

CLINICAL AND PARACLINICAL ASPECTS IN A CASE OF A BOTULINUS INFECTION

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ABSTRACT

We depict the case of a patient who progressively develops a motor deficit and respiratory failure, which eventually proves to be botulinus intoxication, after a series of puzzling results of various investigations leading to a different diagnosis.

CASE PRESENTATION

The patient, aged 31, with no preceding significantly personal pathology, was admitted in a regional hospital for an onset of „blurred“ vision, followed by diplopia, nausea, vomiting, shivering, fever, approximately 12 hours after having eaten – along with his family - fish, sweets, and no alcoholic drinks, followed by a physical effort.

Subsequently the patient's status worsened by the occurrence of lower limb muscular weakness (the patient could not sit or stand up without help) then respiratory failure, which required orotracheal intubation and temporary assisted mechanical ventilation. A botulinus intoxication was suspected, and he received a dose of antitoxin serum.

During the hospitalisation the patient was given corticosteroids, anticholinesterase drugs, antibiotics (cephalosporin, amino glycosides), with progressive recovery of the respiratory function during a week and a partial recovery of the motor deficit.

Testing for seric botulinus toxin was negative. To mention that the tests were performed only for types A, B and D of botulinus toxin.

At this moment the patient was referred to our hospital with the suspicion of myasthenia gravis.

The physical examination revealed fixed mydriasis, lower limb hypotonia with decreased deep tendon reflexes, low blood pressure, bradycardia, constipation. There were no ocular movement disturbances, no deglutition impairment nor sensory impairment, but the patient complained of increased fatigability even on the smallest physical effort.

Laboratory findings showed a moderate transient hypokaliemia, moderately elevated muscular cytolysis enzymes; on spinal tap: clear liquid, with negative Pandy reaction, 0,66 nuclear elements per mmc, 0,33 red blood cells per mmc.

The next day the patient (being on anticholinesterase medication) was subject to an electrophysiological examination.

The motor conduction study revealed normal distal latencies, globally decreased amplitudes, more in lower limbs, normal conduction velocities, prolonged F waves in lower limbs; sensory conduction study: normal amplitudes and conduction velocities, no significant decrement or increment for low frequency neither for high frequency repetitive nerve stimulation, and the needle detection examination revealed poor activity in lower limbs and no fibrillation potentials (the essential data are shown in table 1 and 2 for the right peroneal nerve, but the results were similar in all limbs).

The clinical and paraclinical data of the moment lead to the problem of differentiation between several entities such as periodic hypokaliemic paralysis, a myasthenic syndrome, an atypical variant of a Guillain-Barré syndrome and a botulinus intoxication.

A form of an acute axonal polyradiculoneuropathy comes into one's mind when having a patient developing a relatively sudden motor deficit with decreased deep tendon reflexes, an ascending evolution and a rapid progression to respiratory failure; the electromyographic features of symmetric axonal diffuse polyradiculoneuropathy (prolonged F waves, decreased sensory and motor conduction velocities, generalised low amplitudes). The normal results of the CSF upon spinal tap, the relatively rapid and spontaneous recovery of the respiratory failure – in about two weeks from the onset on the motor deficit, makes an axonal injury improbable.

The hypothesis of a Miller-Fisher syndrome-sustained by the result of the electrophysiological examination-was ruled out by the occurrence of the autonomic disturbances (mydriatic and non reactive

to light pupils, low blood pressure without tachycardia, constipation) and of the motor deficit accompanied by respiratory failure.

A hypokaliemic periodic paralysis was taken into account by the recent sweets ingestion and the hard physical effort made by the patient prior to the onset of the symptoms, by the hypokaliemia found on laboratory testing, and by the transitory association of the respiratory failure that spontaneously recovered. Nevertheless the patient had no positive family history of hypokaliemic periodic paralysis, he did not consume alcoholic drinks (which is a secondary cause for hypokaliemia); moreover, autonomic disturbances are not typical for hypokaliemic periodic paralysis, nor the ascending motor deficit.

Arguments for a myasthenic syndrome were the occurrence of the symptoms (diplopia, muscular weakness) in relation with muscular activity, worsened apparently by the administration of amino glycosides, the improvement of the symptoms after the patient was given anticholinesterase drugs, and also the finding of moderate muscular cytolysis. On clinical testing of repetitive muscular effort there was no evidence of muscular fatigue, and electrophysiological tests did not show any increment or decrement on repetitive nerve stimulation (figure 1).

The botulinus intoxication features were the onset with autonomic pupillary disturbances (causing the „blurred“ vision) and gastrointestinal symptoms of nausea and vomiting in relation to ingestion of food that could be infested (fish – in this case); decreased CMAP in the affected muscles; the progression of the motor deficit and the respiratory failure that has temporarily required mechanical ventilation, followed by the full recovery of the muscular strength as the patient received a dose of antitoxin serum .

To rule out the periodic hypokaliemic paralysis provocative electrophysiological tests were performed after a week; there were no anomalous findings upon the prolonged effort and cold tests. The persistent hypokaliemia may be explained as being a side effect of the cortisone administration without potassium

supplement prior to the hospitalisation in our department.

The interruption of the anticholinesterase medication did not lead to exacerbation of the motor deficit; on the contrary, it improved progressively to its full recovery. Repetitive nerve stimulation tests performed after a week from the interruption of the anticholinesterase medication did not reveal any decrement or increment.

Motor and sensory nerve conduction studies after a week of hospitalisation showed normal latencies, amplitudes, conduction velocities and F waves, thus eliminating the hypothesis of an axonal injury.

The reason why tests for the seric botulinus toxin were negative is that they were not performed for all types but for the most frequently responsible of botulinus intoxications.

Botulism is a rare and potentially lethal disease, caused by one or several neuroexotoxin proteins produced by the bacteria *Clostridium botulinum*.

There are five clinical forms of botulism: classic botulism- food-borne botulism, infantile botulism, hidden botulism, wound botulism and inadvertent botulism. The term „botulism“ comes from the Latin word for sausage (botulus), as one of the sources of the disease was the sausages containing botulinus toxin.

There are eight immunological different types of the botulinus neurotoxin, named in alphabetically order of their discovery: A, B, C1, C2, D, E , F and G. Types A, B, and E are responsible for the majority of the reported cases of food intoxications, but types D, F and G were responsible of more deaths. Type C affects animals and not humans.

Although the botulinus toxin is a very strong inhibitor of the neuromuscular junction, it also inhibits the release of Acetylcholine from the pre- and post-ganglionic nerve terminals in autonomic nerve system.

A patient is classified as having botulism if the reported disease has neurological manifestations of descending paralysis without impairment of the consciousness, accompanied by one of the following

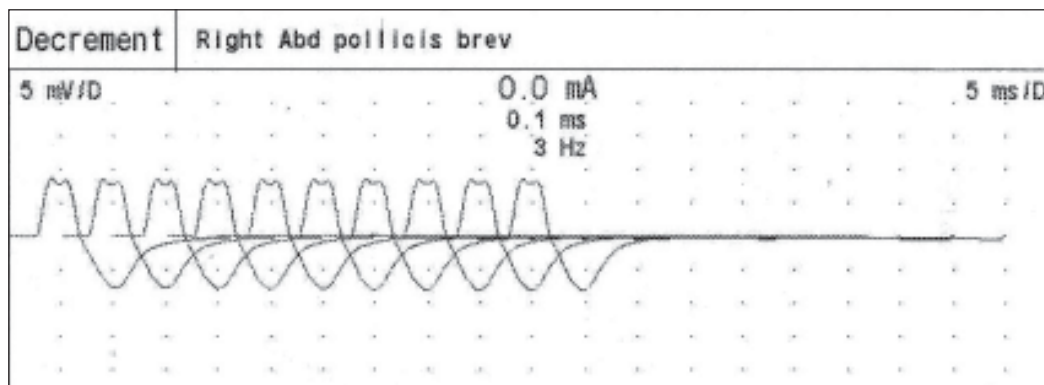


Figure 1

conditions: identifications of the *C. botulinum* in the faeces or in the wound; documentation of the botulinus toxin in the serum, wound, faeces or parts of the food suspected to be infested; or a clinical picture compatible to other cases of food born botulism.

CLASSIC OR FOOD-BORNE BOTULISM

It is important to know that this form of botulism is not an infection but an intoxication from the ingestion of the toxin contained in food. In two thirds of the cases only one person of a group that consumed the infested food develop the symptoms, but the mean number of persons is 2.7. Approximately 54% of the patients are male, the mean age of all the patients being of 44 years. Type A botulinus toxin is frequent in U.S.A (it has been reported in about 60% of cases), type B- more frequent in Europe and in our country, and type E –the less toxic, after fish ingestion; there is a geographic association due to the prevalence in the soil of the specific toxin producing micro organism.

The usual method of the transmission is through poor prepared home vegetable canes; intoxications associated with restaurant food consumption are rare, but having a propensity of affecting more persons.

The number of deaths due to the botulinus intoxication has considerably decreased since the improvement of the emergency care system, the possibility of ventilation support when needed, the possibility of the administration of the antitoxin in the early diagnosis.

Patients over 60 years are prone to more serious complications, less complete recovery and a higher rate of mortality. The first or the only one affected in an intoxication have a death rate of 25% compared to the ones developing the disease symptoms later; this could be related to a shorter incubation rate, the ingestion of a higher amount of the toxin, or a less suspicion of a botulinus intoxication with the first patient (once diagnosed, other patients having similar symptoms are readily diagnosed).

The spores of the bacteria are resistant to heat, and can survive the home cane preparation techniques at temperatures less than 120°C. Acidifying food is recommended in order to ensure a pH < 4.6 which inhibits the growth of *C. botulinum*. Boiling under pressure any home prepared cane before consuming it, inactivates the toxin (which is thermo labile) and disfavours the growth of the bacteria in a lacking oxygen medium.

INFANTILE BOTULISM

Is the most frequent form of botulism in United States having an incidence of 1/100,000 of alive

newborns. The base mechanism is different of that in classical botulism, implying a bacterial enteric infection with an in vivo production of the botulinus toxin, and not the ingestion of a preexisting toxin.

Natural alimentation favors the colonisation of the intestinal tract with a flora relatively inhospitable to the growth of the botulinus spores, comparing to the artificially feed newborns.

HIDDEN BOTULISM

Is a form of infantile botulism in persons aged over one year. The patients have a clinical tableau compatible to botulinus intoxication with positive laboratory tests, but without having an evident food or wound source.

WOUND BOTULISM

The term is used when infecting a wound with *C.botulinum* with in vivo produced botulinus toxin at the wound site. It is the rarest of all the forms of botulism, yet reported with drug consumers. Of great interest is the association between the illicit drug consumers and the site of the injection of the drug and maxillary sinusitis associated with of cocaine snuffing.

INADVERTENT BOTULISM

Is the most recently described form of botulism; connected with iatrogenic causes. Botulinus toxin is used nowadays in the treatment of focal dystonias and of other movement diseases. Rarely, after the local injection of the botulinus toxin, patients can develop muscular weakness at distance or generalised; after local injection of botulinus toxin it may also appear autonomic disturbances.

Clinical manifestations

Adult form. Clinical presentation of the botulinus intoxication in adults is similar whether the disease is acquired through food, wounds, or the hidden form. Despite minimal statistic differences between the symptoms specific to type A or B of the botulinus toxin, in medical practice these differences are inconsistently encountered. Symptoms can be grouped in three categories: neurological, gastrointestinal and other.

The following elements evocate botulism:

- association of symptoms of junction neuromuscular disorders (pupilar constriction, ocular and bulbar weakness than for all the body) and of parasympathic nervous system disorders (dried mouth, abdominal cramps, transit disorders)

- and ingestion of toxin contaminated food in 16 to 60 hours before the clinical signs.

There are some neurological features relatively characteristic to the botulinus intoxication. Patients develop relatively sudden dysphagia, dry mouth sensation, diplopia and dysarthria with progression over 12-36 hours, the period depending of the amount of the toxin ingested. A carefully given anamnesis can show early involvement of superior cranial nerves compared to lower cranial nerves. Gastrointestinal symptoms of nausea, occasionally vomiting and initially diarrhoea followed by constipation appear at the same time or immediately after the signs of cranial nerve disturbance. During the disease evolution the patient can have other symptoms such as: abdominal cramp-like pain, fatigability, dizziness. After these symptoms, the patient progressively develops muscular weakness of the upper limbs initially, than lower limbs also become involved; the patient can also perceive shortened respiration before limb weakness. It is presumed that the initial cranial nerve disturbance anterior to the limb musculature disturbance is due to the higher face temperature.

Clinical examination reveals ptosis, diminished gag reflexes, dysphagia, dysarthria, facial paresis, difficult tongue protrusion and on pressing the tongue against a resistance on the cheek, and occasionally nystagmus. Related to the time by the moment of examination, the patient can also have limb weakness, predominantly upper limb musculature, sometimes slightly asymmetric; deep tendon reflexes are initially normal or decreased, in progression to their abolition in case of severely affected patients. The measurement of the respiratory function is also important. The forced vital capacity is decreased in most cases and mechanical ventilation is needed in 32-81% of cases.

The duration of the assisted mechanical ventilation depends on the severity of the disease and of the serotype of the infecting micro organism, with a mean of 58 days for A serotype and 26 days for B serotype.

Careful examination can reveal autonomic disturbances of both the sympathetic and parasympathetic system; typically there is a loss of the vagal cardiac control, ileus, hypothermia, urinary retention that may require catheterisation; it may also be found a low blood pressure without tachycardia, a lack of motor response to postural changes; pupils may have a temporary decreased response to light. In the possibility of a wound botulism the patient must be carefully examined not only for major wounds, but also for a minor wound, even a scratch with or without local signs of an infection; it must be seed on anaerobic growth mediums; the nasal mucosa must also be examined carefully.

Histopathology

The botulinus toxin produces changes similar to nerve section: loss of the terminal endings; retraction and decrease of the dendritic terminations, the increase of the number of astrocytes, sprouting of motor nerve terminations from the initial arborisation terminals; the denervated muscle can produce a motor nerve growth factor which induces this sprouting. Thus new neuromuscular junctions are formed near the initial neuromuscular junction or at distance from it. The reversibility of the paralysis comes from the producing of new neuromuscular junction, and not from the detachment or degradation of the toxin.

Pathogenesis and pathophysiology

The micro organism responsible for botulinus intoxication is a telluric anaerobic sporulated Gram positive bacillus; it is one of the over 100 anaerobic species of the Clostridium genus. *C. botulinum* is divided in four groups by their capacity of digesting proteins (proteolytic) or sugars (sacharolytic).

The toxin produces by the micro organism is released only after the lysis of the bacteria. After the autolysis of the bacteria, the toxin has a medium toxicity; a proteolytic enzyme either from the bacteria or from human organism, like tripsin, is required to cleave the link between the endings of the „U“-shaped toxin molecule; thus the chains remain connected through a disulphidic bridge. This represents the high toxicity form, the minimal lethal dose in humans being estimated at approximately 0,014 µg/kgb.

The intoxication of the cell by the botulinus toxin involves four stages: cell binding, internalisation, membrane translocation and modifying the cytosol target. The receptors for botulinus toxin are situated on the motor neuron plasmalema at the neuromuscular junction; different types of sialoglycoproteins are specific receptors for each type of botulinus toxin.

Botulinic toxin puts axonal termination unable to liberate acetylcholine with an action in 3 steps: toxin binding to the axonal termination, internalisation (calcium dependant transport of the toxin through the terminal membrane) and finally membrane destruction (Rivner M.H, 1993). Because of calcium effects blockage on the acetylcholine liberation, through the synaptotagmine, the neuromuscular transmission is defected. A similar mechanism is produced by tetanic toxin (Littleton J.T et al., 1995)

Electrophysiologic changes

These are prejunctional disorders of neuromuscular transmission. That means an association of

- Non specific signs: decrement with low frequency, jitter increase in single-fiber EMG

- studies, MUAPs are short or with indentation
- Signs which are linked with the acetylcholine presynaptic liberation defect:
 - CMAP amplitude decrease to a single stimulation (often inferior to 1 to 2 mV) and sometimes for the MUAPs
 - Relevant increase of CMAP amplitudes and surface after the effort (facilitation post exercise superior to 50%)
 - Increments of CMAPs amplitudes and surface to the high frequency repetitive stimulation (superior at 50 to 200 or 400%) (Rivner M.H, 1993; Keesey JC, 1999, Fournier E, 1998)

The major feature of the nerve conduction study is the decrease of the CMAP amplitude; some patients may have normal CMAP amplitude early after symptom onset, which in the next days or weeks gradually decrease with the neuromuscular junction blockade progression. There are normal sensory and motor conduction velocities. At low rates of repetitive stimulation (2-3 Hz) over 50% demonstrate a decremental response; other may not reveal any decrement or may even have an increment.

The needle EMG examination in early stages of the botulinus intoxication may be normal, yet in severely affected muscles fibrillation potentials and positive sharp waves may be found; the motor unit action potentials (MUAPs) have a myopathic appearance.

Abnormal increase in jitter can be observed very early in the disease in 40% to 50% of single-fiber EMG studies.

Treatment

The great challenge in treating botulinus intoxication is prompt recognition of the disease, because of its rarity. The combination of rapidly progression of descending paralysis, starting with cranial nerves, preserved consciousness, absence or minimal sensory complaints, autonomic disturbances are suggestive for acute botulinus intoxication. Antibiotics which can alter the neuromuscular transmission – such as amino-

glycosides – are to be avoided, as they may worsen the clinical manifestations.

The administration within 24 hours from the onset of the symptoms of the polyvalent antitoxin serum (which contains actually A and B antitoxins in various amounts according to the product) is beneficial. The mechanism of the antitoxin is neutralising the toxin before all the amount of the toxin binds and enters the nerve terminals. There is a potential for side effects to the administering of the antitoxin, taking the form of an allergic reaction or a serum sickness type response (the antitoxin is prepared from heterologous serum - immunized horses), as a long time sensitisation to the antitoxin in the case of another administration needed (having the disease once does not leave immunity to it). The mainstay of care is supportive from the perspective of maintaining adequate ventilation and being prepared for prompt mechanical ventilation intervention; the control of the secretions and of the nutrition, monitoring intestinal transit to avoid constipation, psychological support and concise explanations regarding the patient's disease evolution, especially if the patient is a child.

Recovery is usually satisfying for all the patients, on condition they are hospitalised since the first symptoms of the disease. In elderly patients, the associated complications may lead to death. Long term sequelae are fatigability and moderate decrease of the respiratory capacity in some patients. Guanidine and some aminopiridine derivates (4-aminopiridine and 3,4 diaminopiridine-3,4 DAP) may lead to an increase of the amount of Acetylcholine released from the nerve terminals affected by botulinus toxin in vitro, but studies in humans gave controversial results, and many side effects. Future potential treatment is the administration of a drug to antagonise the effects of the botulinus toxin.

We considered this case presentation useful because it's driving our attention towards a less frequent situation with some atypical aspects. Early diagnosis in this case is of vital importance for these patients. So it is important to have in mind botulinus intoxication as a differential diagnosis.

MOTOR CONDUCTION VELOCITIES

Table I

Date	Right Peroneus	Lat (ms)	AMP (mv)	CV (m/s)
25.10.2006	ANKLE - REC POS	4,3	0,5	
	COL PER - ANKLE	11,6	0,6	50,7
	KNEE - COL PER	14,8	0,5	40,6
02.11.2006	ANKLEB - REC POS	4,0	4,2	
	COL PER - ANKLE	13,2	3,7	40,2
	KNEE - COL PER	14,7	3,2	80,0

Table 2

Date	Right Peroneus	Lat (ms)	AMP (mv)	CV (m/s)
25.10.2006	FORELEG - ANKLE	2,8	20	70
	STIM 2- REC 2	2,8	1,7	
02.11.2006	STIM 1- REC 1	3,1	5,9	52,1
	STIM 2- REC 2	3,1	11	

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