M. M. Mishina, O. V. Kochneva, O. V. Kotsar

LABORATORY DIAGNOSIS OF TORCH INFECTIONS

Educational handbook for students medical and dental faculties educational institutions of the III–IV levels of accreditation

Ed. M.M. Mishina

МІНІСТЕРСТВО ОХОРОНИ ЗДОРОВ'Я УКРАЇНИ
Харківський національний медичний університет

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ЛАБОРАТОРНА ДІАГНОСТИКА TORCH-ІНФЕКЦІЙ

Навчальний посібник для студентів медичних та стоматологічних факультетів навчальних закладів III–IV рівнів акредитації

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The textbook corresponds to the program approved by the Ministry of Health of Ukraine. It contains information about the morphology and ultrastructure of pathogens belonging to the TORCH group, modern taxonomy and classification, considered issues of pathogenesis, epidemiology, clinical symptoms, immune response, described methods of laboratory diagnostics and prevention of infectious diseases, caused by TORCH-infections. It is intended for students of medical and dental faculties of higher educational establishments of the III–IV level of accreditation.

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М71 Лабораторна діагностика TORCH-інфекцій : навч. посібник для студентів медичних та стоматологіних факультетів навчальних закладів III–IV рівнів акредитації / за ред. М. М. Мішиної. – Харків : ХНМУ, 2019. – 24 с.

Навчальний посібник відповідає програмі, затвердженій Міністерством охорони здоров'я України. У ньому міститься інформація про морфологію та ультраструктуру збудників, що відносяться до TORCH-групи, наведені сучасна таксономія і класифікація, розглянуті питання патогенезу, епідеміології, клініки, імунної відповіді, описані методи лабораторної діагностики та профілактики інфекційних захворювань, викликаних TORCH-інфекціями. Призначений для студентів медичних та стоматологічних факультетів вищих навчальних закладів III-IV рівня акредитації.

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INTRODUCTION

Actual problem of modern medicine is TORCH-infections, which present a real threat to the occurrence of serious obstetric and pediatric pathology during ante-and-perinatal infection of the fetus.

The abbreviation TORCH infection was proposed in 1971, and means the following: T (Toxoplasma) - toxoplasmosis, O (other) – other, R (rubella) – rubella, C (Cytomegalovirus), CMV – cytomegalovirus infection, H (Herpesviridae) – herpes. Other infections include diseases such as syphilis, gonorrhea, chlamydia, ureaplasmosis, hepatitis B, HIV infection/AIDS, etc.

Despite the fact that these pathogens belong not only to different species, but even to different classes of microorganisms (among them there are protozoa, and bacteria, and viruses), for all these infections are characterized by:

– possibility of lifelong persistence of their pathogens in the human body in the absence of any clinical manifestations of this process;

– ubiquitousness (omnipresence) – all of them are widespread in all regions of the Earth among different groups of the population;

– extreme variety of clinical manifestations of manifest forms of infection, that is, the absence of a characteristic clinic for each of them (non-pathognomonic), as well as the presence of hidden, asymptomatic, latent forms of infection;

– possibility of vertical transmission of pathogens – from the mother to the fetus through the placenta and the possibility of infecting the child during delivery;

– the main role of laboratory essays in the formulation of the final diagnosis.

These features of these infections clearly determine the leading principle of fighting them. The first of all, the need to control risk groups (pregnant women, women of childbearing age, newborns), the main element of which should be regular laboratory examination (monitoring) of these contingents for the timely detection of active markers and latent infection.

Taking into account the characteristics of pathogens and the course of infections of the TORCH group, the main methods of laboratory examination are serological methods for the detection in serum/blood plasma of specific markers of pathogens – immunoglobulins of classes M and G to their antigens. The main among them today is the enzyme-linked immunosorbent assay (ELISA) on solid-phase carriers, combining high sensitivity and specificity with the possibilities of its practical use, if not in all, in the vast majority of clinical laboratories.

Laboratory diagnosis of TORCH-group infections is carried out during the perinatal period, because that infection of the future mother's body, its fetus, and the newborn is possible. It is necessary to take into account three periods of time for the examination. The first – before conception (5 months), the second – from conception to childbirth (9 months), the third – after childbirth (1 month). At the first (5 months), both parents are examined. On the second (9 months) – the expectant mother is examined, on the last (1 month) – the newborn.Thus, regular laboratory examination of pregnant women, women

of childbearing age, and newborns allowsearly detection and prevention of the development of severe pathology.

The purpose of the study:

– **General:** to master the principles of laboratory diagnosis of infections TORCH-group.

– **Specific:**

a) to know biological properties, epidemiology, pathogenesis, clinical manifestations, methods of treatment and prevention of pathogens of TORCH-infections;

b) tobe able tointerpret the results of laboratory examination of TORCH pathogens.

Material and methodological supportof the topic: museum micro-preparations, microscope, immersion oil, disinfectants, tables, atlas.

TOXOPLASMA GONDII

Toxoplasmosis is a zoonotic infectious disease caused by the intracellular parasite *Toxoplasma gondii*, accompanied by parasitemia, lesions of various organs; in humans.The clinical manifestations are polymorphic, or the disease is asymptomatic.

According to WHO, nearly 25–50 % of the world's population is infected by Toxoplasma, although diseases with severe clinical symptoms are observed, indeed, not often, especially in areas with a warm, humid climate. It is known that the frequency of infection of pregnant women in different regions of the planet, depending on the applied methods and test systems, varies from 22 % (Israel) to 83,5 % (Madagascar), averaging 40 %.

The pathogen belongs to the kingdom of Protozoa, type Apicomplexa, class Sporozoa, genus Toxoplasma. It was opened in 1908 by French scientists S. Nicole and L. Manso.

Morphology and cultivation.The life cycle of Toxoplasma has several morphological stages. A typical form is endozoites (trophozoites) - an orange slice or crescent with a size of 4–7 × 1.5–2 mkm (*Fig. 1*).

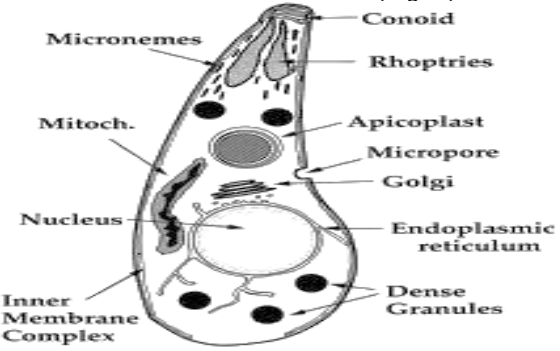

Fig. 1. Structure of Toxoplasma

Resistance. Toxoplasma dies quickly at a temperature of 55 °C, highly sensitive to 50 % alcohol, 5 % solution of NH4OH.

Epidemiology. Toxoplasma is widespread. The source of the invasion is many species of domestic and wild mammals, as well as birds. Infection occurs frequently as a result of the consumption of thermally poorly processed foods (meat, milk, eggs) obtained from animals infected with Toxoplasma. The final owners of Toxoplasma are cats and members of the feline family, which excrete the pathogen (oocysts) with feces. A person infected with Toxoplasma does not release them into the environment (*Fig. 2*).

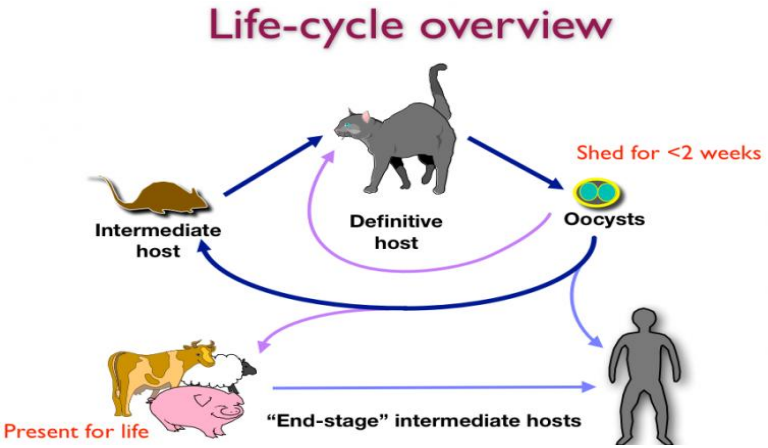

Fig. 2. Life cycle of Toxoplasma

Pathogenesis and clinical manifestation of toxoplasmosis

The pathogens enter the human body through alimentary, less commonly contact (through damaged skin and mucous membranes) or by air and dust. In congenital toxoplasmosis, the pathogen enters the fetus through the placenta.

Toxoplasma that have penetrated into the body, reach with lymph flow regional lymph nodes, multiply and penetrate into the blood, spread throughout the body, entering the cells of the reticuloendothelial and central nervous systems, where they form cysts that have been preserved for decades. It forms hypersensitivity of the delayed type.

The incubation period is about 2 weeks. The clinical picture is diverse and depends on the localization of the pathogen. In congenital toxoplasmosis, fetal death, spontaneous miscarriage or stillbirth, birth of children with developmental defects is possible.

Immunity. The cellular and humoral immunity prevents the new infection with Toxoplasma.

Microbiological diagnosis. Diagnosis is carried out by microscopy of a smear obtained from pathological material, stained by Romanovsky-Giemsa. The cytoplasm will blue, and the nucleus will ruby red (*Fig. 3*).

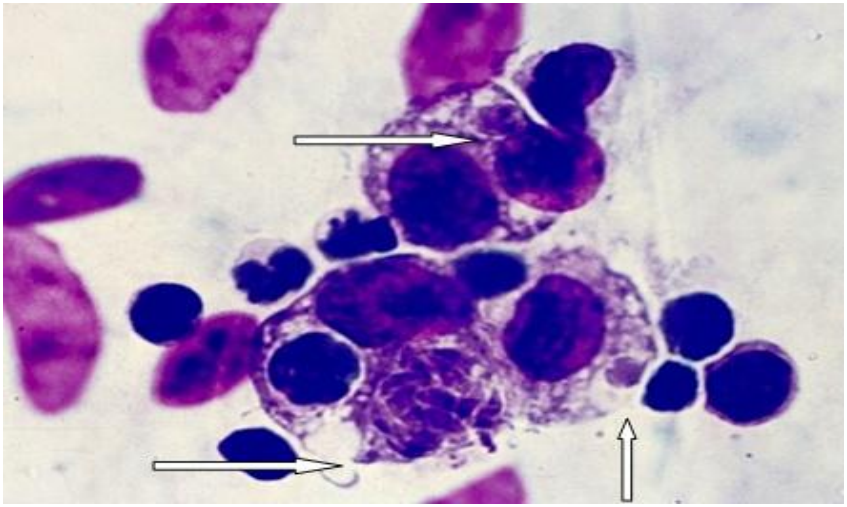

Fig. 3. Toxoplasma is stained by Romanovsky-Giemsa

Less commonly used biological method of infection of mice. The main diagnosis baseonthe serological method. Apply RIF, ELISA, CFT, PCR. An allergic method is used – setting up an intracutaneous test with toxoplasmin.

Toxoplasma infection (carriage) and toxoplasmic disease (toxoplasmosis) should be distinguished. Therefore, the main thing in laboratory diagnostics is not the fact of detecting a positive immune response (antibodies), but the clarification of the nature of the course of the process – carrier state or disease.

Laboratory diagnostics is based on the identification of serological markers (IgMimmunoglobulins, IgA, IgG and IgG avidity) and molecular methods (PCR detection of the virus in various biological materials).

Immunological diagnosis is the main method of diagnosis of toxoplasmosis. It allows you to establish the duration of infection, the intensity of immunity.

Toxoplasma gondii, IgM antibodies. IgM antibodies are usually found in patients with a primary recent infection, and can also be observed in people with secondary infection or reactivation of the infection. IgM antibodies to *Toxoplasma gondii* appear in the blood during the acute period of infection (in the first week of the disease), reach a peak by 2–3 weeks of the disease; they can persist in low titers up to 2 years. In some patients, IgM antibodies may disappear within 3 weeks after the onset of the disease. The detection of Ig M cannot be as a reliable criterion for acute primary infection or reactivation of the infectious process.

The diagnosis of primary infection can be established in the presence of IgM antibodies, the presence of low-avid IgG and an increase in the concentration of IgG antibodies over time (4-fold increase with an interval of 10–4 days).

In pregnancy, detection of toxoplasmosis IgM in previously seronegative women may indicate a recent infection that can lead to congenital toxoplasmosis. When using IgM and IgG antibodies, false positive reactions are often observed during pregnancy. IgG in dynamics with an interval of 10–14 days, as well as the determination of the avidity of IgG.

Avidity is a characteristic of the binding strength of specific antibodies with the corresponding antigens (determined by the number of binding sites and the strength of binding).

In patients with reduced immune status, the results should be interpreted with caution, as they are characterized by low IgG titers, and IgM antibodies may not be detected.

Toxoplasma gondii, IgA antibodies. IgA serum antibodies to *Toxoplasma gondii* are produced 2–3 weeks after infection and persist in the blood for a long time. A decrease in the IgA antibody titer indicates a transition of the disease to the latent phase, and an increase indicates that the process is activated. A small fraction of infected antibodies does not detect IgA.

Toxoplasma gondii, IgG antibodies. IgG antibodies are produced by the body several weeks after the initial infection and can persist for a long time in the blood. IgG levels increase as the infection progresses. The test can be used along with an IgM assay to confirm the presence of acute or past infection with *Toxoplasma gondii*. The increase in titers 4 or more times will confirm the presence of an acute infection.

To avoid complications during pregnancy, it is recommended to assess the immune status before conception. Before pregnancy, seropositive women are protected from the point of view of future fetal infection,seronegative women are at risk of infection during pregnancy.

Toxoplasma gondii, avidity of IgG antibodies

This test allows you to evaluate the duration of infection: low-avid antibodies circulate about 3 months after the initial infection and then are replaced by high-avid antibodies. Detection of low-avid IgG antibodies is not an unconditional confirmation of fresh infection, but serves as an additional confirmatory serological test for suspected toxoplasmosis in pregnant women or in difficult clinical situations associated with the need to determine the phase of the pathological process. When the infection is reactivated, antibodies of the IgG class against *Toxoplasma gondii* of high avidity are detected.

Diagnosis of congenital toxoplasmosis

IgG antibodies reach the child from the mother (vertical transmission). Their concentration sharply decreases by the 4th month of life. Therefore, an increase in the level of IgG can be considered as a diagnostic marker only after the 6th month of life. However, as a rule, even in the presence of clear clinical

signs, an increase in IgG titer is not pronounced or is not observed. The concentration of IgM can quickly increase as the fetus independently produces these antibodies. However, the absence of IgM on the background of IgG does not give grounds for excluding intrauterine infection, since IgM lose their diagnostic value 3 months after the development of the infection.

Treatment and prevention of toxoplasmosis

Chloridine is used in the treatment. The most effective is the use of a combination of pyrimethamine (daraprim) with sulfa drugs. If pregnancy is recommended instead of pyrimethamine apply spiramycin, which does not pass through the placenta.

Prevention measures include hygienic requirements, such as washing hands before meals; careful heat treatment of meat is necessary; should avoid contact with stray cats.

RUBELLA VIRUS

Rubella (German measles) is an acute viral infection disease characterized by a rash, enlargement and tenderness of the lymph nodes, fever and mild intoxication. When rubella virus infects women in early pregnancy, it may be transmitted to the fetus and cause birth defects (congenital rubella syndrome).

Taxonomy, morphology of rubella virus. Rubella virus belongs to the family Togaviridae, genus Rubivirus. It has spherical shape, enveloped, with size 40 to 80 nm, positive-sense, single-stranded RNA virus with spikes from glycoproteins (*Fig. 4*). Unlike other Togaviruses, the rubella virus contains neuraminidase.

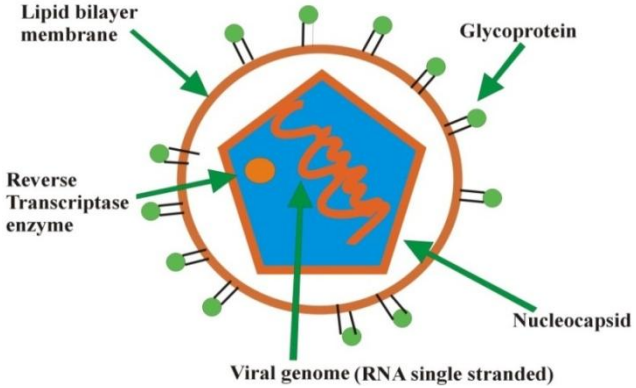

Fig. 4. Structure of Rubella virus

Cultivation The virus multiplies in primary and transplantable cell cultures with the formation of cytoplasmic inclusions, sometimes with a cytopathic effect.

Resistance Rubella virus

It is relatively heat-labile, inactivated for 30 minutes at 56 °C for 4 minutes. at 70 °C and for 2 min at 100 °C. With normal freezing at -20 C, the rubella virus quickly collapses, but remains stable at -60 °C and lower, as well as with freeze drying in the presence of a stabilizer. In the presence of protein as a stabilizer, the rubella virus can be re-frozen and thawed without losing the titer of the virus. Fat solvents, weak acids and alkalis, as well as ultraviolet rays inactivate the rubella virus. The virus is also sensitive to a wide range of disinfectants, it can be inactivated with 1 % sodium hypochlorite solution, 70 % ethanol or formaldehyde.

Epidemiology of rubella

Rubella is a highly contagious infection, it is widespread, affecting mainly children aged 3–6 years. Adults can also hurt. The maximum number of diseases is recorded in April–June. During an epidemic outbreak, not only children but also adults get sick, especially in organized groups. Rubella is particularly dangerous for pregnant women due to fetal intrauterine infection. The rubella virus is released into the external environment a week before the rash appears and within a week after the rash. The source of infection is a patient with a clinically severe or asymptomatic form of infection. Viruses are excreted in mucus from the upper respiratory tract, with feces and urine. The mechanism of pathogen transmission is aerogenic (airborne droplet), in pregnant women – transplacental.

Pathogenesis

The rubella virus in a natural infection enters the body through the mucous membranes of the respiratory tract. It was known the virus caused disease with the intradermal administration of the virus in an experiment on volunteers. Thenviremiacomes. By blood the virus spreads throughout the body, has dermatotropic properties and causes changes in the lymph nodes, which increase already at the end of the incubation period. At this time, the virus can be isolated from the nasopharynx. With the appearance of a rash, the virus in the blood and in the nasopharynx is not detected, but in some cases, its excretion lasts 1–2 weeks. after the rash. Serum antibodies appear 1–2 days after the rash. In the future, their titer increases. After suffering the disease antibodies persist throughout life. The immunity is lifelong (*Fig. 5*).

Rubella virus has a tropism for embryonic tissue. It destroys the development of the fetus. The frequency of fetal lesions depends on the duration of pregnancy. Rubella disease at the 3–4th week of pregnancy causes congenital deformities in 60 % of cases, at the 9–12th week – in 15 % and at the 13–16th week – in 7 % of cases. When pregnant women become ill with rubella, the virus enters the placenta, multiplies and infects the fetus. Infection causes a defect of mitotic activity, chromosomal changes, which lead to a retard in physical and mental development. About half of women who has rubella in the first three months of pregnancy may have a miscarriage or may have a child with very severe developmental defects, such as heart defects, blindness,

deafness and mental retardation. In congenital rubella, despite the presence of antibodies to the rubella virus in the serum, the pathogen is stored for a long time (up to 31 months) in the child's body. A child during this time can be a source of infection for other children.

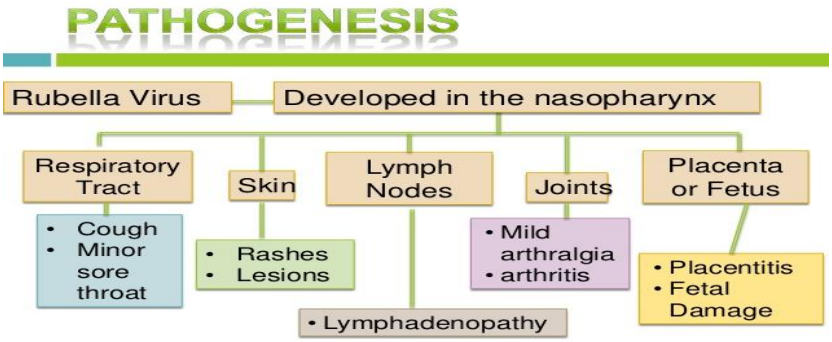

Fig. 5. Pathogenesis of rubella

Clinical manifestations of rubella

The incubation period lasts 11–24 days. The general condition of patients with rubella suffers little. The first symptom that attracts attention is rash. Patients note a slight weakness, malaise, mild headache, and sometimes pain in muscles and joints. Adults have more severe disease with brief prodrome period. Body temperature often remains subfebrile, although sometimes it reaches 38–39 °C and lasts 1–3 days. An objective examination noted mild symptoms of catarrh of the upper respiratory tract, a small hyperemia of the pharynx, injection of the conjunctival vessels. From the first days of the disease the generalized lymphadenopathy appears. Especially pronounced increase and pain of the posterior cervical and occipital lymph nodes. Sometimes all these symptoms are mild, and the disease attracts attention only when a rash appears. The disease can occur in different forms. There is no generally accepted classification of clinical forms of rubella.

A characteristic manifestation of rubella is rash. Often the rash appears on the first day of the disease (40 %), but it may appear on the second (35 %), third (15 %) and even on the fourth day (in 10 % of patients). In some cases, it was the rash that attracted attention, since a slight malaise before the rash was not considered any disease. Most often, a rash is first noticed on the face, and then during the day it appears on the trunk and extremities. Unlike measles, there is no staging of the rash. The rash is more abundant on the extensor surfaces of the limbs, on the back, lower back, buttocks. On the face, the rash is less pronounced than on the body (unlike for measles). In contrast to scarlet fever, the elements of the rash are located on the background of normal (non-hyperemic) skin. The main element of the rash is a small spot (5–7 mm in

diameter) that does not rise above the skin level and disappears when pressed on the skin or when it is stretched. A typical is a small-spotted rash (95 %), although in some patients it may be large-spotted (spot diameter 10 mm or more). Along with spots, flat roseola with a diameter of 2–4 mm can occur, papules are less common. Elements of the rash are usually separate, but some of them can merge, forming larger spots with scalloped edges, but never form extensive erythematous surfaces (as is the case with measles or infectious erythema), rarely revealed individual petechiae (in 5 %).

Congenital rubella (CR) is a maternal infection in early pregnancy can lead to fetal infection. CR develops as a result of intrauterine infection of the fetus in the first trimester of pregnancy, which can cause spontaneous abortion, miscarriage, stillbirth, or having a child with multiple developmental defects. Theoretically, all organs and systems can be affected. Deafness is the most common and often the only manifestation of CR. If intrauterine infection of the fetus has not led to the development of congenital malformations, then this condition is called congenital rubella infection.

The risk of fetal malformations due to rubella infection of the mother ranges from 10 to 90 %. The severity and nature of these disorders depend on the duration of the pregnancy in which the infection occurred. The most dangerous period is the first 12 weeks of pregnancy. When infected after 20 weeks, malformations are rare. In general, the specificity of organ damage depends on the gestation stage. However, the relationship between fetal developmental disorders and the time of infection is not always clearly defined. If an infection occurs, the infection can spread and cause damage to various organs. Eye and heart pathology usually occurs with rubella in the first 8 weeks of pregnancy, while brain damage and deafness occur with infection in the first 18 weeks of pregnancy (*Fig. 6*).

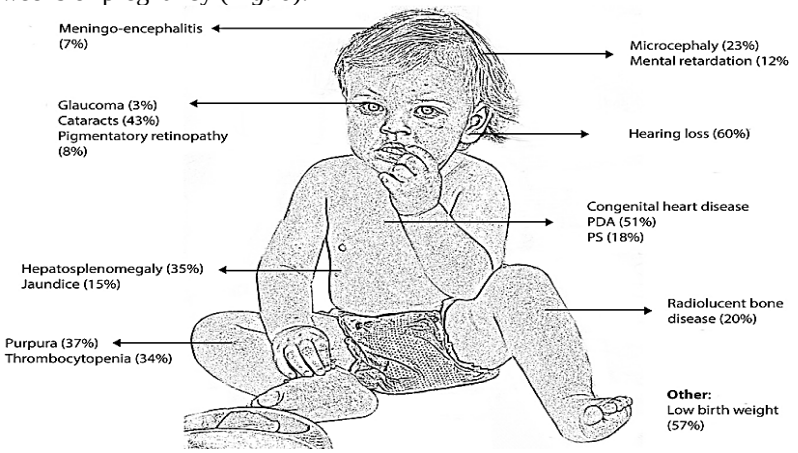

Fig. 6. Congenital rubella in children

Despite the fact that rubella vaccination is contraindicated in pregnant women, no cases of CR were observed in more than 1 000 susceptible women who inadvertently received rubella vaccine in the early stages of pregnancy. The observation of nearly 19 000 susceptible pregnant women who were unintentionally vaccinated during mass immunization campaigns also did not reveal a single case of CR. Thus, unintended rubella vaccination during pregnancy is not an indication for interruption of it.

Immunity. After infection, immunity is lifelong In case of rubella infection, both humoral and cellular immunity develop. IgG and IgM antibodies appear 14–18 days after infection, almost simultaneously with the appearance of the rash. The level of rubella IgM antibodies drops rapidly and after 2 months they, as a rule, are not detected, while IgG antibodies remain. Specific cellular immunity against rubella appears 1 week after humoral and persists for life. Although transferred rubella usually leads to the formation of lifelong immunity, rare cases of repeated, serologically confirmed, infection in previously ill (or vaccinated) individuals have been noted. In addition, there were cases of rubella due to reinfection of pregnant women who had post-infectious or post-vaccination rubella immunity, but such cases are extremely rare. Maternal antibodies provide protection against rubella in the first months of a child's life and may interfere with the immune response to vaccination if the vaccine is given at a very early age.

Laboratory diagnosis of rubella

The rubella virus can be isolated from the discharge of the nasopharynx, blood, urine and cerebrospinal fluid from rubella patients or CRS. From the throat, the virus can be isolated within 1 week and up to 2 weeks after the appearance of the rash. For laboratory confirmation of a CR case, detachable nose and throat samples, blood, urine, cerebrospinal fluid, as well as biopsy or autopsy samples can be used.

Serological tests include detection of IgM antibodies to the rubella virus or detection of IgG antibodies during the period when passively obtained maternal antibodies disappear. In patients with CRIgM antibodies are sometimes detected within a year after birth, and the persistence of IgG antibodies over 6 months of age has been detected 95 % of cases examined. The virus is isolated in cell cultures. Rubella diagnosis can be confirmed either by isolating and identifying the virus, or by increasing the titers of specific antibodies. To identify the isolated virus we can use hemagglutination inhibition reaction (RIHA).

Also the reaction of immunofluorescence (RIF), enzyme-linked immunosorbent assay (ELISA), RIHA are used. Serological reactions set with paired sera with an interval of 10–14 days. Diagnostic is the increase in antibody titer 4 times or more. Isolation and identification of the virus are quite complex and are practically not used in practical work.

Treatment and prevention of rubella

Unless bacterial complications occur treatment is symptomatic.

The main goal of vaccine is to protect pregnant women and, as a result, to prevent fetal infection and birth of children with congenital rubella syndrome.

Vaccination is carried out in many countries. There are foreign rubella live attenuated vaccines, produced in the form of mono-, as well as di- and trivaccines (mumps – measles – rubella, MMR). In the majority of countries they carry out double immunization of children of primary and school age (12 months and 6 years old).

The use immunoglobulin in women who have been expose to rubella in early pregnancy is of no proven value.

The ***prognosis*** for rubella is favorable, with the exception of rubella encephalitis, in which mortality reaches 50 %. In congenital rubella, some developmental defects (for example, deafness) may develop later (after a year).

HERPESVIRUSES

The diseases caused by herpesviruses occupy the second place (15,8 %) after hepatitis (35,8 %), as the cause of death from viral infections. The frequency of genital herpes varies from 7 to 35 %. Herpes simplex virus 1 antibodies are found in 99 % of the adult population, type 2 – in 73 %. However, most researchers believe that the true situation with genital herpes is unknown because of the significant frequency of its asymptomatic forms. It is estimated that about 90 % of the world's population is infected with one or more types of viruses belonging to the Herpesviridae family.

IgG for cytomegalovirus infection (CMVI) in the blood of various populations in Europe, Asia, America and Africa are detected with a frequency of 40 to 100 %. In children, this figure varies from 13 to 90 %. In the UK and the USA, 40–60 % are seropositive among the adult population with medium and high socioeconomic levels, compared with 80 % in the population with low social status. In the United States, 1–2 % of all neonates with CMVI are detected in urine at birth. By the age of 1, the number of such children increases to 10–20 %, by the age of 35, 40 % of adults have seroconversion with the appearance of CMV antibodies, and by the age of 50 almost all adults are infected with CMV. **Seroconversion** is the time period during which a specific antibody develops and becomes detectable in the blood. According to researches CMV excretes from a cervical canal in 3,5–20 % of practically healthy women and from sperm about 30 % of practically healthy men.

The unique biological properties of herpesviruses are an ability to persist and latency in the body of an infected person. Persistence is the ability of herpes viruses to continuously or cyclically multiply (replicate) in infected cells of tropic tissues, which creates a constant threat of an infectious process. The latency of herpes viruses is the lifelong preservation of viruses in morphologically

and immunochemically modified form in the nerve cells of regional (relative to the site of herpesvirus adsorbtion) ganglia of sensory nerves. Strains of herpesviruses have different capacity for persistence and latency and sensitivity to antiherpetic drugs due to the peculiarities of their enzyme systems. Every herpes virus has its own persistence and latency rate.

Herpes is one of the most common human viral infections, characterized by fever and blistering rashes, which are most often localized on the skin and mucous membranes. Important features of a herpetic infection are life-long carrier of the virus and frequent recurrences of the disease.

Structure of herpes virus

Herpes virus has an icosahedral shape, linear double stranded DNA, large size (120-200 nm), enveloped DNA virus, with glycoproteins.

Also herpes virus has tegument is protein – filled region (for replication) (*Fig. 7*).

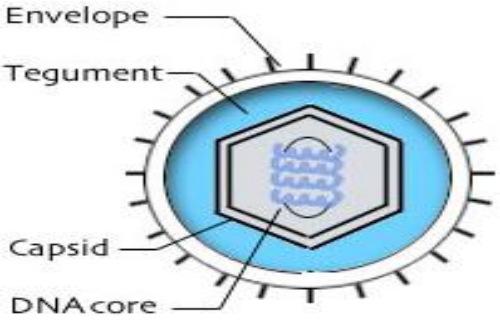

Fig. 7. Structure of herpes virus

Cultivation. Herpes simplex virus (HSV) is cultured in chicken embryos, cell cultures, and laboratory animals. On the chorioallantoic envelope of chick embryos, the virus forms small white dense nodules-plaques; in infected cultures causes a cytopathic effect: the formation of giant multinucleated cells with intranuclear inclusions.

Antigenic structure. The virus contains a number of antigens that are associated with both internal proteins and glycoproteins of the outer shell. The last one is the main immunogens inducing the production of antibodies and cellular immunity. There are two serotypes of the virus: type HSV1 and type HSV2.

Resistance. The virus can survive on the surface of objects at room temperature for several hours, it is sensitive to UV rays, common disinfectants, thermo labile.

Susceptibility of animals. The herpes simplex virus is pathogenic for many animals. It causes encephalitis when the pathogen is introduced into the brain or a local inflammatory process when infected into the eye of animals. Under natural conditions, animals do not get sick.

Epidemiology. Herpes simplex is one of the most common infections that affects different age groups of people, most often in the autumn-winter period. There are sporadic cases of the disease, sometimes small outbreaks in families, children's groups, hospitals. Epidemics are not observed.

The source of infection is sick and carriers. The main transmission mechanism is a contact, aerogenic. Infection occurs when viruses enter damaged skin or mucous membranes.

The epidemiology of herpes caused by types 1 and 2 viruses is different. HSV1 is transmitted through saliva, hands and household items are infected with saliva, and can also affect the genitals. HSV2 is sexually transmitted. Possible infection of the fetus through the placenta.

Pathogenesis. The entrance gates of the pathogen during primary herpetic infection are damaged areas of the skin and mucous membranes of the mouth, eyes, nose, urinary tract, where viruses are reproduced. Then, through the lymphatic vessels, viruses enter the bloodstream and are carried to various organs and tissues (*Fig. 8*).

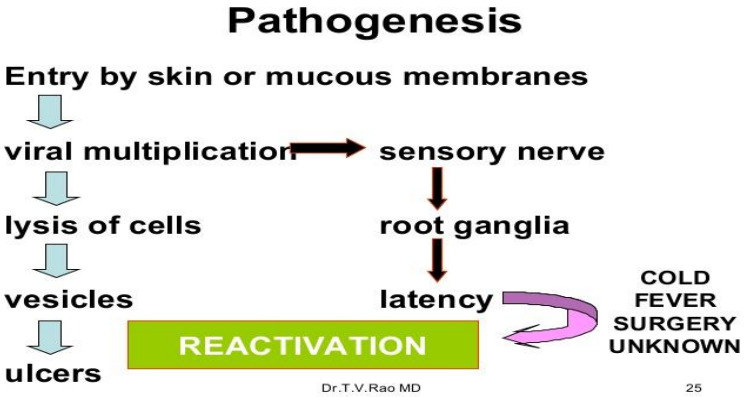

Fig. 8. Pathogenesis of herpesviruses infection

Clinical manifestations of herpes.

The incubation period for primary herpes is on average 6–7 days. The disease begins with a burning sensation, itching, redness, swelling in limited areas of the skin and mucous membranes, then on this spot appear bubble eruptions filled with liquid. Sometimes the disease is accompanied by fever and a violation of the general condition. When the bubbles dry the scars are not formed. Primary herpes in newborns is difficult and often ends in death. However, in most people, the primary infection remains unrecognized, as it is asymptomatic.

After the primary infection (overt and asymptomatic), 70–90 % of people remain lifelong carriers of the virus, which is latent in the nerve cells of the sensitive ganglia. Often, carriers of the disease appear relapses as a result of hypothermia, overheating, menstruation, intoxication, various infectious diseases, stress, and neuropsychiatric disorders. Recurrent herpes is characterized by repeated rashes on the skin and mucous membranes, often in the same places. The most frequent localization of recurrent herpes caused by HSV1 are the lips, nose wings, oral cavity, conjunctiva of the eyes. HSV2 affects the urogenital system and causes herpes in newborns. The role of HSV type 2 in the development of cervical cancer has been proven. The generalized forms of recurrent herpes such as damage to the nervous system and internal organs are rare.

Immunity. As a result of the primary herpes infection antibodies are formed in the body, which cause immunity to the primary herpes, but do not prevent the virus from retaining and the occurrence of relapses. Recurrent herpes occurs when there is a high level of antibodies to the herpes virus. The state of cellular immunity is of major importance in the development of recurrent herpes.

Laboratory diagnosis. The material for the analysis are the contents of herpetic vesicles, saliva, scrapings from the mucous membrane, cornea, blood, cerebrospinal fluid, in lethal cases, pieces of the brain and spinal cord.

Express diagnostics consists in the detection of giant multinucleated cells with intranuclear inclusions in smears imprints of eruptions stained by Romanovsky–Giemsa. For differentiation from other viruses belonging to this family, use RIF, ELISA, RIA, PCR. Virus isolation is carried out on chicken embryos, cell culture and on laboratory animals (suckling mice). In recent years, monoclonal antibodies have begun to be used in the diagnosis of herpes simplex, which makes it possible to determine the serotype of the virus. CFT, PH, RIF, ELISA are used for serodiagnosis of the disease.

Methods of laboratory diagnosis of herpes viral infections are divided into two groups: direct and indirect. The direct methods aimed at identifying the pathogen (tissue culture, laboratory animals), identifying antigens, early proteins (immunofluorescence reaction – RIF) and/or detecting the viral genome – DNA (by PCR) in different clinical material (duration of virus detection during primary infection is up to 1 month, with relapse – 7–10 days). The cultural method is not used in practical health care institutions at the present time because of its duration, laboriousness, high cost. Cytology and electron microscopy are also not applied due to low sensitivity and specificity. By sensitivity, specificity, availability, relatively low cost and speed of obtaining PCR results, it is preferable to other methods for use in diagnostic purposes. Indirect serological methods: enzyme immunoassay (ELISA) and RIF are aimed at detecting antibodies in serum, plasma, cerebrospinal fluid. With the help of these methods it is possible to establish the form and stage of infection diseases.

Diagnosis of herpes infection during pregnancy

In case of genital herpes, the diagnosis is made according to the clinical picture, taking into account prodromal pain symptoms and typical small vesicular rash in a pregnant woman. When in doubt, the diagnosis of these vesicles can be detected by DNA of the virus by PCR.

Forms of the active stage of herpes infection

1. Primary infection. It develops after infection with a virus of a previously uninfected person. In uninfected patients, antibodies are not detected, the primary infection is accompanied by seroconversion, the appearance of specific IgM, low avidity IgG, an increase in antibody titers, the detection of virus DNA in clinical specimens. The primary infection may develop with pronounced clinical manifestations in the form of vesicular rash or with asymptomatic secretion of the virus.

1. In case of the primary genital herpetic infection in a pregnant woman, the risk of transmission to the fetus is especially high, therefore, it is important for the obstetrician-gynecologist to focus on identifying cases of primary infection.

2. Recurrent herpes: re-registered clinical manifestations of herpes. The following laboratory markers are detected: high-avid anti-HSV IgG + anti-HSV IgM ± detection of virus DNA in clinical specimens.

3. The first episode of herpes: the first identified clinical manifestations in previously infected individuals. Laboratory markers: high-avid anti-HSVIgG+, anti-HSV IgM + detection of virus DNA in clinical specimens.

4. Asymptomatic herpes infection. In pregnant women with a history of genital herpes or in a partner with a herpes, serological markers of activation and/or DNA of the virus are detected in clinical specimens.

5. Inactive stage of herpes infection - latent infection. Infectious virus is not detected in clinical samples. The virus is stored in the form of DNA - a protein complex in the ganglia of the sacral nerves. Highly avid anti-HSVIgG are detected.

Treatment and specific prevention

For the prevention of severe forms of recurrent herpes during remission, repeated administration of inactivated, cultured herpetic vaccine is used. Vaccination, immunomodulators, for example, reaferon, prolong the interrecurrence period and facilitate the course of subsequent relapses. In the acute period, chemotherapeutic drugs (virazole, acyclovir, oxolinic, tebrophenic, florenal ointment, bonafton), interferons and interferon inductors are used for therapeutic purposes. In the acute period, chemotherapeutic drugs (virazole, acyclovir, oxolinic, tebro-phenic, florenal ointment, bonafton), interferons and interferon inductors are used for therapeutic purposes.

CYTOMEGALOVIRUS

Cytomegalovirus infection is an infectious disease caused by the cytomegalovirus (CMV) (from the Greek. Cytys – cell, megas – large). Infection is characterized by damage to almost all organs (mainly salivary glands) with the formation of giant cells with intranuclear inclusions, occurring in various forms - from asymptomatic carriage to a severe generalized form, ending in death. Cytomegalovirus causes severe birth defects.

Structure of CMV

It is known in humans as HCMV (HHV5), double stranded DNA enveloped virus with icosahedral symmetry. The structure of the genome of CMV is similar to other herpesviruses.

Cultivation. Cytomegalovirus is replicated in a limited number of primary and transplantable cell cultures, causing a characteristic cytopathic effect – the formation of giant (“cytomegalovirus”) cells with intranuclear inclusions (a symptom of the "owl eye") (*Fig. 9*). The virus is pathogenic for monkeys.

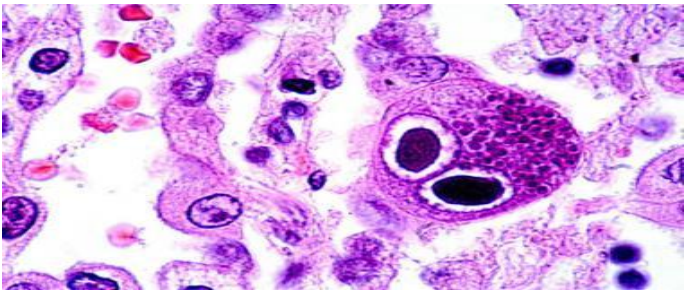

Fig. 9. Cytopathic effect of CMV «owl eye»

Epidemiology. Cytomegalovirus infection is widespread throughout the world. CMV takes the 2nd place in the development of immunodeficiency after the HIV virus! The source of the virus is a sick person or carrier. In other words, this disease called «kissing disease».

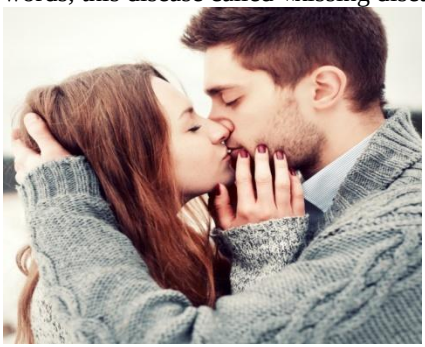

Fig. 10. Cytomegalovirus infection or «kissing disease»

The virus is secreted with saliva, urine, secretions of the body, rarely feces. It is assumed that the leading mechanism of transmission of infection is contact-related; aerogenic and fecal-oral mechanisms of transmission are possible. The cytomegalovirus has a high ability to cross the placenta (vertical transmission), causing fetal pathology of the fetus. CMV has neurotropic, epitheliotropic, hepatotropic, cardiotropic actions. It depresses all immunity links (decreases account of interleukin, T-killers, interferon, macrophages)

Pathogenesis and clinical manifestations of CMV infection

Pathogenesis is not fully understood. Infection is associated with long-term carriage of the virus, which in a latent state is preserved in the salivary glands, kidneys and other organs. Activation of latent infection occurs in immunodeficient states, immunosuppressive therapy. The virus infects the central nervous system, bone marrow, kidneys, liver, blood cells. In pregnant women, cytomegaly can lead to prematurity, stillbirth, the development of fetal abnormalities (*Fig. 11*).

Fig. 11. Congenital cytomegalovirus infection

Immunity. In patients, regardless of the clinical form of the infection, as well as carriers, antibodies are formed, which, however, do not prevent the virus from preserving in the body and excreting it into the environment. The intensity of the disease is controlled by the host's cellular immune system.

Laboratory diagnosis of CMV infection

Children with lesions of the central nervous system and congenital deformities, as well as women with dysfunctional pregnancy are examined on cytomegalovirus. The test material are saliva, urine, sputum, cerebrospinal fluid, blood, liver puncture.

The diagnosis is based on the identification of cytomegalic cells under the microscope in the material under study, as well as the detection of IgM class antibodies using RIF, ELISA, and RIA. The virus is isolated in the cell culture, identified by the morphological changes of the infected cells and using RN on tissue culture. Express diagnostics is RIF. Also PCR-methods is used.

Diagnosis of cytomegalovirus infection (CMVI) during pregnancy

Routine serological screening is currently not recommended. Diagnosis of CMVI is carried out in cases where a pregnant woman has had a flu-like illness or a disease similar to infectious mononucleosis, as well as with the appearance of suspicious echo-graphic data. In CMV, active and inactive stages are distinguished. An uninfected woman who has no clinical or laboratory markers of infection is at risk, as she can be infected during pregnancy. Therefore, it is necessary to re-examine such women once every 3 months to identify a primary infection that is dangerous to the fetus.

Active forms of CMVI

1. Primary CMVI. The incubation period is 2–12 weeks. The process of reproduction of the virus is slowly increasing, the duration of the active stage of the primary infection from 2 to 6–8 months. Laboratory markers indicating CMV reproduction: detection of CMV DNA in clinical samples (blood, in genital smears, urine, saliva, cerebrospinal fluid), detection of anti-CMV IgM and IgG to super-stored EIA proteins, anti-CMV IgM to structural proteins L, low avoid anti-CMV IgG, 4-fold increase in G-antibody titers in paired sera, seroconversion. Anti-CMVIgG titers can fluctuate, their increase or decrease does not always allow to evaluate the activity of the virus. Anti-CMV IgM may be absent in patients with severe cytomegaly and, conversely, be determined in the absence of other signs of virus activation. Anti-CMV IgM can be detected in the presence of rheumatoid factor, the active replication of other herpes viruses. Low avid anti-CMV IgG detected only during primary infection. As the immune response develops, the virus multiplies. The infection moves from the active to the inactive stage.

2. Reactivation of latent infection. With recurrent infection under the influence of various exogenous and endogenous factors (intercurrent infections, pregnancy, the use of immunosuppressants, radiation, hematological diseases, diabetes, AIDS) there is a decrease in cellular immunity. In this case, the persistent virus moves from the inactive (latent) to the active phase (activation, reactivation, reproduction). Activation markers appear (except for low-avid IgG). Activation can occur both asymptomatically and with clinical manifestations.

3. Inactive form of CMVI or latent infection. At this stage, the virus does not multiply, persists in the epithelial cells of the ducts and the parenchyma of the salivary glands, the lymphoreticular cells of the kidneys, and leukocytes of the peripheral blood. Virus replication markers are not detected.

Only anti-CMV IgG is detected, the latter circulate in the blood throughout life and tend to decrease in titer. The effectiveness of antiviral drugs can be realized only in the phase of viral replication, with active forms. The program of management and treatment of pregnant women is made up taking into account the forms and stages of herpes infections.

Specific prevention and treatment of CMV infection. Developed live attenuated vaccine. For treatment, chemotherapeutic drugs are used (ganciclovir, fcornis sodium), immunomodulators, interferon.

O (others) of the TORCH infection are other infections that affect the development of the fetus, create conditions for intrauterine infection and affect the viability of the newborn, as well as older children. These include such infections as chlamydiosis, ureaplasmosis, hepatitis B, hepatitis C, etc. HIV infection is also related to this group. Screening for other infections is indicated for pregnant women who are at risk if they are suspected.

Theoretical questions:

1. Characteristics of the causative agent of toxoplasmosis.
2. Pathogenesis, clinical manifestations, laboratory diagnostics of toxoplasmosis.
3. Morphological characteristic of the genus Rubivirus
4. Epidemiology, pathogenesis and clinical forms of rubella.
5. Methods of laboratory diagnosis of rubella.
6. Laboratory diagnosis of rubella in pregnant women and newborns.
7. Methods of treatment and prevention of rubella.
8. Morphology and antigenic structure of the Herpesviridae family.
9. Methods of laboratory diagnosis of herpes viruses and cytomegalovirus infection.
10. Methods of treatment and specific prevention of herpes viruses and cytomegalovirus infection.
11. The others of infection: what microorganisms belong to this group?

Practical tasks for the class:

1. The study of demonstration smears.
2. Analysis of the scheme of laboratory diagnosis of toxoplasmosis.
3. Analysis of the scheme of laboratory diagnosis of rubella.
4. Analysis of the scheme of laboratory diagnosis of herpes viruses and cytomegalovirus infection.
5. Registration protocol.

Control tests:

1. A pregnant woman came to the doctor with complaints typical of toxoplasmosis. To confirm the clinical diagnosis of her blood was taken. What serological test is needed in this case?

A. ***Complement fixation test****.* *C. Neutralization.* *E. Wasserman.*
B. Precipitation. *D. Vidal.*

2. A man is ill with a protozoan disease characterized by cerebral affection and loss of sight. Blood analysis revealed halfmoon-shaped unicellular organisms with pointed ends. This disease is caused by:

A. ***Toxoplasma gondii****.* *D. Amoeba histolyticum.*
B. Leishmania. *E. Trichomonada vaginalis.*
C. Lamblia intestinalis.

3. A 32-year-old woman with an asymptomatic disease has a second-time dead child with severe microcephaly. What disease should a doctor first think about?

A. ***Toxoplasmosis.*** *C. Brucellosis.* *E. Listeriosis.*
B. Syphilis. *D. Histoplasmosis.*

4. The patient has a preliminary diagnosis of toxoplasmosis. What material was used to diagnose this disease?

A. Sputum. *C. Duodenal contents.* *E. Urine.*
B. ***Blood****.* *D. Feces.*

5. A 2 y.o. girl has been ill for 3 days. Today she has low–grade fever, severe catarrhal symptoms, non-abundant maculopapular rash on her buttocks and enlarged occipital glands. What is your diagnosis?

A. ***Rubella****.* *C. Measles.* *E. Pseudotuberculosis.*
B. Scarlet fever. *D. Adenoviral infection.*

6. A pregnant woman was registered in an antenatal clinic and underwent complex examination for a number of infections. Blood serum contained IgM to the rubella virus. What is this result indicative of?

A. Of primary infection.
B. Of a chronic process.
C. The woman is healthy.
D. Of exacerbation of a chronic disease.
E. Of recurring infection with rubella virus.

7. A pregnant woman was detected with IgM to rubella virus. An obstetrician-gynecologist recommended therapeutic abortion due to the high risk of teratogenic affection of the fetus. Detection of IgM was of great importance as it is these specific immunoglobulins that:

A. Indicate recent infection.
B. Penetrate placental barrier.
C. Have the largest molecular weight.
D. Are associated with anaphylactic reactions.
E. Are the main factor of antiviral protection.

8. A 36 y. o. woman is in the 12-th week of her first pregnancy. She was treated for infertility in the past. She contacted a child who fell ill with rubella 2 days after their meeting. Woman doesn't know if she has ever been infected with rubella. What is the adequate tactics?

A. Monitoring of the specific IgG, IgM with the ELISA.

B. Fetus wastage.

C. Immunoglobulin injection.

D. Cyclovin prescription.

E. Interferon prescription.

9. A 27-year-old sexually active female complains of numerous vesicles on the right sex lip, itch and burning. Eruptions regularly turn up before menstruation and disappear 8–10 days later. What is the most likely diagnosis?

A. ***Herpes simplex virus****.*

B. Bartholinitis.

C. Primary syphilis.

D. Cytomegalovirus infection.

E. Genital condylomata.

10. A pregnant woman found Ig M to the rubella virus, on the basis of which the obstetrician-gynecologist recommended terminating the pregnancy because of the high probability of teratogenic effects on the fetus. It was important to identify IgM, since the immunoglobulins of this class are:

A. Indicates of fresh contamination.

B. Can cross the placental barrier

C. Have the highest molecular weight

D. Associated with anaphylactic reactions

E. Are a major factor in antiviral protection.

11. For the diagnosis of generalized herpes virus infection, blood serum was examined to identify specific antibodies of a particular class. What class of antibodies did indicate the initial stage of a viral infection?

A. Ig G. *B. Ig A.* *C. Ig E.* *D.* ***Ig M****.*

12. An HIV-positive patient's cause of death is acute pulmonary failure resulting from pneumonia. The pathohistological investigation of lungs has revealed transformed cells resemble «owl's eye». Name the pneumonia causative agent:

A. ***Cytomegalovirus****.*

B. Pneumococcus.

C. Influenza virus.

D. Candida fungi.

E. Toxoplasma.

13. A woman with diagnosis of cervical cancer. What virus can this pathology be associated with?

A. ***Herpes simplex virus type 2****.*

B. Varicella-Zoster virus.

C. Cytomegalovirus.

D. Papilloma virus.

E. E. Arenavirus.

14. The patient went to the doctor with complaints of stomatitis, which was treated with antiseptic agents. What drug should be prescribed to the patient if the lesions of the mucous membrane are herpetic in nature?

A. Acyclovir. *C. Furazolidone*. *E. Clotrimazole.*
B. Remantadin. *D. Biseptol.*

17. The patient turned to the dentist with complaints about the appearance of bubbles with liquid on the lips, localized at the border of the skin and mucous membrane. What microorganisms can cause such pathology?

A. Mycobacterium. ***C. Herpesviruses***. *E. Staphylococcus.*
B. Streptococcus. *D. Orthomyxoviruses.*

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