**LESSON 22.
Microbiological diagnosis of wound and septic infections**

**LESSON PLAN:**

1. Inflammatory diseases of the skin. Complicated infections during skin lesions.

2. Wound infections, their causes. Rules for taking pathological material, microbiological diagnosis.

3. Understanding of sepsis, septic infections.

4. Bacteriological examination of blood.

5. Automated systems for obtaining hemoculture.

**Microbiological diagnosis of wound infections**

• Wound infections develop as a result of traumatic disruption of the integrity of the skin and mucous membranes.

• Wound infections are manifested in different ways, depending on the character of the wound and its localization, its dimensions, the state of the organism, the character of the agent that caused the trauma, etc. depends on many factors.

• Various medical manipulations can develop wound infections, especially after surgical interventions.

• During wound infections, examination material is obtained from the depth of the wound with a sterile swab.

• Exudate from cavities is collected by a specialist doctor by aseptic puncture with a syringe and sent to the laboratory in an anaerobic transport environment.

• The abscess cavity is punctured. The material from the drained wound is obtained with a sterile syringe, transferred to a sterile test bottle or anaerobic transport media following aseptic rules.

• Seed pieces and foreign bodies taken from the wound area can also be examined.

• Swabs are prepared from the material taken from the wound contents with a swab.

• If the exudate from the cavities (pleural exudate, empyema pus, synovial fluid, ascites fluid, etc.) is clear, it is centrifuged, and the obtained sediment is used to prepare a plaster.

• When the exudate is purulent, thin plasters are prepared directly from it. After Gram staining, the morphology and quantity of microorganisms are recorded during microscopy.

• The contents of the wound are inoculated from the tampon into nutrient media - MPA, blood and glucose agar, Saburo's medium, glucose broth, media for anaerobes.

• Solid samples are inoculated with a 4-sector loop into a solid medium. Samples are incubated at 370C under aerobic and anaerobic conditions. The obtained cultures are identified.

• When the association of microorganisms is obtained from the contents of the wound, the quantitatively predominant species are considered to be microorganisms with an etiological role.

• Any type of microorganism obtained from the materials taken from sterile body cavities and from the depth of purulent wounds following the rules of aseptic is considered as the causative agent of the purulent-inflammatory process.

• Pieces of seeds are crushed with a sterile lancet, 1 gram of seeds is "dissolved" in 1 ml of nutrient broth.

• Ten times rinses are prepared from the obtained mixture, and 0.1 ml of each rinse is inoculated into solid nutrient media by means of a spatula.

• The number of microorganisms per 1g of seed (CFU/g) is calculated based on the number of colonies developed after incubation and the degree of rinsing.

• The number of microorganisms in 1 g of wound seeds is 105 or more is a diagnostic indicator.

 **Principles of microbiology and diagnosis of septic infections**

• Pathological processes related to the entry of microorganisms into the blood and their multiplication there can manifest as bacteremia and sepsis.

• Bacteremia (virusemia, fungemia, parasitaemia, etc.) refers to the entry of microorganisms into the blood. Microorganisms can enter the blood by exogenous means (for example, as a result of trauma) or from the infection centers in the body. The last case is observed during bacteremic infections.

• During sepsis (Latin, sepsis - suppuration), microorganisms stay in the blood for a long time and multiply there. If the entrance door of infection in septic processes is unknown, it is called septicemia, and when secondary purulent foci appear in internal organs, it is called septicopyemia.

• Bacteriemic infections can be caused by practically most bacteria. Bacteremias caused by gram-negative and gram-positive bacteria differ according to their specific characteristics.

• Gram-negative bacteremias are mainly caused by Enterobacteriaceae (E. coli, Klebsiella, Proteus, Serratia, Proteus, Enterobacter, etc.) and P. aeruginosa. In most cases, the infection enters the gastrointestinal tract, genitourinary tract, and skin.

• Gram-positive bacteremias are mainly caused by S. aureus and coagulase-negative staphylococci (S. epidermidis and S. saprophyticus). During staphylococcal bacteremia, causative agents enter from the skin coverings, as well as from any source of infection in the body.

• The main causative agents of bacteroid septicemia - Bacteroides fragilis and Prevotella melaninogenica are often found in association with other bacteria.

• P. melaninogenica enters the blood mainly from the oral cavity, and B. fragilis from the primary site in the gastrointestinal tract.

* During septicemia caused by clostridia, the causative agents are often detected in association with other anaerobic and aerobic bacteria. The main causative agent, C. perfringens, enters the blood from the intestinal tract and bile ducts, and in some cases, from the mucous membranes of children after abortions.

• Microbiological diagnosis is based on bacteriological examination of blood.

• Blood should be taken from the elbow vein, strictly following aseptic conditions, until the start of antibacterial treatment, or after a certain period of time has passed for the elimination of the used drug from the body.

• Bacteriological examination of blood is based on obtaining the causative agent from blood - taking hemoculture.

• For the purpose of examination, the blood is immediately added to the nutrient medium, and in its absence, to a sterile vial containing reagents that prevent blood coagulation (sodium citrate, heparin, etc.).

• In order to neutralize the effect of the bactericidal factors contained in it, it is inoculated into a liquid medium that is 5-10 times more than the volume of blood taken (usually 5-10 ml of blood is inoculated into 50-100 ml of liquid medium). Special nutrient media are used when stomach flu and other infectious diseases are suspected.

• Samples are incubated at 370C for 10 days with daily observation.

• When growth occurs in the nutrient medium (broth turbidity, sediment, etc.), it is transferred to blood agar, a pure culture is obtained, identification is made, and sensitivity to antibiotics is determined.

• Obtaining any microorganism from the blood is considered as bacteremia and sepsis, regardless of the quantity.

• A single blood test does not always ensure that a blood culture is obtained.

• If the result is negative, it is advisable to carry out the examination no less than three times with an interval of one day.

• Recently, automatic culture systems have been applied to speed up the acquisition of hemoculture, as well as to facilitate numerous examinations. For this purpose, Bactec automatic hemoculture system is used more.

• The principle of the method is based on the detection of carbon dioxide formed as a result of the development of microorganisms.

• Blood samples containing labeled carbon isotope (e.g 14 C isotope) glucose, amino acids, etc. Cultivated in supplemented nutrient media with continuous mixing. In case of development, CO2 produced as a result of the life activity of microorganisms is detected in detectors with computer analysis.