CLOSTRIDIAL NEUROTOXINS

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iii35

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The genus *Clostridium* comprises a number of spore forming Gram positive, rod shaped bacilli. They are found in the intestines of numerous mammalian species including domestic animals, horses, chickens, and humans. They are also widely distributed in the soil and in marine and freshwater sediments. Many clostridial species produce medically important toxins but the species of neurological interest (*Clostridium tetani* and *Clostridium botulinum*) produce neurotoxins. The toxins responsible for these neurotoxic syndromes are tetanus toxin (sometimes known as tetanospasmin) and the botulinum toxins.

THE TOXINS

Tetanus and botulinum toxins share several important features: they are produced as a single polypeptide of 75 kb which undergoes post-translational cleavage to form a heavy (H) chain and a lighter (L) chain of 100 kDa and 50 kDa, respectively, linked by a single disulfide bond. The H chain facilitates binding to gangliosides on the plasma membrane of peripheral nerve terminals before internalisation via receptor mediated endocytosis. Protonation of the endosome results in the reduction of the disulfide bond. The H chain forms a transmembrane pore across the endosome and the L chain then enters the nerve terminal cytosol. The L chains of both tetanus and botulinum toxins are zinc activated proteases. Their targets are a number of specific proteins involved in synaptic vesicle docking—synaptobrevin (also known as VAMP), SNAP-25, and syntaxin.¹

The toxins selectively target individual proteins (table 1), but the result is always the same—the hydrolysis of the target protein, blockade of transmitter release, and a resultant flaccid paralysis. While the botulinum toxins remain in the nerve terminal the tetanus toxin is transported by retrograde axonal transport into the cell body and then by transsynaptic exchange into the terminals of inhibitory neurones in the spinal cord and brain stem. The resultant inhibition of inhibitory transmission by the tetanus toxin results in the dominant clinical features of hyperexcitability combined with clinical weakness between spasms and the paralysis of cranial nerves (as seen in cephalic tetanus). Both tetanus and botulinum toxins affect both somatic and autonomic nervous systems, but autonomic features (for example, labile hypertension and tachycardia) are more common in tetanus.

TETANUS

C. tetani form resilient spores capable of surviving household disinfectants and boiling in water for several minutes. In conditions of low oxygen tension (for example, wounds) the spores germinate and the resultant bacteria multiply and produce a neurotoxin responsible for the clinical features of tetanus.

Generalised tetanus is the most common presentation in which muscles throughout the body are affected, with the head and neck being usually affected first followed by a caudal spread of spasms. Tetanus following intramuscular injections of quinine has a particularly poor prognosis; the low pH of quinine may facilitate the entry of tetanus toxin into nerves. Quinine is often mixed with heroin as it has a bitter taste that resembles heroin. The effects of quinine may therefore also explain the poorer prognosis of tetanus in drug abusers.

Localised tetanus occurs if the rigidity and pain remain localised to the site of injury, and is usually associated with a better prognosis. An exception is cephalic tetanus following a head or neck injury, and which is associated with a high mortality. A unilateral lower motor neurone facial weakness is the most common involvement in cephalic tetanus, though other lower cranial nerves and the oculomotor nerve may also be affected.

Tetanus neonatorum is rare in the developed world. The condition arises from poor umbilical hygiene, and is prevalent in communities that employ traditional midwifery practices such as cutting the cord with grass or dirty scissors, or rubbing manure on the umbilical stump. The affected neonate presents within a week of birth with a failure to feed, vomiting, and

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Table 1 Clostridial neurotoxins and their target proteins

Toxin	Target
Tetanus toxin	VAMP (synaptobrevin)
Botulinum toxin	SNAP-25
	A VAMP (synaptobrevin)
	B SNAP-25, syntaxin
	C VAMP (synaptobrevin)
	D SNAP-25
	E VAMP (synaptobrevin)
	F VAMP (synaptobrevin)
	G VAMP (synaptobrevin)

“convulsions”. The disease may be prevented by improved hygiene and by maternal vaccination, even if the latter is administered during the pregnancy.

Despite the World Health Organization's intention of eradicating tetanus by 1995 there are still 800 000 to 1 million deaths worldwide each year from tetanus, with half these deaths caused by neonatal tetanus. Most fatal cases occur in Africa and South East Asia, where the problem remains endemic. In these regions mortality is related to limited access to artificial ventilation—for neonatal tetanus mortality is 65–90% without ventilation and drops to 10% with ventilation. There are 12–15 cases in Britain and 50–70 cases of tetanus in the USA each year. In such countries with access to assisted ventilation the mortality is negligible in young adults, but increases to over 50% in patients over 60 years of age. The age related differences in mortality may be caused by waning immunoprotection.² Death may occur due to autonomic dysfunction or the complications (for example, sepsis, thromboemboli) of prolonged critical illness. Although tetanus is easily preventable by immunisation (the first effective vaccine was produced over 100 years ago), it is tragic that many people still remain unprotected.

Clinical features

Tetanus usually occurs following a deep penetrating wound where anaerobic bacterial growth may occur, particularly if the wound is contaminated by soil, manure or rusty metal. It may also occur by other mechanisms such as in burns, ulcers, septic abortions, circumcisions, intramuscular injections, acupuncture, ear piercing, tattooing, poor dentition, chronic otitis media, and following snakebites. In up to 30% of cases the portal of entry cannot be identified.

The incubation period (time of inoculation to the first symptom) is usually 7–10 days (range 1–60 days), and the period of onset (time from first symptoms to the start of spasms) is 1–7 days. The severer forms of the disease have shorter incubation periods and periods of onset.

The clinical picture is dominated by muscle spasms, rigidity, and autonomic disturbances.^{3,4} Neck stiffness, sore throat, and difficulty opening the mouth are the earliest features. Masseter spasm causes trismus (“lockjaw”), with the spasms extending to the facial muscles to cause a characteristic facial expression (“risus sardonicus”). Muscle spasms may lead to laryngeal obstruction as well as decreased chest wall compliance, resulting in respiratory compromise. Involvement of axial muscles leads to neck extension, and truncal rigidity may lead to opisthotonus. Back pain, trismus, muscle stiffness, and dysphagia are common. The episodic spasms tend to be extremely painful and affect agonist and antagonist muscle groups together, and may be spontaneous or stimulus sensitive (triggered by

touch, visual, auditory, or emotional stimuli). These spasms may appear convulsive in nature and be violent enough to cause fractures or tendon avulsions. Rigidity is most prominent in muscles adjacent to the portal of entry. The autonomic disturbances can lead to labile hypertension, tachycardia, pyrexia, profuse sweating, excessive bronchial secretions, gastric stasis, and diarrhoea. The hypertension is predominantly caused by increases in systemic vascular resistance secondary to raised concentrations of circulating catecholamines similar to those seen in phaeochromocytoma.

Increasing muscle spasms and rigidity characterise the first week of illness. Autonomic disturbances usually start a few days after the spasms and reach a peak during the second week, and persist for 1–2 weeks. Spasms start to subside after 2–3 weeks, but the muscle rigidity may continue long after the spasms and autonomic involvement have subsided. Muscle rigidity may last up to 6–8 weeks in severe cases.

Various grading systems of severity of tetanus have been described, one of the most commonly used being the Ablett system (table 2). Other scoring systems (for example, Dakar and Phillips scores) have also been devised to assess overall prognosis.³

Diagnosis

The diagnosis is made clinically as there is no specific confirmatory investigation. A positive culture of *C tetani* from a wound would be supportive evidence for the diagnosis.

The differential diagnosis for tetanus may be diverse. Intense muscle rigidity may be mistaken for acute dystonic reactions. Rigidity of abdominal muscles may mimic an acute abdomen. Poisoning by strychnine (a competitive antagonist of glycine) may mimic tetanus. Cephalic tetanus may be difficult to differentiate from other causes of cranial nerve palsies—helpful clinical pointers include impaired mouth opening and complaints of dysphagia which are commonly seen in tetanus. The “spatula test” (stimulation of the pharynx with a spatula provokes an intense spasm of the masseters resulting in the patient biting the spatula) may aid in diagnosis, though this should be performed with great care as intense pharyngeal and laryngeal muscle spasm may occur leading to respiratory arrest. Neonatal tetanus may need to be differentiated from hypocalcaemia, hypoglycaemia, neonatal seizures, and meningitis.

Management

Wounds from which the infection originated should be surgically debrided and an antibiotic administered. Penicillin has been the worldwide antibiotic of choice. However, the structure of penicillin is similar to γ aminobutyric acid

Table 2 The Ablett classification of tetanus severity

Grade	Clinical features
I	Mild-moderate trismus; general spasticity; no spasms; no respiratory embarrassment; little or no dysphagia
II	Moderate trismus; well marked rigidity; mild-moderate but short spasms; moderate respiratory embarrassment with RR >30/min; mild dysphagia
III	Severe trismus; generalised spasticity; reflex prolonged spasms; RR >40/min; severe dysphagia; HR >120/min
IV	Grade III and severe autonomic disturbances affecting cardiovascular system (for example, severe hypertension and tachycardia alternating with relative hypotension and bradycardia, either of which may be persistent)

HR, heart rate; RR, respiratory rate.

(GABA); it therefore acts as a competitive GABA antagonist, and in high doses may cause central nervous system (CNS) hyperexcitability and convulsions. In tetanus this potential side effect of penicillin may act synergistically with the toxin to block GABA neuronal activity. Metronidazole is therefore considered to be the antibiotic of choice in the treatment of tetanus. Comparative studies in human tetanus have demonstrated the superiority of metronidazole over penicillin. Antibiotics should be administered for 7–10 days. Alternative agents that may be used include erythromycin, tetracycline, vancomycin, clindamycin, doxycycline, and chloramphenicol.

Passive vaccination with anti-tetanus immunoglobulin shortens the course and may reduce the severity of the illness. Anaphylactic reactions occur in approximately 20% of cases receiving the equine antitoxin, and in 1% may be severe enough to require the use of adrenaline (epinephrine), steroids, and intravenous fluids; such reactions occur much less frequently with the human antitoxin. For prophylaxis against tetanus passive immunisation should be given as soon as possible after an injury.

Active immunisation (that is, tetanus toxoid) also needs to be given to add to the short term immunity provided by anti-tetanus immunoglobulin, as well as providing longer term humoral and cellular immunity. The toxoid and the anti-tetanus immunoglobulin need to be given at different sites of the body to prevent interaction at the injection site. Also, if they are to be administered at the same time the dose of anti-tetanus immunoglobulin needs to be modified, as higher doses may neutralise the immunogenicity of the toxoid. Reactions to the toxoid occur in approximately one in 50 000 of injections. Reactions include local tenderness, oedema, flu-like illness, and a low grade fever. Severe reactions to the toxoid are rare, and include a Guillain-Barré type syndrome.

External stimuli provoke muscle spasms and may worsen the autonomic disturbances in tetanus. Unnecessary stimuli should therefore be minimised, and all patients should therefore receive adequate sedation and be nursed in a darkened and quiet room. The benzodiazepines (which augment GABA activity) are the sedatives most commonly used. Diazepam is given initially as intravenous boluses (total daily doses of up to 200 mg are common) followed by oral administration in the recovery phase. Midazolam is a suitable alternative with a shorter half life. Parenteral boluses of morphine can also prove beneficial for sedation. Morphine also induces peripheral venous and arteriolar dilatation, probably by reducing sympathetic discharge centrally, an effect which can offset some of the autonomic disturbances seen in tetanus.

If muscle spasms persist despite adequate sedation, muscle relaxants may be required. Traditionally the long acting agent pancuronium has been used to achieve muscular paralysis. However, this agent inhibits catecholamine reuptake and may therefore worsen autonomic instability and cause hypertension and tachycardia in the more severe cases. Pipercuronium and rocuronium are newer longer acting agents with fewer cardiovascular effects, but are relatively expensive. Vecuronium similarly has fewer cardiovascular effects but is shorter acting. Baclofen and dantrolene tend to be less effective agents in relieving muscle spasms in this context. If muscle spasms are severe enough to require such agents the patients also usually require assisted ventilation.

Excessive bronchial secretions and hypersalivation resulting from autonomic overactivity, in conjunction with laryngeal spasms and dysphagia, make aspiration a particular complication that needs to be guarded against in tetanus. Protection of the airway may require percutaneous tracheostomy. Assisted ventilation may be required if there is any respiratory impairment. Autonomic disturbances (for example, labile hypertension, tachycardia, vasoconstriction, excess sweating) may need to be corrected. The routine use of agents that block autonomic transmitters and receptor blockers (for example, magnesium, α blockers, β blockers, atropine) have not shown consistent benefits in tetanus; therefore, autonomic functions should be monitored and any specific complications treated as they arise.

Most adults start to recover once the muscle spasms have subsided. In most cases recovery is complete. Some of the long term sequelae of tetanus include limb contractures, bed sores, seizures, myoclonus, and sleep disturbances. The prognosis is generally worse in neonates, especially if prolonged hypoxic periods had occurred during the illness. Overall the prognosis is dependent on disease severity and the medical facilities available, with particular regard to access to ventilation.

BOTULISM

Human botulism occurs in a variety of forms. Food-borne botulism occurs after the ingestion of food contaminated with *C botulinum* containing the pre-formed toxin. This form typically occurs when susceptible foods are exposed to room temperatures for prolonged periods. Wound botulism occurs in wounds contaminated with *C botulinum* spores. Increasing numbers of cases of wound botulism have been reported in drug addicts in the USA following the subcutaneous injection of black tar heroin ("skin popping").⁵ ⁶ Infant/adult intestinal botulism occurs when spores are ingested and then germinate in the intestinal tract. Infants less than 1 year of age (95% of infants being younger than 6 months) and adults with a history of gastrointestinal abnormalities or antibiotic use may contract this form. Infant botulism is the most reported form of botulism. In the USA approximately 110 cases of botulism are reported annually, with infant botulism accounting for 72% of cases compared to 25% caused by food borne botulism. The caecum is thought to be the initial site of activity, and paralysis of the ileocaecal valve may allow the colonising bacteria to extend into the terminal ileum. Soil and the ingestion of honey are the two well recognised sources of spores in infant botulism.⁷ *C botulinum* may be present in 10% of honey supplies in the USA where it has been linked with 20–35% of known cases of infant botulism. As a preventative measure honey should not be fed to infants younger than 12 months of age. The infant's intestinal tract lacks the protective bacterial flora of the adult, allowing colonisation by *C botulinum*. Once colonisation occurs the toxin produced is absorbed through the intestinal tract.

The role of breastfeeding in infant botulism is unclear. Breastfeeding occurs in 70–90% of infants with botulism, and in a prospective case-control study was found to be a risk factor for the development of the disease in infants younger than 2 months of age.⁷ However, breast feeding may delay the disease such that infants reach medical attention in time for supportive care, and may therefore be a protective feature.⁸

Inhalational botulism occurs from the absorption of a man made aerosolised version of the toxin from the lung mucosa, and may occur in the context of a bioterrorist attack.⁹

Inadvertent/iatrogenic botulism occurs in patients being treated with botulinum toxin.

Of the seven distinct serotypes of botulinum toxin (types A–G), most forms of human poisoning are caused by types A, B, E, and F. Food borne botulism results from the absorption of toxins A, B, E, and F, whereas intestinal and wound botulism results from toxin type C originating from dead tissue. Infant botulism is caused by types A and B. Type A toxin is typically found in home preserved and canned foods that have not been heated to the correct temperature. Home preserved vegetables, fish, and meat are recognised sources of infection. Type A toxin is usually associated with more severe disease and a higher mortality rate than type B or E toxin. Type E toxin is often associated with the ingestion of contaminated seafood, but can occur with other foods.

Clinical features

All forms of botulism produce similar effects, and should always be considered in afebrile and alert patients presenting with a descending flaccid paralysis with intact sensation. The incubation period and time to onset of symptoms is determined by the amount of toxin absorbed. In food borne botulism the time to onset of neurological symptoms varies from 2–36 hours, but can be up to eight days.

An initial involvement of cranial nerves (resulting in blurred vision, diplopia, dysarthria, dysphonia, or dysphagia) is usually followed by an acute, symmetric, descending flaccid paralysis that may lead to respiratory failure. Enlarged or sluggishly reactive pupils are common. There are no sensory or autonomic features, and the central nervous system is rarely involved. In food borne botulism gastrointestinal effects (for example, nausea, vomiting, abdominal cramps, diarrhoea, and constipation) may occur in up to half the cases, and may precede the neurological features. Infant botulism typically presents with lethargy, poor feeding, and loss of head control. Constipation is a classical presentation, and may precede weakness by several weeks. Hypotension, neurogenic bladder, and other autonomic features may occur early. Weakness typically starts with cranial nerve involvement and loss of head control. The infant may also have a weak cry, poor sucking ability, impaired gag responses, pooling of secretions, and decreased oral intake.

Diagnosis

The diagnosis is predominantly a clinical one, and is confirmed by testing for the toxin or organism. Toxin isolation from serum occurs in 35% of cases, but this figure drops if the sample is collected more than two days after ingestion. Only 35% of stool cultures are positive after three days.¹⁰ The most reliable test for the *C botulinum* toxin is the mouse inoculation test, whereby mice exposed and non-exposed to type specific botulinum anti-toxin are injected with the patient's serum. A test is deemed positive if the non-exposed mice die within 24–48 hours. Neurophysiological investigations may aid in the diagnosis and show features of a pre-synaptic neuromuscular junction deficit, with reduced amplitude compound muscle action potentials (CMAPs) but with normal motor conduction velocities and completely normal sensory studies. A diagnostic triad of (1) decreased amplitude of CMAPs in at least two muscle groups, (2) tetanic or post-tetanic facilitation (defined by a CMAP amplitude of more than 120% of baseline) after at least

Clostridial neurotoxins: key points

- ▶ Tetanus is easily preventable by active immunisation with the tetanus toxoid
- ▶ The clinical picture in tetanus is dominated by muscle spasms, rigidity, and autonomic disturbances
- ▶ The diagnosis in tetanus is made clinically as there is no specific confirmatory investigation. A positive culture of *Clostridium tetani* from a wound would be supportive evidence for the diagnosis
- ▶ Wounds from which the infection causing tetanus originated should be surgically debrided. Metronidazole is considered to be the antibiotic of choice in the treatment of tetanus, but penicillin is a suitable alternative. Passive vaccination with anti-tetanus immunoglobulin shortens the course and may reduce the severity of the illness. Active immunisation (that is, tetanus toxoid) also needs to be given to add to the short term immunity provided by anti-tetanus immunoglobulin, as well as providing longer term humoral and cellular immunity
- ▶ All forms of botulism produce a flaccid paralysis with intact sensation
- ▶ In food borne and infant botulism gastrointestinal effects often precede the neurological features
- ▶ The diagnosis in botulism is predominantly a clinical one, and is confirmed by testing for the toxin or organism. Neurophysiological investigations may aid in the diagnosis and show features of a pre-synaptic neuromuscular junction defect
- ▶ Specific treatment in botulism historically consists of the early use of a trivalent antitoxin that neutralises toxin serotypes A, B, and E
- ▶ The early use of antibiotics and surgical debridement should be considered in wound botulism

10 seconds of tetanic stimulation at 50 Hz, and (3) prolonged post-tetanic facilitation of more than 120 seconds and absence of post-tetanic exhaustion has been described for diagnosis.¹¹ As studies may be normal in the early stages repeat testing after an interval of 7–10 days may be required.

The differential diagnosis for botulism includes acute inflammatory demyelinating polyneuropathy (that is, Guillain-Barré syndrome), porphyria, poliomyelitis, diphtheria, tick paralysis, myasthenia gravis, and magnesium toxicity. Onset with a bulbar weakness followed by a descending paralysis in a patient with sluggish pupillary reactions and intact sensation is suggestive of botulism. Diagnosis of infant botulism may be delayed because of the relatively non-specific symptoms that may occur in this condition at onset. Tick paralysis tends to occur in older and more mobile children than in botulism.

Management

Treatment is mainly supportive. As respiratory muscles may be involved rapidly, patients suspected of botulism should be initially managed in an intensive therapy unit. In patients at risk of respiratory failure elective intubation and ventilation should be undertaken. Gastric lavage or induced emesis should be considered if contaminated food was ingested recently. The early use of antibiotics and surgical debridement should be considered in wound botulism. Neuromuscular junction blocking agents (for example, aminoglycosides, magnesium containing compounds) should be avoided.

Specific treatment historically consists of the early use of a trivalent antitoxin that neutralises toxin types A, B, and E. Significant side effects to the antitoxin may occur in more than 20% of cases. The equine derived antitoxin has now been superseded by a human derived antitoxin. A repeat dose of antitoxin may be given 2–4 hours later, with doses repeated at 12–24 hour intervals if required. The antitoxin is traditionally thought to be relatively ineffective against infant botulism as the type A toxin implicated in this form of botulism is rarely found in the blood of the infant affected. However, a recent five year study of human derived antitoxin in infant botulism showed a reduction in the time spent in hospital and the need for assisted ventilation and tube feeding.¹² Benefits from anti-toxin are most likely with type E botulism. A heptavalent antitoxin is available to the US Army in the event of a bioterrorist attack.

With improved supportive care the overall mortality rates in botulism have dropped to less than 10%, with rates of less than 2% in infant botulism and higher rates in patients above 60 years of age. Recovery typically takes place over a period of weeks to months, and is dependent on re-innervation following the growth of new motor neuronal sprouts. Respiratory support may therefore be required for months, and weakness and autonomic dysfunction may persist for more than one year.

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