrate

4) during therapeutic plasmapheresis (in justified cases, especially in patients with thrombotic thrombocytopenic purpura).

Do not overfill the NWP:

1. to replenish the volume of circulating blood, unless there is a deficiency of coagulation factors;

2. as a source of immunoglobulins;

3. as a source of protein in exhausted patients;

4. in patients with allergy to plasma proteins;

5. in case of clotting factor deficiency, if the appropriate concentrate is available.

Cryoprecipitate is a protein preparation of isogenic human blood plasma, which contains antihemophilic factors - a cold-insoluble fraction of donor plasma, which remains after defrosting of the CPP at a temperature of 1 to 6 °C. This cryoglobulin precipitate contains about 50% of factor VIII, 20-40% of fibrinogen, impurities of factor XIII, fibronectin. Antihemophilic factor VIII can be in the form VIII:C and in the form VIII:FV (von Willebrand factor). One dose of cryoprecipitate contains at least 70 IU per dose.

Indications:

1) deficiency or qualitative changes (hereditary or acquired) of fibrinogen (e.g. after fibrinolytic therapy), if its concentrate is not available (fibrinogen concentration <1.5 g/L in patients with clinically significant bleeding or <1 g/L in patients undergoing invasive interventions associated with the risk of clinically significant bleeding);

2) ICE syndrome and other complex coagulation factor deficiencies;

3) congenital plasma coagulation disorders (von Willebrand's disease, hemophilia A, factor XIII deficiency), only if the appropriate concentrate is not available

4) bleeding associated with hemostatic disorders in acute kidney injury (efficacy is controversial).

Canned donor blood

1 dose = 450 ml of blood ($\pm 10\%$) with preservative, volume ≈ 500 ml. It does not contain functional platelets and labile clotting factors (V and VIII). Transfusion of 1 dose usually increases the concentration of hemoglobin (Hb) ≈ 1 g/dl, hematocrit in the range of 3-4%.

Indications:

Simultaneous erythrocyte deficiency and significant decrease in circulating blood volume, if no appropriate blood components and blood substitutes are available.

Granulocytes, apheresis (granulocyte concentrate)

Granulocytes, in plasma ($\geq 1.2 \times 1010$), obtained from one donor by apheresis. The preparation contains many impurities of other cells: other leukocytes, erythrocytes and 3-7 \times 1011 platelets. If necessary, it can be stored at

20-24°C for 24 hours after the leukapheresis procedure.

Indications:

Rarely used, in life-threatening bacterial or fungal infections in patients with agranulocytosis (neutrophils $<500/\mu$ L) or granulocyte dysfunction.

Not recommended for prophylaxis.

Transfusions should be repeated daily until the patient's bone marrow function is restored, the infection is controlled, or there is no improvement despite the administration of high doses of granulocyte concentrate or in case of serious adverse reactions.

Blood transfusion, absolute and relative indications and

contraindications.

Basic principles of modern transfusiology:

It is considered erroneous to transfuse whole blood, since blood transfusion is, as mentioned above, an operation - tissue transplantation. When transfusing blood, sensitization of the body, post-transfusion complications are possible. Therefore, a new tactic has been put forward: component hemotherapy, that is, transfusion of only those blood components that are needed in each case. Transfusion of whole blood is justified only in cases of massive blood loss.

The principle of "one donor - one recipient", that is, for the treatment of 1 patient it is necessary to use blood components from 1 or a minimum number of donors, which reduces the likelihood of transfusion complications.

Transfusion of the same group of blood, that is, the patient is transfused with blood of the same group and the same Rh factor. Only in exceptional cases, Rh-negative blood of group 1 can be transfused to a patient with any blood group in the amount of up to

500 ml.

Blood transfusion is performed only by a physician: a doctor, a doctor on duty, a doctor of the transfusion department, and during surgery - by an anesthesiologist or surgeon who is not involved in anesthesia or surgery.

Transfused blood must be tested for HIV, hepatitis, syphilis!

Indications for blood transfusion are determined by the need:

- replacement of the volume of lost blood (substitution);
- activation of the body's defenses (stimulation);
- reduction of intoxication (detoxification);
- increase blood clotting to stop bleeding.

Absolute indications are conditions in which the use of whole blood cannot be replaced by other methods of treatment, and the refusal of blood transfusion can lead to a sharp deterioration in the patient's condition or even death.

Such conditions include:

- 1. acute blood loss (more than 21% of the circulating blood volume).
- 2. traumatic shock of II-III degree.
- 3. severe traumatic operations.
- 4. blood diseases.

Relative indications:

- 1. anemia (hemoglobin < 80 g/l, hematocrit < 30 %).
- 2. preoperative preparation.
- 3. chronic anemia.
- 4. intoxication in purulent-septic pathology.
- 5. ongoing bleeding.
- 6. disorders of the blood coagulation system.
- 7. decreased immune status of the body.
- 8. some types of poisoning.

Contraindications to blood transfusion

I. Absolute contraindications - acute cardiopulmonary insufficiency (pulmonary edema). However, in case of significant blood loss and traumatic shock of II-III degree there are no absolute contraindications to blood transfusion, blood should always be transfused.

II. Relative contraindications.

- 1. fresh thrombosis and embolism.
- 2. severe disorders of cerebral circulation.
- 3. ischemic heart disease.
- 4. septic endocarditis.
- 5. myocarditis with circulatory failure of the third degree.
- 6. severe functional disorders of the liver and kidneys.
- 7. hypertension of the third degree.
- 8. allergic diseases (bronchial asthma, polyvalent allergy).
- 9. acute and disseminated tuberculosis.
- 10. rheumatism.

Tests performed before blood transfusion. The order of blood transfusion

When transfusing blood, the doctor is obliged to conduct:

Determination of the blood group and Rh factor of the patient and donor blood, despite the marks in the passport and in the patient's medical history and on the donor blood label. Group compatibility test: 2 - 3 drops of the patient's (recipient's) blood serum are applied to the Petri dish, a small drop of the donor's blood is added, they are mixed and the result is observed for 5 minutes. There should be no agglutination of erythrocytes.

If agglutination appears, this blood is incompatible!

Polyglucin method - 2 drops of patient's serum, 1 drop of donor's blood and 1 drop of 33% polyglucin solution are added to the test tube. The contents are mixed, the tube is rotated so that the contents spread along the walls. After 5 minutes, 3 - 4 ml of saline is poured into the tube. Agglutination should not appear.

1) Determination of donor and recipient group belonging by AB0 system (see above for the principle). It is allowed to transfuse only blood of the same group according to the AB0 system.

2. determining the Rh blood type of the donor and recipient (see below for the principle). It is allowed to transfuse only blood of the same group according to the Rh system.

In addition to the main blood group systems (AB0 and Rh), there are about 20 non-main blood group systems

(M, S, KK...). The blood belonging must be determined by the main systems. Incompatibility of donor and recipient blood by non-major group systems is excluded by means of compatibility tests: the recipient's blood plasma should not contain agglutinins to the agglutinogens of the donor's red blood cells (therefore, the recipient's plasma is mixed with the donor's blood in the ratio of 10

3. test for individual group belonging of donor and recipient blood. It is performed by mixing the recipient's plasma with the donor's blood on a clean, dry surface at room temperature without adding colloids. Under these conditions, total antibodies react (cold agglutination). The reaction is recorded by the absence or presence of agglutination. Its presence indicates the incompatibility of the donor and recipient blood - transfusion should not be performed.

1. Rh-compatibility test of donor and recipient blood. It is performed by mixing the recipient's plasma with the donor's blood, adding a colloid (gelatin, albumin) and placing it in a water bath (46 degrees). Under such conditions, incomplete antibodies react; often such antibodies are antibodies to the Rh factor (hence the name of the test). The reaction is recorded by the absence or presence of an agglutination reaction. Its presence indicates the incompatibility of donor and recipient blood

- blood transfusion cannot be performed.

5. Biological test - the recipient is injected three times intravenously with 10-15 ml of blood, the system is blocked for 3-5 minutes and the patient is observed, the patient is monitored, complaints are asked, blood pressure and pulse are measured. Possible complaints: shortness of breath, high blood pressure, tachycardia, discomfort in the heart and lower back, pale or flushed skin, hematuria.

Legal aspects and documentation of blood transfusion

Transfusion of blood components has a number of risks, which accompanies the use of this therapeutic method with certain legal issues. There are two main causes of legal conflicts related to hemotransfusions: iatrogenic injuries and failure of medical staff to take into account the rights of patients. Iatrogenic injuries are unintentional and inevitable damage to the functions or structure of the body caused by medical intervention. There are three circumstances that allow to systematize iatrogenic injuries associated with hemotransfusion:

1. Foreignness of blood components, which leads to immune reactions of the body (from moderate chills to hemotransfusion shock even with confirmed group compatibility)

2. Possible infection, metabolic and functional inadequacy of preserved blood components

3. Relatively complicated technology of preparation and transfusion of the blood medium.

In the legal aspect, all iatrogenic pathology resulting from hemotransfusion therapy can be associated with each of the following circumstances or their complex:

1. The direct effect of the method included in the hemotransfusion program itself

2 Wrong choice of blood component, dose or transfusion program

Errors of the procedure, often associated with non-compliance or violation of transfusion techniques and requirements of existing instructions.

These three reasons form the basis of legal conflicts that arise in connection with hemotransfusion.

Another reason for ethical and legal conflicts in hemotransfusions is violation or disregard of patients' rights. The rights of patients are regulated in the Law of Ukraine

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"Fundamentals of the Legislation of Ukraine on Health Care", which was adopted by the Verkhovna Rada of Ukraine on 19.11.1992 under No. 2801-XII. This Law with additions and amendments is still in force. This is a consolidated legislative act, the leading principles of which are a clear definition of the rights and obligations of citizens in the field of health care, the establishment of state guarantees and legal protection. This act is based on the articles of the Constitution of Ukraine regarding the rights of Ukrainian citizens. A balanced attitude to the rights of patients should be the norm of everyday medical practice also because hemotransfusion is not free from risk and the patient has the right, stipulated by law, to receive full information about all the problems and adverse effects related to the prescribed transfusion and to know what can threaten his health if he refuses to use hemotransfusion media. This information should be provided in a form that will help the patient to have an adequate idea of the nature of the medical intervention. This is clearly required by the modern European Convention on Human Rights and the law of Ukraine. In addition to providing information, the doctor's duty is to obtain informed consent from patients for hemotransfusions. In particular, Article 39 "Duty to provide medical information" states: "The doctor is obliged to explain to the patient in an accessible form the state of his health, the purpose of the proposed research and treatment measures, the prognosis of possible development of the disease, including the presence of risk to life and health. The patient has the right to get acquainted with his medical history and other documents that may serve for further treatment. In special cases, when full information may harm the patient's health, the doctor may limit it. In this case, he informs the family members or legal representative of the patient, taking into account the personal interests of the patient. The doctor acts in the same way when the patient is unconscious.

The legal principles of obtaining or not obtaining the patient's consent are set out in Article 43 "Consent to medical intervention" of the Law of Ukraine. It should be noted

that there are no mandatory legal documentary forms of such patient consent. Legally, the patient's signature must be obtained only in case of refusal of treatment, if this refusal threatens the patient's life and he is informed about it.

Thus, before each hemotransfusion, the doctor records in the medical history a pretransfusion epicrisis (indications for transfusion, transfused medium, its dose and method of infusion).

The transfusion is recorded in the "Transfusion Media Transfusion Log", and in the medical history - in the form of a blood transfusion protocol, or in the

"Transfusion media transfusion registration sheet".

After hemotransfusion, the patient is monitored, thermometry is performed three times every hour, macroscopic assessment of the color and amount of urine. This information is recorded in the medical history in the observation diary. The next day after hemotransfusion, a complete blood count and urine test are taken.

Consent to blood transfusion

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Protocol of blood transfusion and its components

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Registration sheet for transfusion of transfusion fluids

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The main orders of the Ministry of Health:

1) Orders of the Ministry of Health of Ukraine Order of the Ministry of Health of Ukraine dated 14.12.2010 № 1112 "On approval of the Regulations for blood transfusion institutions (on the organization of management of the quality and safety system of donor blood and its components)" 2) Order of the Ministry of Health of Ukraine dated 09.03.2010 № 211 "On approval of the Procedure for monitoring compliance with safety and quality indicators of donor blood and its components". 3) Order of the Ministry of Health of Ukraine dated 14.02.2012 No. 110 "Transfusion fluid transfusion registration log" and "Transfusion fluid transfusion registration sheet" 4) Order of the Ministry of Health of Ukraine dated 19.02.2013 No. 134 "On approval of the Procedure for screening donor blood and its components for hemotransmissible infections". 5) Order of the Ministry of Health of Ukraine dated 17.12.2013 No. 1093 "On Approval of the Instruction on the manufacture, use and quality assurance of blood components". 6) Order of the Ministry of Health of Ukraine of 29.05.2013 № 435 "Protocol of blood transfusion and its components"

7) Order of the Ministry of Health of Ukraine dated 08.02.2021 No. 207 "On Amendments to the Procedure for Quarantine of Donor Plasma and to the Procedure for Medical Examination of Blood Donors and (or) its Components" Registered with the Ministry of Justice

of Ukraine on March 26, 2021 under No. 404/36026

8) Order of the Ministry of Health of Ukraine dated 07.03.2022 No. 424 "On the organization of meeting the needs of donor blood and blood components in martial law"

Action of transfused blood:

1. Replacement effect - in cases of massive blood loss, chronic anemia. In these cases, the therapeutic effect is associated with an increase in the OCC, an increase in the respiratory surface of erythrocytes, improvement of oxygen metabolism.

2. Hemostatic effect - due to the infusion of blood clotting factors with blood, especially with direct blood transfusion or transfusion of freshly prepared blood.

3. Detoxifying effect - due to the infusion of plasma proteins with blood, absorbing toxic substances.

4. Immunobiological effect - due to the content of antibodies in the blood.

5. Stimulating effect - due to plasma proteins, metabolism is enhanced, tissue regeneration is stimulated.

Massive transfusion

Massive transfusion of blood components - volume equivalent over 24 hours or transfusion of >4 units per hour or replacement of 50% of the circulating blood volume in 3-4 hours in adults and >40 ml of blood components/kg in children.

Ultra-massive transfusion - defined as the use of more than 20 units of blood over 24-48 hours.

Damage control resuscitation - includes the concept of transfusion of a fixed ratio of blood components in order to prevent the development of dilutional coagulopathy, along with the control of factors that can exacerbate the manifestations of hypocoagulation - acidosis, hypothermia, fibrinolysis.

When to perform massive hemotransfusion?

- Severe bleeding, inability to stop quickly and adequately (intracavitary bleeding) + ABC score = immediate activation of the massive hemotransfusion protocol.

Moderate bleeding that continues and is significant, but does not reach the level of severe bleeding (the need for transfusion of more than 4 units of red blood cells or in the presence of drainage, the continuation of bleeding through it more than 200 ml / h)
massive hemotransfusion should be performed.

- Severe bleeding that is stopped effectively - stitching of the scalped wound, compression of venous bleeding from the limbs, tourniquet on arterial bleeding from the limb. Is it necessary to carry out massive hemotransfusion? Not recommended!!!! - Correction is carried out according to clinical and laboratory needs.

Criteria for blood suitability for transfusion:

The presence of a label on the bottle with full donor data.

Shelf life: canned blood is stored in a refrigerator at a temperature of +4 degrees for 21 days. The shelf life can be extended by using new preservatives, freezing blood, etc.

Macroscopically: blood should be three-layered: red blood cells at the bottom, a layer of leukocytes, and plasma on top. There should be no flakes, fibrin threads in the plasma. There should be no hemolysis, i.e. red color of the plasma. In case of accidental mixing of 3 layers, it is necessary to defend the blood.

Preservation of the tightness of the vial. It is not allowed to transfuse blood from 1 vial to several patients, if there are cracks in the vial, from previously hidden vials.

Complications of blood transfusion.

All complications can be divided into 3 main groups: 1) mechanical complications; 2) reactive complications;

3) complications caused by transfusion of infected donor blood. Mechanical complications include acute cardiac dilatation, air embolism, thromboembolism, needle thrombosis, vein puncture, extravascular or intravascular injection.

Reactive complications include fever, allergic reactions, massive transfusion syndrome, hemotransfusion and citrate shock, potassium intoxication, thrombosis.

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Posttransfusion fever (hemotransfusion pyrogenic reactions) is caused by the entry into the blood of pyrogenic substances - decay products of proteins and microorganisms.

Most often, fever occurs as a result of the interaction of the recipient's antibodies with transfused leukocytes, platelets or immunoglobulin.

Fever usually occurs 1.5-2 hours after blood transfusion. The patient has a feeling of heat, chills, sometimes headache, vomiting. Body temperature rises to 38-39 ° C. There are three degrees of fever, and accordingly the severity of the febrile reaction:

In case of a mild febrile reaction, the patient feels general weakness, chills, body temperature rises (within 1 °C).

A moderate febrile reaction is manifested by chills, weakness, headache, mild abdominal and lower back pain, and an increase in

body temperature by 1.5-2 °C.

In case of a severe febrile reaction, severe chills, headache, nausea, vomiting, shortness of breath, low back pain, cyanosis of the lips, and a significant increase in body temperature (more than 2 °C) are observed.

Mild and moderate febrile reactions disappear in a few hours.

Severe febrile reaction lasts longer.

Treatment.

Mild and moderate febrile reactions do not require treatment. The patient should be warmed (cover with a blanket, put a heating pad to the feet, drink hot tea). In case of severe febrile reaction it is necessary to warm the patient and administer painkillers (morphine hydrochloride, norphine, tramal, promedol); drugs that normalize cardiac activity (sulfocamphocaine, caffeine, cordiamine), antihistamines (dimedrol, diazoline, suprastin), corticosteroids (hydrocortisone, prednisolone), antipyretics (acetylsalicylic acid, ascofen, paracetamol). Intravenously administer 10 ml of 10% calcium chloride solution, 500 ml of 5% glucose solution with ascorbic acid.

Allergic reactions

Allergic reactions are caused by sensitization of the body to plasma proteins of donor blood. They occur in case of repeated transfusion of blood and plasma, administration of protein substances and protein preparations, as well as in some diseases (chronic inflammatory processes, malignant tumors, blood diseases).

An allergic reaction can be observed during blood transfusion or 15-20 minutes after its completion. The febrile reaction is accompanied by urticaria, allergic edema, shortness of breath, etc.

Allergic reactions are often mild (except for anaphylactic shock). They disappear in 30-40 minutes. Urticaria, joint pain, itching can last 1-2 days.

Treatment.

If an allergic reaction occurs during blood transfusion, it should be stopped immediately. The patient is intravenously administered 10 ml of 10% calcium chloride solution and 5-10 ml of 5% ascorbic acid solution, diazolin, dimedrol or suprastin, corticosteroids, caffeine, sulfocamphocaine, if indicated.

Massive transfusion syndrome

Transfusion of large amounts of blood is accompanied by massive transfusion syndrome (homologous blood syndrome). This syndrome develops due to the introduction of preserved donor blood containing sodium and potassium citrate into the recipient's body. It can be caused by changes in biochemical constants and form elements that occur during blood storage, immunological incompatibility of donor and recipient blood by erythrocyte, leukocyte and platelet antigens and plasma protein antibodies, which are practically not taken into account during the selection of donor blood.

In massive transfusion syndrome, vasospasm, increased viscosity of the recipient's blood, gluing of red blood cells, capillary occlusion, circulatory disorders, small hemorrhages in the liver and kidneys, blood stasis in the lungs are observed. Blood

does not clot, bleeding occurs.

Prevention.

In order to prevent this complication, only fresh preserved blood should be transfused, and during transfusion, low molecular weight plasma replacement solutions (rheopolyglucin, rheomacrodex, neocompensan, etc.) should be administered intravenously.

Hemotransfusion shock

A dangerous complication of blood transfusion is hemotransfusion shock. It is caused by the transfusion of AB0 and Rh factor incompatible blood, or the transfusion of infected or altered blood (blood that was accidentally frozen and thawed, or blood heated to a temperature above 40 ° C). These complications cause destruction of donor red blood cells, their hemolysis and release of toxic decay products (histamine, bradykinins, catecholamines, etc.). Sometimes hemolysis of the recipient's red blood cells under the influence of donor blood agglutinins is also possible. This happens in the case of blood transfusion 0(I) to recipients with other blood groups. All this eventually leads to shock, severe intoxication, DIC, acute renal failure.

There are three periods in the course of hemotransfusion shock:

I - the period of shock;

II - period of renal failure; III - recovery.

Period of shock.

It can develop even during blood transfusion, after the introduction of 20-40 ml of blood into the bloodstream. The patient becomes restless, complains of feeling hot, tightness in

the chest, headache and pain in the lumbar region, nausea, vomiting. Pain is caused by spasm of cerebral, mesenteric and renal vessels. Pulse becomes frequent, blood pressure decreases. The pain is so severe that patients begin to scream. The patient's face first turns red and later pale, chills appear, body temperature rises. Subsequently, involuntary urination and defecation are observed. Hemoglobinuria and hemoglobinemia begin. Jaundice develops rapidly.

Period of renal failure

Characterized by signs of kidney damage. Urine output decreases or stops altogether. Uremia develops, which is characterized by headache, nausea, vomiting, loss of appetite, diarrhea, adynamia, drowsiness, chills, increased blood pressure. Pallor and jaundice of the skin, pastiness and edema increase. Body temperature rises to 38 °C and more. Anemia develops, blood levels of urea, creatinine, bilirubin, potassium increase. Protein, leukocytes, erythrocytes, cylinders are found in the urine.

Death or recovery.

In case of severe hemotransfusion shock, patients die on the 3rd - 18th day after hemotransfusion. In case of its favorable course, from the 2-3rd week the patients' condition begins to improve and the recovery period begins. The first sign of the recovery period is the restoration of diuresis. It gradually increases and by the 8-12th day can reach 3-4 liters per day. The general condition of the patient gradually improves. However, after the recovery of renal function, general weakness, increased fatigue, and reduced concentration ability of the kidneys may be observed for a long time. Clinical manifestations of hemotransfusion shock in Rh incompatibility have much in common with the shock that develops in AB0-conflict, but it occurs more often after blood transfusion and has a less acute period.

Treatment of hemotransfusion shock in AB0- and Rh-conflict is the same.

It depends on the period of shock.

In case of the first signs of hemotransfusion shock, blood transfusion is stopped, the blood transfusion system is disconnected and a system with saline is connected (the needle is not removed from the vein!).

Treatment of hemotransfusion shock.

Administer 90-120 mg of prednisolone, 10 ml of 2.4% eufiline solution, 100 mg of furasemide. Antihistamines and narcotic analgesics should also be administered. Start infusion therapy. Rheopolyglucin, polyglucin, gelatinol are administered. To obtain an alkaline urine reaction, 4% sodium bicarbonate solution is administered. To remove free hemoglobin, polyionic solutions are transfused. Massive plasmapheresis is highly effective. In order to prevent intravascular coagulation, it is advisable to administer 5000 units of heparin, antienzymatic drugs (Contrical).

After the patient is out of shock and in the presence of acute renal failure, hemodialysis is performed using an "artificial kidney" apparatus. During the recovery period, symptomatic therapy is prescribed.

Citrate shock

Citrate shock develops in case of rapid transfusion of large amounts of blood stabilized with sodium citrate. This is more common in patients with liver or kidney disease. An increase in the level of sodium citrate in the blood serum is accompanied by a decrease in the content of ionized calcium, with which sodium citrate forms a complex, which causes a number of reactions, namely: spasm of the lungs, heart, weakening of myocardial contractile function, electrolyte metabolism (mainly calcium and potassium ions). All this leads to impaired hemodynamics and nervous system function. A dose of sodium citrate of 10 mg/kg per 1 minute is considered toxic, which corresponds to the administration of 2-3 ml of preserved blood per 1 kg of the recipient's body weight per 1 minute.

Clinically, citrate shock is manifested by increased pulse rate, arrhythmia, decreased blood pressure, shortness of breath, convulsions. These signs appear during or at the end of blood transfusion. Citrate shock can cause death of the patient (from cardiac arrest).

In order to prevent citrate shock, it is recommended to inject 10 ml of 10% calcium chloride or calcium gluconate solution for every 500 ml of blood.

Treatment.

In case of citrate shock, blood transfusion should be stopped immediately, 10 ml of 10% calcium chloride or calcium gluconate solution should be administered intravenously, caffeine should be administered subcutaneously.

Acute lung injury associated with blood transfusion

Transfusion-related acute lung injury is an uncommon complication caused by antibodies to HLA and/or to granulocytes in donor plasma that agglutinate and degranulate recipient granulocytes in the lungs. With the development of acute respiratory symptoms, chest radiography has features typical of non-cardiogenic pulmonary edema. This complication is the second most common cause of transfusion-related death. The incidence is between 1 in 5000 and 1 in 10000 blood transfusions, but many cases are mild. It is likely that mild to moderate transfusion-related acute lung injury is often missed. General supportive therapy usually leads to recovery without long-term consequences. The use of diuretics should be avoided. The use of blood from male donors reduces the risk of this reaction. Cases should be reported to the hospital blood transfusion service or blood bank.

The third group of complications

The third group of complications is caused by the transfusion of infected blood. During transfusion, you can get infected with HIV / AIDS, hemocontact viral hepatitis (B, C, D), malaria, syphilis, toxoplasmosis, herpesvirus infections, trypanosomiasis, etc.

During blood storage, potassium leaks from erythrocytes into the plasma, which in case of rapid injection of large amounts of blood can lead to potassium intoxication. This has a harmful effect on the myocardium. This complication often occurs in patients with kidney pathology, as well as in traumatic toxicosis.

During infusion and transfusion therapy, as of 2021, not whole donor blood is used, but its components and preparations in combination with blood substitutes and parenteral nutrition products.

At blood transfusion stations, the following blood components are obtained from whole blood different types of erythrocyte mass (red blood cells, red blood cell mass, red blood cell suspension, washed red blood cell mass, thawed and washed red blood cell mass) platelet mass; native plasma; fresh frozen plasma; immune and hyperimmune plasma; platelet concentrate; granulocyte mass; concentrated plasma; leukocyte mass.

Blood reinfusion. Autohemotransfusion. Blood donation.

Methods of blood transfusion

1. Indirect blood transfusion: infusion of preserved blood, most often intravenously. Less often - intra-arterially, intra-osseous, intra-aortic.

2. Direct blood transfusion: directly from the donor to the patient. Currently, due to the danger of infection with AIDS, hepatitis, it is practically prohibited. Only in extreme situations, especially in DIC syndrome, direct transfusion from a previously examined reserve donor is possible.

3. Exchange transfusion: partial or complete removal of the patient's blood with its replacement with donor blood. In acute poisoning with poisons (mushrooms, salts of heavy metals), in hemotransfusion shock. 4. Reverse transfusion of own blood, there are 2 types:

K4.1 Autohemotransfusion - when blood is taken from the patient before surgery, and

transfused to him during or after surgery.

Blood reinfusion can be performed:

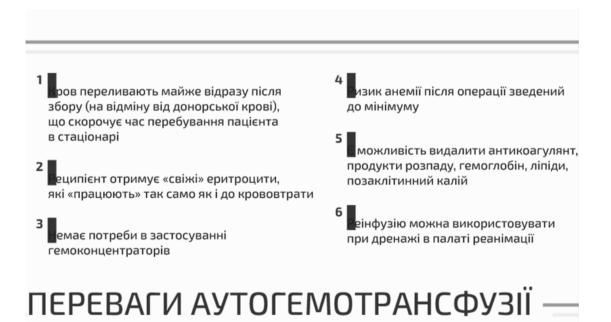
1. Routine method, when the spilled blood is passed through an eight-layer gauze and returned to the patient;

2. In the form of return of washed red blood cells, when the spilled blood is taken away, in the conditions of the transfusion department (station). The blood is washed, and the patient returns the red blood cell mass freed from destroyed blood cells and foreign bodies;

3. Automatic method, when the spilled blood during the operation is taken, immediately cleaned by filtration through the devices (such as "Sell sever") and returned to the patient.

Reinfusion of blood by the routine method is contraindicated in case of damage to the lumen of the gastrointestinal tract with mixing of its contents with altered blood.

4. Plasmapheresis - removal of plasma from the patient's blood with replacement of its volume with plasma replacement solutions and donor plasma. Together with plasma, toxic substances, antibodies, etc. are removed.



Other advantages of autohemotransfusion:

1. Blood is transfused almost immediately after collection (unlike donated blood), which reduces the patient's stay in the hospital

2. The recipient receives "fresh" red blood cells that "work" as well as before blood loss.

3. There is no need to use hemoconcentrators

4. The risk of anemia after surgery is minimized

5. It is possible to remove anticoagulant, decay products, hemoglobin, lipids, extracellular potassium

6. Reinfusion can be used for drainage in the intensive care unit.

Blood donation or blood donation is the voluntary provision of blood or its components for further transfusion to patients in need. Promotion of donation is an integral part of the moral foundations of the donor movement, due to the kindness, humanism, mercy of people who unselfishly give their blood to the sick. One donor dose (up to 470 ml).

There are 3 types of blood donors:

- 1. voluntary unpaid
- 2. family / replacement
- 3. paid

Healthy people aged 18 and over and weighing 50 kg can donate blood in Ukraine (some blood centers accept donors weighing 55-60 kg, for example, Lviv Regional Blood Service Center). Ukrainians and foreigners who have a permanent residence permit in Ukraine can donate blood. If age and kilograms are more or less clear, it is more difficult to understand what the expression "healthy person" means.

A healthy person is a person who has no contraindications. If you have chronic diseases, take medications or are registered, you cannot be a donor temporarily or at all.

Blood substitutes.

Blood substitutes are medications that can improve or replace any of the blood functions. According to the mechanism of action they are divided into the following groups.

Hemodynamic, which include low molecular weight dextran (rheopolyglucin), medium molecular weight dextran (polyglucin) and gelatin preparations (gelatinol). They increase the volume of circulating blood, increase blood pressure, improve microcirculation. The main indications for use are shock, acute blood loss, intoxication. Detoxification (neohemodesis, polidesis, neocompensan). These drugs bind toxins circulating in the blood, including bacterial ones, neutralize them and excrete them in the urine.

Preparations for parenteral nutrition: protein (casein hydrolyzate, aminopeptide, nitrolysine), amino acid (polyamine, infezol, friamine), fat (lipofundin, librolipid) and carbohydrates (glucose, sorbitol, fructose). They are administered to stabilize and normalize all types of metabolism. They are often used in patients who cannot take food by mouth.

Regulators of water-salt and acid-base state: saline solutions

(sodium chloride solution, Ringer-Locke solution, disol, trisol) and osmodiuretics (mannitol, sorbitol). They are administered to stabilize and normalize water-salt balance, in case of shock, acute poisoning. In acute blood loss they are often used to replenish the volume of lost fluid.

Drugs with the function of oxygen transfer (perfluorane, foluosol) - bind (2-3 times better than hemoglobin) and transport oxygen to tissues.

Preparations of complex action (polyfor, rheogluman, lactoprotein, rheosorbilact).

In order to prevent complications when using blood substitutes, it is necessary to follow the general rules of infusion, control of tightness and quality of the solution (in case of sedimentation, turbidity - the drug can not be used), conducting a biological test.

There are a number of recombinant (synthesized by genetic engineering) blood substitutes and blood products:

- Desmopressin - an analogue of the hormone vasopressin, which is used to stop bleeding;

- erythropoietins (epoietin α , epoietin β) stimulate erythropoiesis in chronic anemia;
- aprotinin inhibitor of proteolytic enzymes;

- purified hemoglobin and pyridoxylated hemoglobin polymer - preparations that bind oxygen twice as well as regular hemoglobin.



Shelf life of blood and components.

The shortest for granulocytes is only 24 hours. I store these components at +20...

+24 C.

Fresh plasma should be kept in the refrigerator at +2...+6 C for no more than three days. For platelet storage, room temperature (+20...+24 C) and constant stirring are required. Under such conditions, they can wait for transfusion up to 5 days.

The shelf life of red blood cells varies between 21 and 42 days and depends on the anticoagulant - a substance that prevents blood from clotting.

The temperature is +2...+6 C.

Cryopreservation - low-temperature long-term preservation of biological materials - helps to extend the shelf life of blood and components. Then platelets can be stored for up to 24 months, and plasma - up to 36.

The record holders of cryopreservation are red blood cells. They can be kept in this state for up to 30 years.

Control questions:

- 1. What is meant by the term "ABO blood group"?
- 2. How to conduct a macroscopic assessment of the transfusion medium?
- 3. What is meant by the concept of "Rh factor"?
- 4. What blood is considered Rh-positive"?
- 5. How is the test for determining the Rh blood type performed?
- 6. How is the ABO blood group test performed using standard red blood cells?

7. How is the ABO blood group determination test performed using monoclonal antibodies?

8. What is the method of performing a group compatibility test?

- 9. How is the individual compatibility test performed?
- 10. How is the biological test performed?

11. What are the methods of blood transfusion?

12. What methods do you know to monitor the patient's condition during blood transfusion?

13. How often and for how long is the body temperature measured after blood transfusion?

14. What color are anti-A, anti-B and anti-AB monoclonal antibodies?

15. List the blood products.

16. List the components of blood.

17. List the types of complications of hemotransfusions by nature of occurrence.

18. List your actions in the event of hemotransfusion shock

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