Botulism was originally called “sausage poisoning” because it occurred following the ingestion of poorly prepared blood sausages in Stuttgart. Justinus Kern first described the clinical features of botulism in 1822. Van Ermengem isolated the bacillus from the remains of a meal following which members of a musical party developed symptoms and 3 of them died. This occurred in Ellezelle, Belgium (Sakaguchi 1983). The clinical manifestations include gastrointestinal distress, blurred vision, mydriasis, diplopia, ptosis, dysphagia, loss of sweating and progressive flaccid muscle paralysis leading to respiratory failure and death. There are 5 types of botulism: food borne, wound, infant, hidden and inadvertent botulism (Cherrington). Inadvertent botulism follows therapeutic injections of doses of botulinum toxin causing systemic effects. There are rare reports of generalized weakness following injection with 250 to 900 units of Dysport® with electromyographic evidence of systemic botulism (Bhatia 1999, Bakheit 1997). All patients recovered completely.

Outbreaks of botulism were fairly common in the United States in early twentieth century, attributable to the consumption of canned or preserved foods, or seafood (Meyer 1956). Prior to the development of life support technology, many who contracted the disease died. Although outbreaks continue to be reported (Wainwright), with 724 cases of food borne botulism and 1444 cases of infant botulism in the United States from 1973 though 1996, (Shapiro 1998) survival has improved as a result of the development of antitoxins and advancing medical technology allowing for life support.

**Botulinum Toxins Pharmacology**

Botulinum toxin (BTX) is a large complex protein. An anaerobic, spore-forming bacillus, Clostridium botulinum, produces the toxin. *C. botulinum* is a common bacterium that is widely dispersed in the environment. The spores are heat resistant. The spores germinate, reproduce and produce toxin in the appropriate environment of anaerobic conditions, low acidity and water. If not heated appropriately, the toxin is ingested and absorbed through the gastrointestinal tract. With systemic spread, the toxin exerts its effect at cholinergic synapses. The toxin itself is heat labile and is destroyed by heating at 80°C for 1 minute (Cherington 1998). There are seven antigenic distinct serotypes of botulinum toxin, A-G. Beginning in 1946, the serotypes have been purified. The serotypes most associated with human disease are BTX types A, B, and E (Shapiro 1998). BTX-B may have more autonomic effects than other serotypes. This was first appreciated in an observational report of 9 patients with food-borne botulism type B poisoning. In these cases, there was minimal muscular weakness, but marked reduction in salivary secretion, causing intense dry mouth, and loss of accommodation causing blurred vision as well as pupillary abnormalities. These symptoms lasted as long as 80 days (Jenzer).

Botulinum toxins are produced as single chain polypeptides. The molecular weight of purified BTX is approximately 150 kD. Enzymatic nicking of the polypeptide by bacterial proteases results in the activated di-chain molecule, consisting of a heavy chain and a light chain linked by a disulfide bond. The heavy chain mediates binding to presynaptic cholinergic nerve terminals and internalization of the toxin into the cell. The light chain is responsible for the toxic effects, acting as a zinc-endopeptidase, cleaving specific proteins responsible for membrane fusion (SNARE complex). Each serotype of botulinum toxin binds to a serotype-specific acceptor site on the presynaptic nerve terminal (Black and Dolly 1986). Internalization involves receptor-mediated endocytosis, and translocation of the light chain into the cytosol (Coffield). The light chain then cleaves specific proteins (Table 1). BTX A, C and E cleave SNAP-25 (synaptosomal-associated protein 25), a membrane-associated protein. Serotypes B, D, F and G cleave Vesicular Associated Membrane Protein (VAMP) also called synaptobrevin. Type C additionally cleaves Syntaxin, a membrane protein (Schiavo 1993, Blasi 1993). Each BTX serotype cleaves at a unique amino acid sequence even when acting on the same protein.

The proteins, VAMP, SNAP-25 and Syntaxin, are part of the SNARE complex proteins that are involved in vesicle fusion (Catsicas 1994) (Figure 1). By cleaving these proteins, botulinum toxins prevent the fusion of the vesicle with the presynaptic membrane, thus preventing the release of acetylcholine into the synaptic cleft.
The specificity of botulinum toxin for cholinergic synapses resides in the location of high affinity acceptor sites on these nerve terminals. The acceptor sites have not yet been identified but serve as the location for binding of the heavy chain of BTX.

The action of botulinum toxin at the neuromuscular junction is to interrupt transmission and, in effect, denervate muscle (Montecucco 1996). Currently, there is no known way to reverse the paralytic effects of botulinum toxin. Although antitoxin may neutralize unbound toxin, once the toxin has been bound irreversibly to the neuronal membrane and muscle paralysis occurs, recovery only occurs spontaneously and may take months (Hambleton 1992).

The action of BoNT at the neuromuscular junction is to interrupt transmission and in effect to denervate muscle (Montecucco et al., 1996). This chemodenervation effect persists for weeks to months. The duration of effect may be dependent on serotype (Sloop et al., 1997, Dolly et al., 2002; Dolly, 2003). The mechanism for this extended duration has been hypothesized to arise from either continued protease activity within the cell or from persistent interference by cleaved substrate with normal membrane fusion (Dolly et al., 2002). Currently, there is no known way to reverse the paralytic effects of BoNT after it has been internalized. Both active and passive immunization can inactivate toxin in the circulation, but antibody cannot enter nerves to neutralize internalized toxin.

Recovery from botulinum toxin injection is a process only recently elucidated following use of serotype A. A single injection of botulinum toxin serotype A in mice interrupts neuromuscular transmission. At approximately 3-4 weeks post-injection, there is sprouting of new processes along the nerve axon with formation of temporary synapses and upregulation of the muscle nicotinic receptors (Lee 1999). Subsequently, the neuronal sprouts undergo regression and the original synaptic connection is restored, demonstrating that in the absence of additional injection, the original neuromuscular junction is returned to its original, pre-injection state (Aoki 2001, dePaiva 1999).

Although the effects of botulinum toxins are predominantly at cholinergic synapses outside of the central nervous system, a central effect cannot be excluded. Labeled BTX-A neurotoxin injected into muscle has been shown to penetrate into the spinal cord via retrograde neuronal axonal transport and the ventral roots. This is similar to the pathway of tetanus toxin, but the effect of BTX on the spinal neurons is not marked and does not reproduce the effects of tetanus toxin (Wiegand).

Edward Schantz and Alan B. Scott pioneered the use of botulinum toxin as a therapeutic agent (Schantz 1997) in the 1970’s. In 1979, Shantz and colleagues at the University of Wisconsin prepared 200 mg of twice crystallized botulinum toxin serotype A, which was approved for human use in the United States in 1989 for the indications of strabismus, blepharospasm, and other disorders of the seventh cranial nerve.

The original batch of Botulinum toxin serotype A (Botox® lot 79-11) was the only commercially available botulinum toxin for human use until the year 2000. Botulinum toxin is now available in 2 serotypes. Botulinum toxin type A is distributed by two pharmaceutical companies: Allergan (Botox®) and Ipsen (Dysport®). Botulinum toxin serotype B is distributed by Elan (Neurobloc™, Myobloc™). Since its introduction as a therapeutic modality, the number of disorders treated with botulinum toxin has expanded, now numbering over 50 (Jost, 2001).

The dosage of botulinum toxin varies by preparation and serotype. For the two brands of serotype A commercially available, Botox® dosing for CD ranges from 100 to 300 Units per injection series, and Dysport dosing ranges from 800-1200 (First 1994). A controlled, dose response in cervical dystonia showed that effective dosing for Dysport was between 250 and 1000 Units. Duration of the effect was prolonged with the higher doses, but adverse effects were also more frequent (Poewe 1997). A comparison of outcome in rotational CD patients dosed with a ratio of 3 units of Dysport to 1 unit of Botox® showed no difference in benefit or side effects (Odergren 1998). The reason for the dose difference between these brands of BTX serotype A is attributed to differences in the mouse bioassay used and variability among the different batches of the same toxin (Pickett 1994). Botulinum toxin serotype B as Myoboc™ or Neurobloc™ has a dose range for CD from 2,500 to 15,000 Units, with some extending the maximum dose as high as 25,000 Units.

There have been no direct comparisons of Botox® and Myobloc™ in dystonia patients. In normal extensor digitorum brevis muscle, M wave amplitudes were compared following injections of serotype A (Botox®,
supplied by Allergan) and serotype B (Myobloc or Neurobloc supplied by Elan). In this study, the maximal reduction of M wave amplitude occurred at 2 weeks following a dose of 320 to 480 Units of serotype B and 7.5 to 10 Units of serotype A. The M wave amplitude reduction returned to normal 11 weeks following serotype B, but the effect of serotype A was still present after 57 weeks (Sloop 1997). Whether increased dosing of BTX B would prolong the duration of this effect is not known and cannot be accurately extrapolated from current information.

The FDA approved both Botox® and Myobloc™ for treatment of cervical dystonia in December of 2000. Both have been shown to be safe and effective in alleviating the abnormal movement and pain associated with cervical dystonia. A comparison of the two preparations is shown in Table 2. At this time, there is no definitive information to guide the clinician in choosing one toxin over the other. The labeling for each drug suggests that these two serotypes may have similar effects (Table 3). A large, multicenter study is currently in progress that directly compares the Botox® and Myobloc™ in cervical dystonia patients in a controlled, blinded study to assess maximal effect, adverse effects and duration of clinical benefit.

**Electrophysiological effects of Botulinum toxin**

The electrophysiological findings in systemic human botulism include a reduction in the amplitude of compound muscle action potentials (CMAP) following both single and repetitive supramaximal nerve stimulation. There is minimal reduction in CMAP amplitude following repetitive stimulation at 2 Hz and variable facilitation following brief maximal exercise. These changes persist but steadily improve for at least the 100 day study length (Gutmann 1976, Hamjian 1994). The magnitude of BTX effect on the muscle may be increased by muscle activation either with electrical stimulation or exercise (Eleopra 1997) immediately following injection. Maximal paralysis from a given dose of botulinum toxin depends primarily on the proximity of the injection to the motor end plate, with concentration of BTX and volume of injection being much less important (Shaari 1993). BTX diffuses through muscle, with higher doses increasing diffusion (Borodic 1994). BTX does diffuse through muscle fascia, but spread of the toxin is reduced by approximately 20-25% (Shaari 1991). Taken together, the electrophysiologic data suggest that maximal effect of a given injection is greatest when administered near the motor end plate, and that diffusion can be limited by injecting directly into the belly of the desired muscle, using higher concentrations of toxin in a smaller injected volume. Muscle atrophy, weakness and clinical benefit parallel these electrophysiological changes, although clinical benefit tends to wane prior to restoration of normal electrophysiological muscle activity (Odergren). Chronic reinjection of a muscle does not appear to alter the muscle nor reduce the effect of subsequent injections (Sloop 2001).

Evidence of systemic effects of botulinum toxin following regional injection into facial or cervical muscles has been demonstrated. Single fiber EMG studies show increased jitter in muscles in muscles distal to the injection site (Sanders 1986, Lange 1988, Lange 1991, Olney 1988, Girlanda 1992). For example, Olney showed that CD patients injected into neck muscles for cervical dystonia had increased jitter and fiber density in the biceps brachii muscle. These abnormalities return to normal after approximately 3 to 6 months (Olney 1988). There have also been mild distal effects of injections on autonomic functions, including cardiovascular reflexes and blood pressure (Girlanda 1992). There is one case report of distal muscle atrophy by muscle biopsy (Ansved 1997), but overall, the distant electrophysiologic effects on muscle are not associated with evidence of clinical weakness.

**Immunogenicity of botulinum toxins**

Botulinum toxin is a protein that serves as an antigen when injected into humans. The development of an antibody response to an antigen is dependent on several factors. These include the presence of an adjuvant (a substance that increases the immune response), the persistence of the antigen in the tissues, and the frequency of exposure to and quantity of the antigen (Critchfield 2000). In addition, other factors, such as the genetic make-up of an individual may increase the susceptibility for that individual to develop antibodies to a particular antigen. Antibody formation in response to botulinum toxin is a desirable event in people with frequent exposure to the toxin and risk of contracting botulism. Hence, in endemic areas and in laboratory personnel, vaccinations using toxoid are administered. However, in patients whose clinical condition responds to botulinum toxin, the formation of neutralizing antibodies results in a clinical resistance to the beneficial effects. The factors that predispose to development of antibodies have not been identified. Large doses of botulinum toxin (≥ 250 Units botulinum toxin A), larger cumulative doses, and injections administered at less than 3 month intervals (“booster” injections) have been identified as possible risk factors for the development of resistance (Jankovic 1995, Zuber 1993, Greene 1994). Botulinum toxin resistance primarily affected cervical dystonia patients probably as a result of the larger doses used to treat this disorder.
Initially, BTX resistance was not considered to be common. Early reports suggested a frequency of approximately 5% resistance cervical dystonia patients receiving repeated injections (Kessler 1999). This observation was based on retrospective assessments of patients in a single location. However, the clinical trials of botulinum toxin serotypes A and B published as the package inserts for the drugs when FDA approval was obtained revealed antibody formation to be much more frequent than previously thought (Table 4). Prospective studies of both Botox® and Myobloc™ are underway to assess the frequency and factors associated with resistance.

Antibodies to botulinum toxin may be detected using various methodologies. The Mouse Protection Assay (MPA) is the “gold standard” method. This assay evaluates the ability of increasing dilutions of a patient’s serum to protect experimental mice from lethal test doses of botulinum toxin. Recently, the mouse hemidiaphragm model with phrenic nerve intact has also been described. The Immunoprecipitation Assay (IPA) is a simple, rapid technique for detecting antibodies against botulinum toxin (Rush 1983). IPA has been found to be both sensitive and specific (Palace 1998, Dressler 2001). Compared to the MPA, IPA was shown to be more sensitive to the MPA in detecting botulinum toxin antibodies and shows a positive result earlier than the MPA, suggesting it may predict future unresponsiveness.(Hanna 1999). The in-vitro assays listed above are expensive and have not yet been shown to be useful in an office setting. Until these tests are demonstrated to be sensitive and specific for detecting neutralizing antibodies in a patient population or predictive of antibody formation, they add little to the clinical assessment of a patient who reports a loss of benefit following BTX.

Clinicians often use the more relevant in vivo tests of resistance. The FTA (Frontalis Type A test), the SCM (Sternocleidomastoid) test, and the EDB (Extensor digitorum brevis) test have all been explored as sensitive methods to detect clinical resistance (but not antibody titers) in patients reporting the secondary failure of BTX to improve symptoms. The FTA was proposed by Borodic and involves injections of a small amount of botulinum serotype A (20 Units) into the frontalis muscle. Two weeks following injection, a determination of paralysis of the frontalis muscle in the area of injection is made. If the forehead wrinkles remain symmetric, the patient is thought to be resistant to the toxin, if there is effacement of wrinkling, the patient is thought to be clinically sensitive to the effects of the toxin (Borodic 1995). Similar test injections into the sternocleidomastoid and extensor digitorum brevis denervation using appropriate doses are additional methods of ascertaining resistance to the effects of botulinum toxin in patients (Dressler 2000, Kessler 1997, Birklein 2000, Sloop2001).

Treatment of botulinum resistance is problematic. Depletion of the neutralizing antibodies through plasma exchange or immunosuppression using drugs such as mycophenolate has been suggested (Duane 2000, Naumann 1998). Because each serotype of botulinum toxin is immunologically distinct, replacing one serotype with another may be effective (Brin, 1999) although the long term sensitivity of the patient to an alternate serotype of BTX has not been prospectively evaluated (Sankhla 1998, Brin 1999, Houser 1998, Chen 1998, Truong 1997, Sheean 1995, Greene).

Until more information is available regarding the predictive factors for the development of BTX resistance to any serotype, the generally accepted guidelines for patient treatment include:

1) Careful preparation of botulinum toxin to reduce or prevent degradation of BTX. Denatured or inactivated BTX, if injected, would provide no clinical benefit and serves only to stimulate an immunological response.
   a) Gentle mixing and careful handling of BTX
   b) Injection of BTX within 4 hours of removal from freezer or refrigerator
   c) Avoidance of heating or shaking of the BTX vials or syringes

2) Spacing the BTX injections at the longest interval possible to avoid frequent injections:
   a) Currently, it is recommended that BTX injections occur at least three months apart to avoid frequent, smaller treatments that may serve to immunize a patient.
   b) Avoid “booster” injections. Prior to the recognition that clinical resistance to BTX was not a rare occurrence, patients who had a suboptimal outcome would receive a supplemental injection from 2 to 6 weeks following treatment. This practice has been largely abandoned.
3) Use the minimal dose of BTX for clinical improvement
   a) In some patients, this may not provide the full clinical benefit following the first or second injection sessions (spaced at least 3 months apart). However, because BTX injections are viewed as a long-term treatment for chronic neurological conditions, educating the patient as to the need to limit treatment dose and frequency as much as possible to provide prolonged benefit over the course of repeated injections is necessary.

References


Erbguth FJ, Naumann M. Historical aspects of botulinum toxin: Justinus Kerner (1786-1862) and the "sausage poison". Neurology 1999;53:1850-3.


Table 1: Botulinum toxin serotypes and substrates (Montecucco 1996)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Protein target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotype A</td>
<td>SNAP 25</td>
</tr>
<tr>
<td>Serotype B</td>
<td>VAMP/ Synaptobrevin</td>
</tr>
<tr>
<td>Serotype C</td>
<td>Syntaxin/SNAP 25</td>
</tr>
<tr>
<td>Serotype D</td>
<td>VAMP /Synaptobrevin</td>
</tr>
<tr>
<td>Serotype E</td>
<td>SNAP 25</td>
</tr>
<tr>
<td>Serotype F</td>
<td>VAMP /Synaptobrevin</td>
</tr>
<tr>
<td>Serotype G</td>
<td>VAMP /Synaptobrevin</td>
</tr>
</tbody>
</table>

Table 2: Serotype A (Botox®) and serotype B (Myobloc™) Comparison of drugs

<table>
<thead>
<tr>
<th>Description</th>
<th>Botulinum toxin serotype A Botox®</th>
<th>Botulinum toxin serotype B Myobloc™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage</td>
<td>Freezer (&gt; 5°C)</td>
<td>Refrigerate (2-8°C)</td>
</tr>
<tr>
<td>Vial size</td>
<td>100U</td>
<td>2,500; 5,000; 10,000U vials (all 5,000U per ml)</td>
</tr>
<tr>
<td>Preparation</td>
<td>Reconstitute with unpreserved normal saline to desired concentration</td>
<td>None or May dilute with unpreserved normal saline</td>
</tr>
<tr>
<td>pH</td>
<td>Physiologic</td>
<td>Acidic (pH 5.6)</td>
</tr>
</tbody>
</table>
Table 3: Labeling information
Serotype A (Botox®) and Serotype B (Myobloc™)

<table>
<thead>
<tr>
<th></th>
<th>Botulinum serotype A (Botox®)</th>
<th>Botulinum serotype B (Myobloc™)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing for CD</td>
<td>Not specific. Range in the studies from 200-300U</td>
<td>Initial dose 2,500-5,000 Lower doses in de novo patients</td>
</tr>
<tr>
<td>Duration of effect</td>
<td>Up to 3 months (estimated)</td>
<td>12-16 weeks (estimated)</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>17% of self-reported responders had MNA positivity upon entry into pivotal trial</td>
<td>Estimated 18% of patients after 18 months</td>
</tr>
<tr>
<td>Side Effects</td>
<td>Dysphagia (19%) Upper respiratory infection Neck pain Headache</td>
<td>Dry mouth (up to 34%) Dysphagia (up to 25%) Dyspepsia Injection site pain</td>
</tr>
</tbody>
</table>

Proteins involved with vesicle fusion and release of acetylcholine at cholinergic synapses (Sollner 1994)
<table>
<thead>
<tr>
<th>Table 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors which may increase the risk of BTX type A resistance</strong></td>
</tr>
<tr>
<td>Frequent Injections (Intervals less than 3 months)</td>
</tr>
<tr>
<td>Booster injections</td>
</tr>
<tr>
<td>High doses of botulinum toxin (greater than 300 Units of Botox®)</td>
</tr>
</tbody>
</table>
BOTULINUM TOXIN INJECTIONS

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CPT Codes for Botulinum Toxin Injections

64612      Chemodenervation of muscle(s); muscle(s) innervated by facial nerve (e.g., for blepharospasm, hemifacial spasm)
64613      cervical spinal muscle(s) (e.g., for spasmodic torticollis)
64614      extremity(s) and/or trunk muscle(s) (e.g., for dystonia, cerebral palsy, multiple sclerosis)
67345      Chemodenervation of extraocular muscle
J0585      Botulinum Toxin Type A (Botox) Per Unit
J0587      Botulinum Toxin Type B (Myobloc) Per 100 units

Codes 64612 and 64613 describe chemodenervation (to include the use of botulinum toxin) of muscles of the face and neck. CPT code 64614 describes injections of botulinum toxin into the muscles of the limbs and trunk to treat dystonia, spasticity, and muscle spasms, etc. These codes do not include needle EMG guidance (see below) or the drug itself.

If multiple muscles are injected, is that separately reportable?
Codes 64612-64614 are to be reported once, even though multiple injections are performed in sites along a particular muscle and several muscles are typically injected.

If multiple limbs are injected, is that separately reportable?
No, 64614 describes any and all injections into limbs and/or trunk muscles – this code should be reported one time per patient per day, regardless of the number of limbs injected. While some payors allow billing in multiple units or with a modifier (to indicate bilateral limb injections, for example), this is technically incorrect coding, and could expose physicians to allegations of fraud and abuse.

Are there any diagnostic limitations for use of these codes?
CPT codes 64612-64614 are not be reported for botulinum toxin injections to minimize facial wrinkles or to treat hyperhidrosis (excessive sweating). Carrier policies regarding other diagnoses vary, and it is best to check with a given carrier to find out what diagnoses they accept with these procedure codes.

What codes should be used to report EMG-guided botulinum toxin injections?
Changes are pending regarding the use of EMG-guided botulinum toxin injections, check current CCI edits prior to billing.

Further questions about coding and reimbursement for botulinum toxin injections and other procedures may be directed to the AAN’s Center for Health Policy, Medical Economics Administrator Gina Gjorvad, at (651) 695-1940, or ggjorvad@aan.com.