



AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

LESSON 22.

Microbiological diagnosis of wound and septic infections

FACULTY: *General Medicine*

SUBJECT: *Medical microbiology - 2*

Discussed questions:

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.

Purpose of the lesson:

- To acquaint students with the principles of microbiological diagnosis of wound and septic infections.

Microbiological diagnosis of wound infections



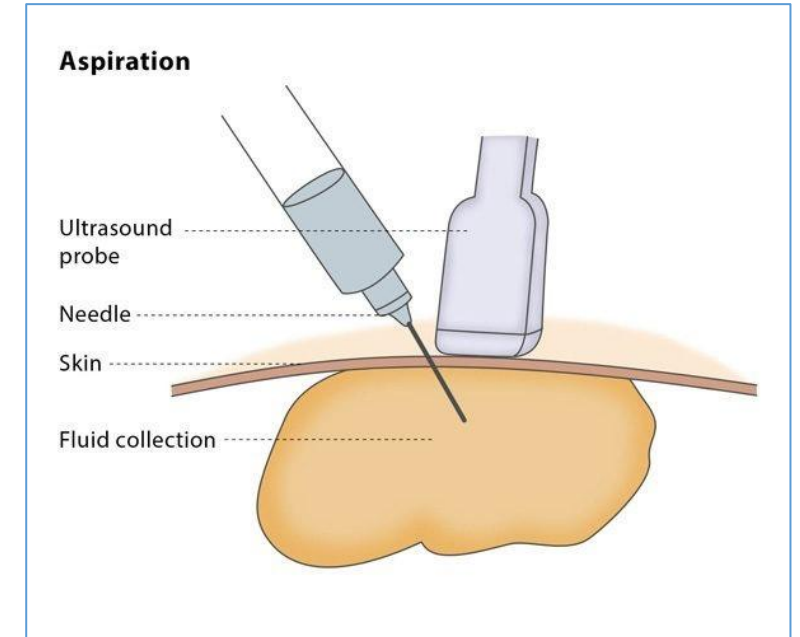
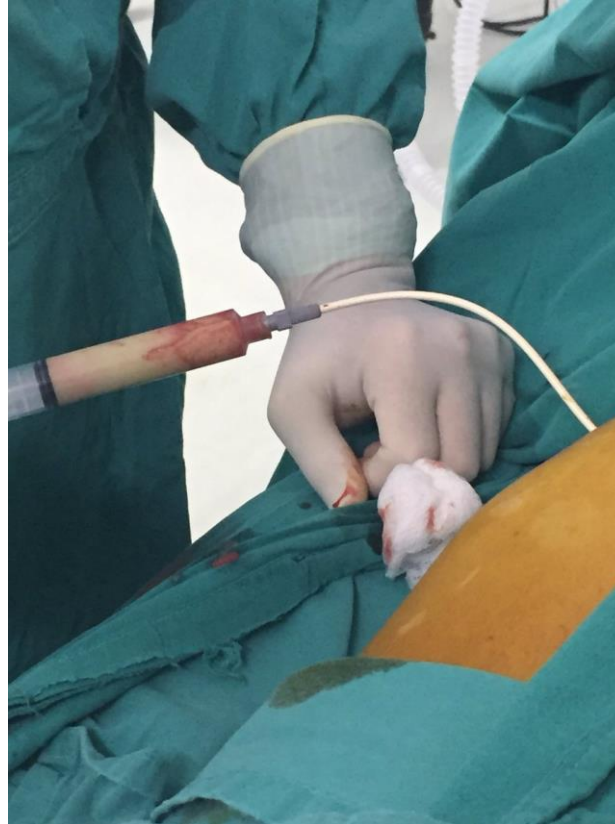
Wound infections

- Wound infections develop as a result of traumatic disruption of the integrity of the skin and mucous membranes.
- Wound infections manifest themselves in different ways, depending on the nature of the wound and its location, size, condition of the body, the nature of the agent that caused the injury, and so on.
- Various medical manipulations, especially after surgical interventions, can lead to the development of wound infections.

Microbiological diagnosis of wound infections (materials for investigation)

- In case of wound infections, the material is obtained from the depth of the wound with a sterile swab.
- Exudate from the cavities is obtained by a specialist doctor by puncture with a syringe in accordance with aseptic procedures and sent to the laboratory in anaerobic transport media.
- The abscess is punctured. Drainage material is obtained from a sterile syringe and transferred to a sterile test tube or anaerobic transport medium following aseptic procedures.
- Tissue fragments and foreign bodies taken from the wound area may also be examined.

Microbiological diagnosis of wound infections (materials for investigation)



Microbiological diagnosis of wound infections (microscopic examinations)

- **Smears** are made from the material taken from the wound contents with a cotton swab.
- If the exudate from the cavities (pleural exudate, empyema pus, synovial fluid, ascites fluid, etc.) is translucent, it is centrifuged, and the sediment is used for preparation of smear.
- When the exudate is purulent, smear are made directly from it. Morphology and quantity of microorganisms are recorded during microscopy after Gram staining.

Microbiological diagnosis of wound infections (cultural examinations)

- The wound contents are inoculated from the tampon into nutrient media – simple media, blood and glucose agar, Saburo medium, glucose broth, anaerobic media.
- Liquid samples are inoculated into a solid medium. Samples are incubated at 37 °C under aerobic and anaerobic conditions. Obtained cultures are identified.
- When an association of microorganisms is obtained from the wound contents, the species that is quantitatively predominant is considered to be a microorganism with an etiological role.
- Any type of microorganism obtained from sterile body cavities and from the depths of purulent wounds, is considered to be a microorganism with an etiological role.

Microbiological examination of wound tissue

- Pieces of tissue are crushed with a sterile lancet, 1 gram of tissue is "dissolved" in 1 ml of food broth.
- The ten-time dilution are prepared from the obtained dilution, 0.1 ml of each dilution is inoculated into solid nutrient media with a spatula.
- Based on the number of colonies growing after incubation and the degree of dilution, the number of microorganisms per 1 g of tissue is calculated.
- The presence of 100 000 or more microorganisms in 1 g of wound tissue is a diagnostic indicator.

The microbiology and principles of diagnosis of septic infections



Bacteremia and sepsis

- The pathological processes associated with the entry and multiply of microorganisms in the blood can manifest themselves as bacteremia and sepsis.
- **Bacteremia** (viremia, fungemia, parasitemia, etc.) refers to the entry of microorganisms into the blood. Microorganisms can enter only through exogenous routes (for example, as a result of trauma) or from sources of infection in the body. The latter case is observed during bacterial infections.
- During **sepsis** (Latin, sepsis - pus), microorganisms stay in the blood for a long time and multiply there.

Infections that accompanied by Bacteremia

- **Bacteremia** can be caused by most bacteria in practice. Bacteremia caused by gram-negative and gram-positive bacteria differ in certain features.
- **Gram-negative bacteremia** is mainly caused by Enterobacteriaceae (E.coli, Klebsiella, Proteus, Serratia, Proteus, Enterobacter, etc.) and P.aeruginosa. Infections are more likely to enter the gastrointestinal tract, urogenital tract and skin.
- **Gram-positive bacteremia** is mainly caused by S. aureus and coagulase-negative staphylococci (S. epidermis and S. saprophyticus). Staphylococcal bacteremia is caused by the skin, as well as any source of infection in the body.

Septic infections

- The main causative agents of bacteroid-induced septicemia, *Bacteroides fragilis* and *Prevotella melaninogenica*, are often found to be associated with other bacteria.
- *P.melaninogenica* enters mainly from the oral cavity, and *B.fragilis* only from the primary hearth in the gastrointestinal tract.
- During septicemia caused by clostridia, the causative agents are often identified in association with other anaerobic and aerobic bacteria. The main causative agent, *C.perfringens*, enters the intestinal tract and bile ducts, and in some cases, after abortions, from the mucous membranes of the uterus.

Principles of diagnosis of septic infections

- Microbiological diagnosis is based on **bacteriological examination of blood**.
- Blood should be taken from the elbow vein in strict adherence to aseptic conditions before the start of antibacterial treatment or after a certain period of time for the elimination of the drug from the body.
- Bacteriological examination of blood is based on the acquisition of the pathogen from the blood - the obtaining of **hemoculture**.

Bacteriological examination of blood

- For this purpose, the blood is immediately transferred to a liquid nutrient medium, and in its absence, to a sterile vial containing reagents (sodium citrate, heparin, etc.) that prevent the blood from clotting.
- To neutralize the effects of bactericidal factors, the blood is inoculated into a nutrient medium 5-10 times the volume of taken blood (usually 5-10 ml of blood is inoculated into 50-100 ml of nutrient medium). Special nutrient media are used when typhoid fever and other infectious diseases are suspected.
- Samples are incubated at 37 ° C for 10 days under daily observation.
- When grown in a nutrient medium (broth turbidity, sedimentation, etc.), it is transferred to blood agar, pure culture is obtained, identified and determined susceptibility to antibiotics.

Bacteriological examination of blood: interpretation

- Obtaining of any microorganism from the blood is assessed as bacteremia and sepsis.
- A single blood test does not always provide a hemoculture.
- If the result is negative, it should be examined at least three times.

Automated Blood Culture System

- Recently, automatic cultivation systems have been used to speed up the obtaining of hemoculture, as well as to facilitate multiple examinations. For this purpose, the **Bactec automatic hemoculture system** is used more and more.
- The principle of the method is based on the detection of carbon dioxide formed as a result of the growth of microorganisms.
- Blood samples are inoculated on nutrient medium with glucose, amino acids, etc. containing the labeled carbon isotope (eg ^{14}C isotope). It is cultivated with continuous mixing in added nutrient media. When microorganisms grow, CO_2 formed as a result of the vital activity of microorganisms is detected in computer analyzers.

Automated Blood Culture System

