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ETIOLOGY, CLINICAL FEATURES AND PREVENTION OF CLOSTRIDIUM TETANI

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Abstract: This article examines the main causes (etiology), clinical signs, and prevention measures of tetanus caused by the bacterium Clostridium tetani. C. tetani is a gram-positive, spore-forming, obligate anaerobic microorganism. It is mainly found in soil, dust, and animal intestines. Bacterial spores are resistant to the external environment; upon entering the body through a wound, they become active and produce a toxin.The neurotoxin secreted by the bacteria influences the development of tetanus. This toxin affects the nervous system, causing intense muscle contractions and spasms. Clinically, the disease manifests as triism, muscle rigidity, and general spasms. In preventive measures, primary attention is paid to active immunization. Anatoxin-based tetanus vaccines are utilized as highly effective prophylactic agents in the prevention of the disease. Furthermore, timely and proper treatment of wound surfaces and strict adherence to sanitary and hygienic standards are important factors in disease prevention.The article briefly outlines the general microbiological characteristics of tetanus, the course of the disease, and preventive measures based on scientific sources.

Keywords: Clostridium tetani, tetanus, etiology, spore-forming bacterium, anaerobic microorganisms, neurotoxin, muscle spasm, trismus, immunoprophylaxis, toxoid vaccine, active immunization, infection.

Relevance of the topic

Tetanus is an acute infectious disease associated with Clostridium tetani spores, and its relevance is determined by the inability to completely rid oneself of the microorganism. Spores of these bacteria persist for a long time on the surface of soil, dust, ash, the intestinal tract of animals and humans, as well as various metal objects. Due to their resistance to heat and many chemicals, they do not lose their vitality for years. Therefore, completely eliminating the source of infection is epidemiologically impossible, and the disease remains a constant threat. (Yen LM, Thwaites CL. Tetanus. The Lancet.

The disease can be observed in various age groups, but individuals with an insufficiently developed immune system or not fully covered by active immunization (vaccination) are considered high-risk groups. The disease is particularly severe among pregnant women and newborns. Neonatal tetanus is often associated with non-compliance with hygiene requirements during umbilical cord incision or care using non-sterile instruments. Carrying out the labor

process in violation of sanitary rules also significantly increases the risk of infection. (World Health Organization, Tetanus Fact Sheet, 2023)In global health practice, the relevance of tetanus is manifested in its close connection with socio-economic inequalities. In regions with low vaccination coverage, morbidity rates remain high. In 2018, approximately 25,000 newborns worldwide died from tetanus, indicating that the disease has not been brought under full control, despite a decrease in this figure compared to previous decades. A decrease in immunization rates or insufficient use of booster doses is noted as a risk factor for certain age groups, particularly among adolescents and older men.For this reason, tetanus remains one of the infections facing the global healthcare system today, requiring strict adherence to preventive measures.

Epidemiological observations in recent years indicate that tetanus is not only characteristic of low-income regions but also persists in developed regions. In 2023, 73 cases of tetanus were registered in the European Union and the European Economic Area, which is slightly higher compared to 69 cases in 2019. Although the number of cases decreased to 32 in 2020, an increase was observed again in subsequent years, with 50 cases identified in 2021 and 53 in 2022. These dynamic indicators indicate sporadic but stable persistence of the disease. (European Centre for Disease Prevention and Control (ECDC), Annual Epidemiological Report on Tetanus, 2024)

Analysis of the age structure shows that the incidence is predominant among individuals aged 65 and older. Cases were recorded relatively more often, especially among elderly women. In 2023, 13 fatalities were identified, and out of 63 patients with a known clinical outcome, 21% were fatal. The majority of deaths occurred in women over the age of 79. This figure is significant because it is higher than the annual number of deaths recorded in 2019-2022 (ranging from 5 to 11).

The epidemiological situation is explained by a decrease in immunity or insufficient reimmunization in the elderly population. Three-dose immunization (DTP3) coverage for diphtheria, tetanus, and pertussis among one-year-old children was 92.8% in 2023, and the overall level remained high despite a slight decrease between 2019 and 2022. Nevertheless, an insufficient level of protection among adults, especially in older age groups, creates a prerequisite for the persistence of the disease.

Data on neonatal tetanus are relatively more accurate, as the disease manifests within the first 28 days of life and is often directly linked to maternal immunization. This form develops when sterile conditions are not provided during childbirth or postpartum care. The incidence of tetanus in newborns is regarded as a crucial indicator of the effectiveness of preventive measures within the healthcare system. (World Health Organization, 2023).

Environmental distribution of C. tetani

C. tetani is a widespread microorganism in soil samples from all regions of the world. Its insulation frequency varies depending on different studies. In studies conducted in Japan, Canada, Brazil, and the United States, 30–42% of positive samples were identified [5]. The frequency of C. tetani isolation from the soil is influenced by a number of factors, including pH, temperature, humidity, organic matter content, and their type. Thus, the germination and reproduction of C. tetani is favorable in neutral or alkaline soils, in conditions with temperatures above 20°C and humidity of at least 15%[1].

The geographical distribution of C. tetani is higher in hot and humid regions. The highest rates of tetanus are observed in West and Central Africa, Southeast Asia, India, the Pacific

Islands, and the southern United States. In cooler regions of the world (e.g. Canada, Norway, England, Finland, Sweden, etc.),[1] the prevalence of C. tetani is relatively low.

This bacterium can be detected in the intestines of animals, but it does not constitute a significant part of the normal digestive flora. For example, in the Durban region of South Africa, the total contamination rate of soil samples with C. tetani was estimated at 28%. In 118 stool samples taken from horses in this region, the detection rate of C. tetani was 5.9%.[6]

A 2009 study in southern India found C. tetani in 2.6% of 115 soil samples, but this microorganism was not found in 59 water samples.[7]In Okinawa Prefecture, Japan, C. tetani was detected in 18.6% of 290 soil samples examined in 1992.[8] In Kanagawa Prefecture, Japan, this bacterium was recorded in 22.9% of 35 soil samples. Samples from mountainous areas had higher levels of pollution compared to samples from fields, private parks, or public roads.[9].

Previous studies have shown a high prevalence of C. tetani in soil samples in Japan: 20% in roadsides, 30% in school and hospital areas, 53% in household yards, and up to 85% in fields, ponds, rivers, and wet shores.[10] Outside of Japan, according to available data, systematic research on C. tetani in soil at the national level has not been sufficiently conducted. However, due to the worldwide prevalence of tetanus, it is assumed that this pathogen may be present in almost all soils.C. tetani may also be present on various surfaces and objects contaminated with soil particles, dust, or feces. In hospital settings, toxic strains of C. tetani were also isolated from katgut, cotton, dust, and air samples, as well as human skin and wounds[2][4].

Clostridium tetani

C. tetani is a gram-positive rod, 0.3-0.6 μm wide and 3-12 μm long. Gram-positive staining is more pronounced in young cultures, but in cultures incubated for more than 24 hours, C. tetani can partially or completely remove the Gram stain. Most strains have peritrichous flagella, which causes them to grow in swarms in an agar medium. Some strains may be flagellumless and immobile.

When grown under anaerobic conditions, mobile strains spread widely across the agar surface, forming a transparent film. Discrete colonies (2–5 mm) can be obtained in a medium with a content of 3–4%. In a blood agar, colonies are slightly elevated, semi-transparent gray, with uneven and rough borders, and are surrounded by a narrow hemolysis zone[1]. C. tetani thrives in complex nutrient media containing peptones or tissue extracts. (Murray et al., 2021).

The spores of C. tetani are round and terminal, forming a "drumstick" shape characteristic of the bacterium. Spore formation varies depending on the strain. At pH 7 or higher and a temperature close to 37°C, sporulation begins within 24 hours after cultivation and typically lasts 4-12 days. At temperatures above 41°C, sporulation does not occur, while at pH <6, the process slows down significantly [2]. The degree of sporulation also depends on the composition of the nutrient medium.The spores are resistant to moderate heating (10 minutes at 75–80°C) and can survive in such conditions. However, at a temperature of 100°C, they usually die within 1 hour.C. tetani spores can germinate under anaerobic or aerobic conditions in complex environments such as liver broth. Spore germination occurs in a wide range of redox potentials (Eh) from −100 mV to +580 mV, but further vegetative growth is not observed under Eh conditions above +300 mV[3].

The optimal temperature for growth is 37°C. At 30°C, moderate growth is observed, while at 25°C or 45°C, incubation stops or significantly slows down growth. Depending on the strain's characteristics, medium or large amounts of gas can be formed in the pepton-yeast extract-glucose medium.

[4]https://pmc.ncbi.nlm.nih.gov/articles/PMC7081504/?utm_source

C. tetani toxins

C. tetani produces two main toxins: neurotoxin — TeNT (tetanospasmin) and hemolysin — tetanolysin. Tetanolysin is a toxin belonging to the cholesterol-dependent cytolysins (CDC) family that forms a hole in the cell membrane. A typical representative of this family is perfringolizin derived from C. perfringens. CDCs form large pores on the surface of the target cell membrane; in this process, 40–70 toxin monomers are oligomerized, leading to membrane damage. Tetanolysin can enhance bacterial colonization in local tissues and increase resistance to the protective effects of macrophages[11][12].

TeNT is initially synthesized as a single-stranded protein with a mass of about 150 kDa, and in this state, it is less active. Upon release from the bacterium, the toxin is proteolytically activated by clostridial proteases or exogenous proteases of the host. Proteolytic cleavage occurs at approximately one-third of the molecule.Active TeNT consists of two parts: a light chain (L; N-terminal part, about 50 kDa) and heavy chain (H; C-terminal part, approximately 100 kDa). The L and H chains are connected by a disulfide bridge. The overall structure of TeNT is similar to Botulinum neurotoxin type E (BoNT/E). (Rossetto et al., 2014).

BoNT and TeNT molecules have three distinct functional domains:

1. L-chain - contains α-spirals and β-threads and contains a special motif that binds with catalytic zinc (Zn2+);

2. The N-terminal part of the H-chain (HN) consists of two long and twisted α-spirals;

3. The C-terminal part of the H-chain (HC) consists of two subdomains (HCN and HCC).

The HC region is involved in the recognition of cell membrane receptors and the process of toxin entry into the cell. Similar to BoNT/E, the L-chain and HC domains are located on the same side of the HN domain, which performs the translocation function[13].

Method of action of tetanus toxin and pathophysiology of tetanus

The ulcer develops after contamination with C. tetani and the bacterium begins to secrete a toxin. Bacteria-contaminated wounds are sometimes so small that they can be difficult to identify. Tetanus can manifest even after the wound has healed. In particular, wounds in parts of the body that frequently come into contact with the soil pose a high risk of developing tetanus. These include, for example, accidental injuries to the extremities, lower body, and abdominal area, or puncture wounds with thorny plants.

Umbilical cord infections are a predisposing factor for the development of tetanus in newborns, especially in lambs and foals. Surgical wounds can also be contaminated with C.

tetani spores; this condition is often observed after castration, caudal ligation, and ear surgery. Contaminated vaccination or injection sites, as well as contaminated wounds resulting from the shearing process, are also factors contributing to tetanus. Postpartum tetanus is caused by contamination of the vaginal mucosa and uterus during difficult childbirth[14][15][16][17].

The presence of an environment with minimal exposure to air and necrotic (dead) tissue creates anaerobic conditions. This condition promotes spore germination, growth of C. tetani, and subsequent production of TeNT. C. tetani is not an invasive bacterium, meaning it cannot penetrate healthy cells. However, if there are damaged and decaying cells inside the wound, it is a favorable medium (substrate) for bacterial growth and toxin production. In experimental conditions, tetanus in laboratory animals is induced by the intramuscular administration of C. tetani spores in combination with a tissue-necrosing agent such as calcium chloride. (Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 2020).

TeNT spreads locally at the site of infection and interacts with the damaged nerve endings. It recognizes specific receptors on the cell membrane of a neuron; these receptors consist of parts of gangliosides and membrane proteins. TeNT binds with high affinity to gangliosides (especially GT1b and GD1b) through two carbohydrate-binding regions located at the C-terminus of the heavy chain.[18][19] TeNT also recognizes the extracellular matrix protein nydogen in a neuromuscular junction. However, specific receptors on the cell membrane of a neuron have not been fully identified[20].

Receptor-bound TeNT enters the cell through receptor-mediated endocytosis. The toxin is then directed into the vesicles of endocytosis, which are not yet acidified. These vesicles are transported via retrograde transport to the cell bodies of neurons in the central nervous system (CNS). In the central nervous system, TeNT is released into the extracellular space and enters inhibitory interneurons that control motor neuron activity [21][22][23][24][25][26].

TeNT enters target inhibitory neurons through acidic vesicles. When the internal environment of the vesicle is acidified, a conformational change occurs in the toxin structure, and the light chain (chain L) transitions into the cytosol. The L-chain blocks the release of glycine and gamma-aminobutyric acid (GABA) in the cytosol. The exact mechanism by which the L-chain passes into the cytosol is not yet fully understood. The disulfide bond between the L and H chains is important in the process of translocation; this bond is then reduced, and 22–24,35 TeNT The L chain is a zinc-dependent metalloprotease that allows the L chain to be freely released into the cytosol.[27]

The L-chain of TeNT is a zinc-dependent metalloprotease that breaks down one of the most important SNARE complex proteins, VAMP (synaprotein), which is involved in neurotransmitter secretion. As a result, the release of glycine and GABA stops. This eliminates the inhibitory control exerted by inhibitory interneurons on motor neurons. Motor neurons become overactive and the muscles become in a state of constant contraction.

Thus, tetanus is characterized by spastic paralysis and, in severe cases, can be fatal due to paralysis of the diaphragm and respiratory muscles.[28][29][30][31][32]

The aforementioned pathogenetic mechanisms indicate that the disease-causing process of C. tetani is associated not with direct invasiveness, but with the production of a potent neurotoxin. In particular, the retrograde transport of TeNT through the nervous system to the central nervous

system and the blocking of the release of inhibitory neurotransmitters - glycine and GABA - form the primary clinical signs of tetanus.

The loss of inhibitory control over motor neurons leads to persistent and painful muscle spasms. This condition scientifically justifies the manifestation of tetanus with spastic paralysis. Especially life-threatening complications develop when the respiratory muscles and diaphragm are damaged. (Kandel et al., 2021).

Clinical signs of tetanus

Tetanus is an acute neurological disorder resulting from the effects of the neurotoxin tetanospasmin (TeNT), produced by Clostridium tetani, on the central nervous system and is characterised by predominantly voluntary muscle hyperactivity. Inhibition of the release of inhibitory neurotransmitters—glycine and gamma-aminobutyric acid (GABA) —plays a crucial role in the pathogenesis of the disease. As a result, inhibitory control over motor neurons is lost, and the muscles remain in a constant state of excitation. Clinically, it manifests as tonic muscle stiffness and recurrent spasms. Muscle stiffness is observed in the form of prolonged, involuntary (tonic) contractions. Spasms, on the other hand, are usually short-lived but intense and painful, often occurring reflexively under the influence of external stimuli - light, noise, touch, or medical manipulations. Spastic paralysis is a characteristic clinical sign of tetanus, and the diagnosis is often made based on typical symptoms. In the early stages of the disease, it can be confused with myopathies or other neuromuscular disorders, which complicates differential diagnosis. (Guyton & Hall, 2021).

Clinically, tetanus is divided into generalized (systemic) and local forms. Generalised tetanus is the most common form, initially manifesting as a spasm of the masticatory muscles known as trismus (the inability to open the mouth). The subsequent contraction of the facial muscles gives the appearance of a "risus sardonicus" (forced sarcastic smile). The process gradually spreads to the muscles of the neck, back, and limbs. As a result of severe back muscle spasms, the body is sharply turned back, a condition known as opistotonus. Muscle spasms are generalized and may involve the respiratory muscles. (Harrison's Principles of Internal Medicine, 2022).In newborns, tetanus often manifests in a generalized form and occurs mainly in the period up to 1 month of age. Neonatal tetanus is usually associated with infection of the umbilical wound and is accompanied by loss of sucking ability, muscle stiffness, and generalised spasms.In local tetanus, symptoms are limited to the muscles near the site of infection. Pain and spasticity are observed in the affected area. This form is more common in certain animal species, particularly in dogs that are relatively resistant to the toxin, and can sometimes transition into a generalized form.

The autonomic nervous system is also affected during the course of the disease. At the same time, episodes of palpitations (tachycardia), hypertension, and excessive sweating are observed; these conditions sometimes alternate with bradycardia and hypotension. Vegetative dysfunction is one of the factors that worsen the prognosis in severe tetanus. (Adams & Victor's Principles of Neurology, 2021).

In severe cases, acute respiratory failure develops as a result of spastic paralysis of the diaphragm and other respiratory muscles, which is the primary cause of death. Clinical symptoms may persist for several weeks, as the binding of the toxin to nerve endings is an irreversible process, and functional recovery is associated with the formation of new synaptic connections. (Kandel et al., 2021).

Epidemiology

Most cases of tetanus occur in developing countries with low vaccination rates, especially in areas affected by natural disasters.[33] In such areas, the disruption of medical services and the fact that the population is not fully vaccinated increase the risk of the disease. In economically developed countries, people who have not been vaccinated at all or whose vaccine effects have decreased over time are at risk. According to observation data, people who inject drugs and patients with insulin-dependent diabetes are particularly vulnerable[34].

The causative agent of tetanus, Clostridium tetani, is a spore-forming bacterium whose spores can be found in soil and dust in almost all regions. They enter the body through an open wound or a damaged area of the skin. Especially deep and dirty wounds with dead or damaged tissues create favorable conditions for the development of the disease. Therefore, a person of any age can be susceptible to infection. If the patient is not provided with high-quality medical care, the mortality rate can be very high, reaching almost 100%. Under conditions of modern and correct treatment, this figure ranges from 10 to 20%[35].

Newborns are a high-risk group, especially in areas with scarce resources. If childbirth is performed without adherence to hygiene rules, if the umbilical cord is incised with a non-sterile instrument, or if a dirty bandage is applied to the umbilical cord, tetanus may develop in the newborn. According to the World Health Organization,[36] an estimated 34,000 newborns died from neonatal tetanus in 2015.[37]

Tetanus is a disease that can be prevented with a vaccine. Vaccines containing tetanus toxoid are included in the routine vaccination schedule for children. Between 2001 and 2008, the average annual rate of tetanus in the United States was 0.01 per 100,000 population. [34] Between 2009 and 2015, 197 cases of illness and 16 deaths were recorded. The highest risk group includes newborns and the elderly.

The tetanus toxoid vaccine was first developed in 1924 and was widely used among soldiers during World War II. Currently, the five-component vaccine (DTP-Hib-HepB), which protects against diphtheria, tetanus, pertussis, hemophilia (Hib), and hepatitis B, is one of the most widely used pediatric vaccines in the world. [35] It should be noted that contracting tetanus does not form protective immunity in the future, and the disease is not transmitted from person to person[38].

Treatment/Management

Treatment of tetanus requires the cooperation of infectious disease specialists and intensive care physicians. The primary goal of treatment for acute tetanus is to neutralize toxins freely circulating in the body and to strictly monitor severe symptoms [39] [40]. Immediate cleaning and surgical treatment of tetanus-prone wounds is crucial, as it helps eliminate spores and reduce the passage of toxins from tissues into the bloodstream. Patients with symptoms of tetanus should be kept under constant supervision in the intensive care unit (ICU) whenever possible. It is necessary to reduce irritating factors such as noise, bright light, and frequent contact with the patient. The risk of airway narrowing or obstruction is constantly monitored, and if necessary, the patient is prepared for intubation for artificial respiration [41].

The tetanus toxin, tetanospasmin, binds firmly to the junction of nerve and muscle. Therefore, treatment is aimed at neutralizing toxins that are not yet bound in the body and are

freely circulating in the blood. When tetanus is suspected, human tetanus immunoglobulin (HTIG) is administered intramuscularly. This neutralizes the toxin using ready-made antibodies and reduces the severity of the disease [40] [42].In countries with limited resources, if human immunoglobulin is unavailable, horse tetanus serum can be administered intravenously. However, this method is used with caution, as it may cause an allergic (anaphylactic) reaction, and a skin test is conducted before administration.

There are differing opinions regarding the benefits of intravenous administration of antiserum [40], so there is no clear recommendation. If a specific tetanus serum is unavailable, a standard human immunoglobulin can be used. Data on intravenous administration of magnesium sulfate are limited[39][40]. It is used alone or in combination with benzodiazepines to reduce muscle spasms and the release of stress hormones [41]. Some studies have shown benefit, especially in milder forms of the disease, but the optimal dosage and treatment duration have not been clearly defined.

Baclofen, administered into the cerebrospinal fluid, was also used to reduce spasms. Some clinical observations have shown a positive result, but its exact dose and duration have not yet been fully determined. Furthermore, it is used only in intensive care settings, as it can cause respiratory distress and cardiac instability.

For wound treatment, metronidazole or penicillin is usually prescribed for 7–10 days. These antibiotics are used to eliminate Clostridium tetani bacteria present in the wound, but they do not directly reduce muscle spasms or nervous system disorders. If the wound is infected with mixed microbes, antibiotics with a wider range of action can also be used. It has been suggested that high-dose intravenous penicillin may interfere with the effects of inhibitory substances in the nervous system, according to some theories[40].

There are few studies comparing metronidazole and penicillin in the treatment of tetanus ulcers. The available data do not indicate a clear advantage of one of these two antibiotics over the other.

[38] https://www.ncbi.nlm.nih.gov/books/NBK482484/.

In my opinion, treating tetanus is very complex and requires a highly qualified medical approach. Because the severity of the disease depends not only on the infection itself, but also on the effect of the toxin on the nervous system. Therefore, in the treatment process, it is not enough to simply destroy the bacteria, but it is important to constantly monitor the patient's respiratory activity, cardiovascular system, and muscle spasms.

Conclusion

Tetanus is a severe infectious disease associated with the tetanospasm toxin produced by Clostridium tetani, which enters the body primarily through wounds and affects the central nervous system. The disease is more common in immunocompromised individuals, newborns, pregnant women, and the elderly. The long-term storage of spores in the external environment causes tetanus to become a constant threat. Clinically, it is accompanied by muscle spasms, rigidity, and autonomic disorders; in severe cases, respiratory failure can lead to death.

Active immunization is the most effective method for the prevention of tetanus. Furthermore, proper wound treatment, passive immunization, and early treatment reduce the severity of the disease. Overall, expanding vaccination and strict adherence to sanitary and hygienic measures are of great importance in controlling tetanus.

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