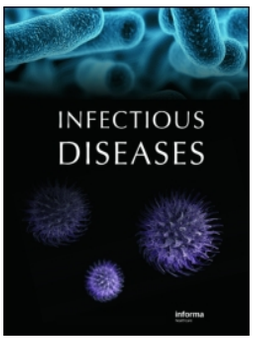

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REVIEW ARTICLE

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Infant botulism: an underestimated threat

Luca Antonucci[a], Cristian Locci[b], Livia Schettini[a], Maria Grazia Clemente[b] and Roberto Antonucci[b]

[a]Academic Department of Pediatrics, Children's Hospital Bambino Gesù, University of Rome 'Tor Vergata', Rome, Italy; [b]Pediatric Clinic, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

ABSTRACT

Infant botulism (IB) is defined as a potentially life-threatening neuroparalytic disorder affecting children younger than 12 months. It is caused by ingestion of food or dust contaminated by *Clostridium botulinum* spores, which germinate in the infant's large bowel and produce botulinum neurotoxin. Although the real impact of IB is likely underestimated worldwide, the USA has the highest number of cases. The limited reporting of IB in many countries is probably due to diagnostic difficulties and nonspecific presentation. The onset is usually heralded by constipation, followed by bulbar palsy, and then by a descending bilateral symmetric paralysis; ultimately, palsy can involve respiratory and diaphragmatic muscles, leading to respiratory failure. The treatment is based on supportive care and specific therapy with Human Botulism Immune Globulin Intravenous (BIG-IV), and should be started as early as possible. The search for new human-like antibody preparations that are both highly effective and well tolerated has led to the creation of a mixture of oligoclonal antibodies that are highly protective and can be produced in large quantities without the use of animals. Ongoing research for future treatment of IB involves the search for new molecular targets to produce a new generation of laboratory-produced antitoxins, and the development of new vaccines with safety and efficacy profiles that can be scaled up for clinical use. This narrative literature review aims to provide a readable synthesis of the best current literature on microbiological, epidemiological and clinical features of IB, and a practical guide for its treatment.

KEYWORDS
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Botulism Immune Globulin
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CONTACT
Roberto Antonucci
rantonucci@uniss.it,
cristianlocci.413@gmail.com
Pediatric Clinic, Department of Medical,
Surgical and Experimental Sciences,
University of Sassari, Sassari, Italy

Introduction

Infant botulism (IB) is defined as a potentially life-threatening neuroparalytic disorder affecting children younger than 12 months that is caused by ingestion and germination of *Clostridium botulinum* spores, resulting in the production of botulinum neurotoxin (BoNT) in the colon [1]. The first diagnosis of IB was made in 1976, but the first known case of this disease is believed to have been in 1931 in the USA [2].

The route of exposure to botulism toxin has led to the following classification system [3]: (1) foodborne botulism, caused by the ingestion of foods containing the preformed toxin; (2) IB that occurs when the infant gut is colonised by *C. botulinum*; (3) wound botulism, a consequence of wound contamination by *C. botulinum* spores; (4) iatrogenic botulism, which results from injection of excess therapeutic BoNT; (5) inhalational botulism, which can result from inhaling accidentally aerosolized botulinum toxin (potentially due to a bioterrorist attack); (6) 'adult' intestinal colonisation and toxaemia, observed in children aged >1 year and adults susceptible to the infant-type botulism, harbouring *C. botulinum* within the gastrointestinal tract. 'Adult' intestinal botulism usually occurs in the presence of inflammatory bowel disease and broad-spectrum antibiotic treatment [4,5], Meckel diverticulum [6], or after intestinal surgery or bone marrow transplantation [7].

This narrative literature review aims to provide a readable synthesis of the best current literature on microbiological, epidemiological and clinical features of IB, and a practical guide for its treatment.

Search strategy and selection criteria

References for this review were identified through searches of MEDLINE/PubMed, EMBASE and Google Scholar for articles in English published between January 1976 and December 2020, using the key words 'infant botulism', '*Clostridium botulinum*', 'botulinum neurotoxin', 'treatment', 'BIG-IV', 'equine antitoxin', and 'vaccine'. A total of 56 original articles, 35 review articles, 20 case reports, 13 web references, 6 book's chapters, and few commentaries were selected for inclusion in this review.

Clostridium botulinum strains and toxins

Clostridium botulinum is an obligate anaerobic Gram-positive sporulating bacillus that can be found in soil, water or air [1]. Currently, different species of bacteria including *C. botulinum* Groups I–IV, some strains of *C. baratii*, *C. butyricum*, *C. argentinense,* and neurotoxin-producing *C. sporogenes* are known to form BoNT [8–10] or BoNT-like toxins (*Weissella oryzae*) [11,12]. *C. botulinum* is classified into 4 main Groups (I-IV) based on phylogeny, biochemistry and serotype [13]. At least seven 'classical' serotypes (A-G), and more than 40 subtypes of BoNT exist [14]. In addition, BoNT serotype FA (previously called 'serotype H') [15,16] and BoNT serotype X (BoNT/X) have been also identified. BoNT/X is not recognised by antisera against known BoNTs, and is identified by genomic sequencing and bioinformatics approach [17]. A, B, E, and F are the main serotypes harmful to humans [18]. *C. botulinum* Group I strains include all subtypes of serotype A, almost all subtypes of serotype B (B1-B3, B5-B7), F (F1-F5), and H [18]. Moreover, it has been reported that some BoNT/B-producing strains may be neurotoxigenic strains of *C. sporogenes* [19,20]. *C. botulinum* Group II strains include subtype B4, almost all subtypes of serotype E (E1-E3, E6-E7), and F6 [18,21]. In addition, BoNT serotypes F and E are produced by some strains of *C. baratii* (F7) and *C. butyricum* (E4 or E5) [22]. On the basis of its genetic and phenotypic traits, Group IV *C. Botulinum* (strains of type G) has been proposed to be renamed *C. argentinense* [10]. It has been increasingly reported that each strain of *C. botulinum* is able to form more than one type of BoNT [23,24].

Generally, the genes of type A, B, E, and F BoNTs are located on the cell chromosome, but on a large plasmid in type G. In types C and D, BoNT production is governed by bacteriophages: the toxin genes are transmitted from the toxigenic strains to the non-toxigenic ones by specific phages [25].

With a lethal dose of 10^{-9} mg/kg of body weight, BoNT is the most powerful poison in nature [26,27]. This neurotoxin is a di-chain metalloproteinase composed of: (1) a heavy chain C-terminal receptor-binding module (100-kDa); (2) a light chain N-terminal Zn^{2+}-metalloprotease (50-kDa); (3) a translocation domain with a central helical protein-conducting channel [26–28]. The main BoNT target is the presynaptic cell membrane of voluntary motor and autonomic cholinergic neuromuscular junctions. At the neuromuscular junctions, the heavy chain binds to the presynaptic membrane through specific membrane glycoproteins, which include either the isoforms of the vesicle protein SV2 for BoNT A, C, E and F, or synaptotagmin I or II for BoNT B and G [29,30], a ganglioside GD1b, GT1b, and/or GD1a [31–34]. This binding leads to the endocytosis of toxin *via* a clathrin-dependent process. Once in the endosome,

translocation domain acts as a transporting channel for the light chain N-terminal Zn^{2+}-metalloprotease allowing it to reach neural cytosol [28,35]. This metalloprotease is responsible for the intracellular activity, and acts as zinc-dependent endopeptidase [36] on the soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex. This complex is a polymeric system by which the cholinergic synaptic vesicles are excreted in the intersynaptic space. BoNT blocks this transfer so that acetylcholine cannot reach the postsynaptic receptor and depolarise the neuromuscular junctions, thus preventing muscular contraction. Depending on serotype, BoNT impairs a different component of soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex: serotypes B, D, F, and G bind vesicle-associated membrane protein (synaptobrevin), serotype C binds syntaxin, and serotypes A, C, and E bind synaptosomal-associated protein 25 (SNAP- 25) [37].

Pathogenesis

When ingested *via* food or dust, *C. botulinum* spores as well as BoNT pass through the stomach and reach the intestine. Human studies have demonstrated that *C. botulinum* spores and toxin reach their highest concentrations in caecum, transverse and recto-sigmoid colon, most likely due to the relatively anaerobic colonic environment [38]. Once in the large bowel, spores begin to germinate, multiply and produce the toxin, which then is transferred across the intestinal membrane in two possible ways. The first route is paracellular and involves perturbing the E-cadherin of the intestinal epithelial barrier. The second route is transcellular and occurs by the heavy chain C-terminal receptor-binding module binding to specific receptors on the apical surface of epithelial cells that actives transcytosis allowing the toxin to reach the basolateral side of intestinal epithelial cells undissociated and functionally active.

Once diffused into extracellular fluid and absorbed by blood and lymphatic vessels, BoNT reaches neuromuscular junctions of voluntary motor and autonomic muscles [35,39], where it binds irreversibly to cholinergic receptors on presynaptic cell membranes, causing flaccid paralysis [40]. Constipation is an early sign of IB, possibly due to the proximity of the intestinal muscular layer to the source of toxin. The musculature of head, face and throat are also involved early in the course of illness, leading to a bulbar palsy. A progressive descending flaccid paralysis follows that involves the respiratory muscles and diaphragm and may lead to respiratory failure and arrest, when more than 90-95% of muscular receptors are blocked [36,40]. BoNT does not cross the blood-brain barrier, however, evidences from clinical and experimental use of BoNT/A support a direct influence of the neurotoxin on central circuits [41]. It has been hypothesised that BoNT/A may undergo long-range axonal transport [42], followed by a transcytosis process by which it may gain access to second-order neurons in the central nervous system [43,44].

Epidemiology and risk factors

Since 1976, IB has been recorded in all inhabited continents except Africa, although with great variation according to geographic region. IB represents the most common botulism form in the US, with approximately 70% of total cases [45], and in Argentina [46]. Conversely, in Europe, the most represented form is foodborne botulism [47]. During the period from 1976 to 2006, the countries with the highest number of cases were the US (2419 cases in the 1976–2006 period) and Argentina (366 cases in the 1982–2005 period) [46], followed by Australia (32 cases in the 1978–2006 period), Canada (27 cases in the 1979–2006 period), Italy (26 cases in the 1984–2006 period) [48], and Japan (22 cases in the 1986–2006 period) [49]. In the recent years, literature data are available only for few countries. The US continues to be the country with the highest number of IB cases, with a mean of 131 cases annually from 2011 to 2017 [50]; in particular, California accounts for about 40% of US cases, with 1345 laboratory-confirmed cases in the period 1976–2016 [51]. In Europe, Italy has one of the highest burden of IB cases, with 36 laboratory-confirmed cases from 1986 to 2015 [52]. In France, 15 cases were reported from 2004 to 2016 [53] while, in the UK, 19 cases were reported for the 1978–2018 period [54]. IB affects infants ranging from less than 1 week to 1 year of age, with up to 88.5% cases occurring in infants younger than 6 months [51]. In the US, the age at onset is comparable to that of the rest of the world (mean, 13.8 vs 14.3 weeks, respectively) [55]. A recent study in California [51] reported a mean age at onset of 3.8 ± 2.2 months, with two different age distributions according to the kind of feeding: 1.7 months for formula-fed infants and 4.4 months for breast-fed infants. Cases are equally distributed between males and females [51,55,56]. Regarding seasonal or monthly distribution, no typical pattern was observed [45]. BoNT serotypes A and B account for almost all IB cases in the USA and Europe [45,47]; BoNT/A cases have a later onset

than BoNT/B cases (median, 3.8 vs 2.9 months, respectively; $p < .001$) [51]. Botulinum-like toxins produced by *Clostridium baratii* and *Clostridium butyricum* serotypes E and F are rarely involved in IB [57].

Environmental risk factors

Traditionally, honey is considered the main avoidable risk factor for IB. In fact, a high percentage of affected children has a history of consumption of honey before the onset of illness in the US, [55] and other countries [58–60]. In Europe and in other geographic areas, around 60% of IB cases continue to have a positive history of exposure to honey [55,58]. Therefore, according to the Advisory Committee on the Microbiological Safety of Food, honey should not be given to children under one year of age [61]. However, it is probably not the main environmental source [55] as *C. botulinum* spores have been found in just 2–24% of honey samples [62,63]. The correspondence between the species of *C. botulinum* isolated in patients and those present in the domestic or soil dust supports the role of other spore sources [64–66]. Indeed, living near or having a parent who works in a farm, building site, windy and dry area where there is potential for soil disruption are major risk factors for IB [67]. Additional food sources of *C. botulinum* include formula milk powder [68,69], and herbal infusions such as chamomile, anise and mint [64].

Host risk factors

After birth, the gut microbiota undergo complex changes predisposing an infant's intestine to opportunistic pathogens. Thus, factors perturbing intestinal microbiota may potentially predispose to *C. botulinum* colonisation. Shirey et al. [70] found a higher prevalence of *Proteobacteria* and *Enterobacteriaceae* and lower abundance of *Firmicutes* and *Lactobacillus* spp in gut microbiota of 14 IB cases. However, the authors could not determine whether this faecal microbiota profile was predisposing to or resulting from botulism colonisation [70]. The food regimen has a crucial role in selecting the composition of gut flora [71]. The majority of infants at the onset of illness are entirely or primarily breast-fed [51,52]. This subset of infants shows a later onset of illness compared to formula-fed infants [51], possibly because of the presence of several immune factors (IgA, lysozyme, lactoferrin) in human milk [6] that may delay intestinal colonisation and BoNT production [51]. In contrast, formula-fed infants may manifest illness at earlier ages, with a more fulminant course [6]. Thus, current recommendations support breastfeeding in patients affected by IB [72]. A recent case-control study found that, in infants <2 months of age, birth order >1, caesarean delivery, ≤ 1 bowel movement/day, windy residence area, and pacifier use were significant risk factors for hospitalisation; in contrast, breastfeeding, ≤ 1 bowel movement/day, dust exposure and pets were significant risk factors in infants $>$ 2 months of age [73].

The slower intestinal transit could create a favourable environment for spore germination, multiplication and toxin production [73–75], even though it is still debated whether constipation is a risk factor or an early sign of illness.

Clinical manifestations

Botulism infection can cause a spectrum of clinical presentations in infants [76,77]. Infants who display *mild and non-specific symptoms* such as apparent sleepiness, poor feeding, reduced frequency of bowel passage, lasting only for a few days, can be diagnosed by expert physicians familiar with IB [21]. Infants who present with *classical manifestations* are usually sufficiently hypotonic and weak to require hospitalisation; the picture is mainly derived from this category [21]. Finally, *severe IB* ('catastrophic manifestation') can be abrupt and rapid enough to lead to infant death within hours to days. The catastrophic form has been associated with some cases of sudden infant death syndrome [21,38,40,76,78]. In general, the clinical picture is more severe in patients with type A than in patients with type B botulism [40]. Usually, the incubation period is at least 3 days [79], although cases with very rapid gut colonisation and toxin production in less than one day have been reported, typically resulting in fulminant IB [80,81].

Before the introduction of Human Botulism Immune Globulin Intravenous (BIG-IV or BabyBIG®), the natural course of the illness took place in three phases: (I) signs' progression in the first 1–2 weeks; (II) the nadir of muscle function is expected for 2–3 weeks; (III) slow motor recovery in months [36]. Usually, constipation is the main symptom at presentation, followed by a variable combination of apparent sleepiness, decreased spontaneous activity, and poor feeding. Subsequently, bulbar palsy (ptosis, diminished gag reflex, bifacial weakness, weak cry, and drooling) develops, followed by a descending bilateral symmetric paralysis that manifests with loss of head control, descending hypotonia and weakness ('floppy baby'). Ultimately, palsy can involve

Table 1. Neurologic signs to assess fatigability after repetitive muscle contraction.

Neurologic assessment	Method to elicit fatigability
Pupillary reflex	Repeatedly shine a light into the eye, in a dark room, for 1 to 3 min. Assess whether the pupillary constriction becomes sluggish/minimal.
Ocular movements	Repeatedly shine a light into the eye, for 1 to 3 min. Assess whether a diminishing effort to avoid the light or an ophthalmoplegia are induced.
Sucking reflex	Place a finger in the infant's mouth. Assess whether the suction is weak and poorly sustained.
Gag reflex	Place a tongue depressor in the infant's mouth in order to stimulate trigger zones (anterior and posterior faucil pillars, base of tongue, palate, uvula and posterior pharyngeal wall). Assess the strength of gag reflex.

Adapted from Arnon [82].

respiratory and diaphragmatic muscles, leading to respiratory failure and, in turn, respiratory arrest [40].

Typically, there is a muscular fatigability with repetitive activity [40] so that, in the early course of the disease, it can be useful to perform tests for muscular fatigue [56,82] (Table 1). Muscle stretch reflexes are usually preserved at the beginning of the disease [78]; decreased heart rate variability and other autonomic disturbances can be present in advanced illness [40]. Cognitive functions are preserved through the course of illness because BoNT does not cross the blood-brain barrier [3]. Regaining function is related to nerve firing rate to affect musculature and how complete is the block. Smile and facial expression have early return while head control and lifting extremities from prone position are late [36,40].

The main complication described is respiratory arrest with secondary hypoxic brain injury. In the absence of this complication, IB does not have long-term consequences or neuromuscular effects [21]. Classically, the clinical syndrome of IB does not recur. Therefore, a relapse in symptoms may suggest the onset of a complication [83]. The current mortality rate from IB is about 1%, thanks to specific therapy and modern supportive care [21].

Diagnosis

IB should be suspected in a clinically floppy infant, or in the presence of a history and physical examination with consistent findings. In diagnostic workup, general laboratory or imaging findings are usually normal at the beginning of the disease, but they can become essential in the case of complications during the course of the disease. Nerve conduction studies and electromyography (EMG) are painful and rarely performed for diagnosis. They show characteristic but not diagnostic patterns, whose absence does not rule out IB diagnosis, especially at the symptom onset [40,67,75,84]. The typical features found in the nerve conduction studies include the reduced amplitude of action potentials in at least two muscle groups, and tetanic and post-tetanic facilitation with an amplitude above 120% of baseline and lasting longer than 120 s. EMG recordings may show fibrillation potentials and positive sharp waves with low amplitude and short duration potentials [85,86].

Laboratory confirmation

Several studies have shown that *C. botulinum* is not part of normal resident flora in the human intestine [76,87–89]. However, Thompson et al. [67] have detected *C. botulinum* in the stool of healthy infants and infants with neurological but not botulism disease ('asymptomatic carriers'). These findings suggest that the simultaneous presence of both microbiological identification of *C. botulinum* and/or its toxin, and typical clinical signs, is needed to confirm the diagnosis of IB [36,56,86,90]. Laboratory confirmation is also essential to determine prognosis. Hospital length of stay and clinical course are longer and more severe in untreated BoNT/A cases compared to untreated BoNT/B cases [55,82]. Microbiological identification consists of BoNT detection in either stool or serum. Isolation of *C. botulinum* or other BoNT-producing clostridia (*C. butyricum* and *C. baratii)* from stool is usually obtained through a small saline enema [48,91,92]. Regarding BoNT detection in serum, only 13% of IB case sera are positive for BoNT in the US [93]. In general, BoNT detection takes only 24–48 h, while a culture of *C. botulinum* needs at least 5 days [36].

The standard method for culture is based on egg yolk agar [94]. When partially modified, this method can detect both lipase-positive colonies of *C. botulinum*, and lipase negative colonies of other neurotoxigenic clostridia strains (*C. baratii*, *C. butyricum,* and *C. argentinense*) [36]. However, nowadays, culture is performed only in research laboratories for epidemiological studies.

Regarding BoNT detection, the only standard method accepted is a mouse bioassay in which the mortality of mice is monitored for 96 h after injection of a sterile supernatant of stool sample [95]. This method requires laboratory animals and is time consuming. To address this challenge, an endo-peptidase mass-spectrometry (Endopep-MS) *in vitro* activity assay was developed [96]. This assay is based on the specific endo-peptidase

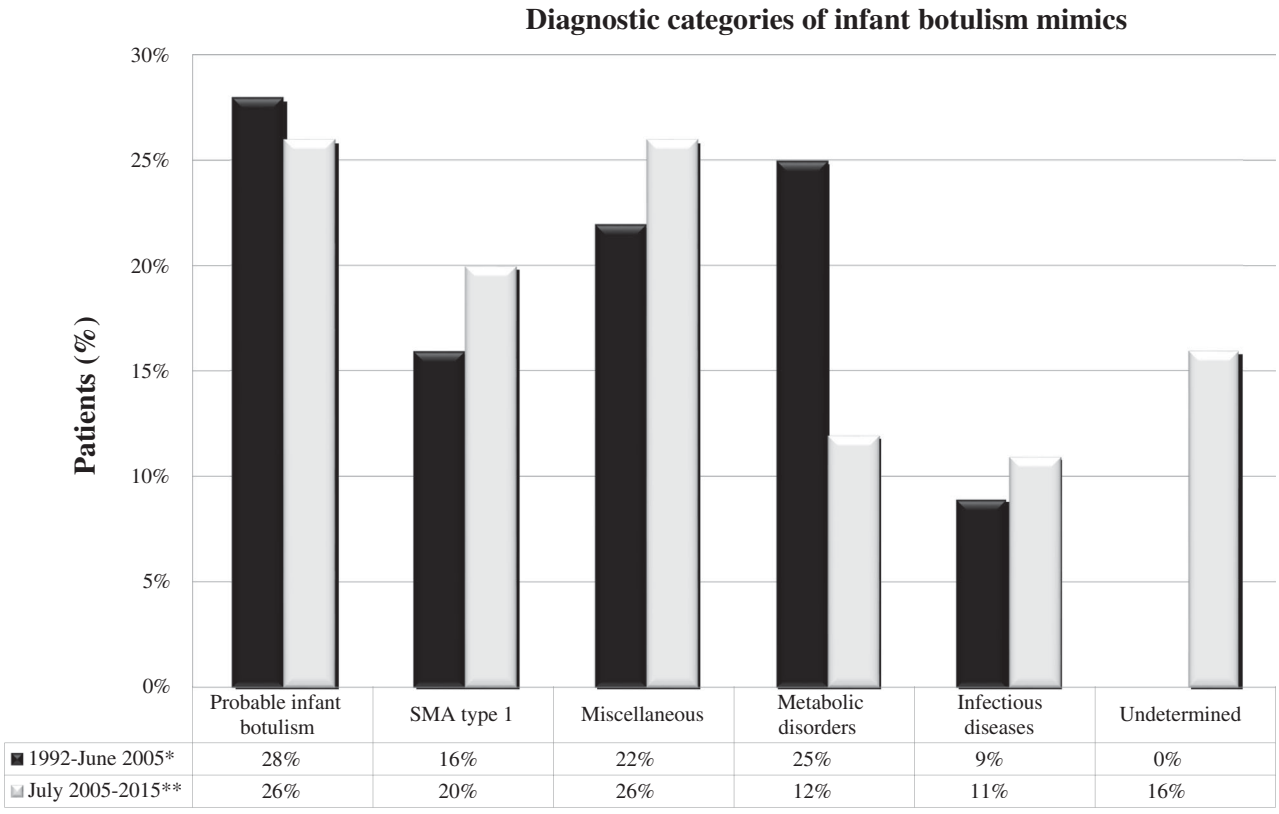

Figure 1. The graphic shows diagnostic categories treated with BIG-IV because they closely mimicked IB. Data are obtained from studies by Francisco and Arnon [102] and Khouri et al. [103]. *Francisco and Arnon [102]. **Khouri et al. [103].

activity of each BoNT serotype, and allowed for the detection of all BoNT serotypes in different matrices including serum, stool, food and milk [97–100]. The entire process takes a few hours, has high specificity and is more sensitive than the *in-vivo* mouse bioassay [101].

In 2016, Wang et al. [100] developed a multiplex Endopep-MS assay to simultaneously detect BoNT serotypes A, B, E and F11. However, the authors found a 90% reduced BoNT/A activity in the multiplex assay compared to the single substrate system. Subsequently, Rosen et al.. [101] developed a multiplex Endopep-MS assay for the simultaneous detection of BoNT/A, B and E, using a new peptide substrate for BoNT/A. This new multiplex assay has a sensitivity similar to that of the single substrate system, and requires less sample volume and a shorter timeframe for completion. These tests for IB are currently in development, and are not used in routine analyses.

Differential diagnosis

The most common IB misdiagnoses at the hospital admission include sepsis [4], dehydration and viral syndromes [102], which may be ruled out by blood and cerebral fluid exams. In a typical diagnostic workup, genetic, metabolic, neuromuscular, and infectious diseases are considered. However, even large clinical studies report 5–6% of cases where an IB diagnosis was not confirmed [102,103]. In a clinical report of cases of suspected IB from 1992 to 2005, the most prevalent cause for misdiagnosis was a metabolic disorder [102]. In a more recent treatment study of cases of clinically suspected IB, spinal muscular atrophy type 1 was the most common misdiagnosis [103]. Other rarer differential diagnoses to be considered include early-onset myasthenia gravis, Guillain-Barré syndrome and its variant Miller–Fisher syndrome, poliomyelitis, diphtheria, and atypical tetanus [3,102–104]. Figure 1 shows the main diagnostic categories that may mimic IB. Of note, in most states and countries, but not in the US, botulism, even when only suspected, requires mandatory reporting to national public health services [82].

Botulism therapy and management

Historically, the cornerstone of IB management was supportive care with special attention to feeding and breathing needs (Table 2) [40,104–107]. BoNT binding to nerve endings is irreversible, and neurologic recovery

Table 2. Supportive care interventions to manage infant botulism.

Respiratory monitoring
- Careful observation for signs of airway obstruction
- Assessment of gag reflex and dysphagia
- Transcutaneous oxygen (O2) monitoring
- Transcutaneous carbon dioxide (pCO2) monitoring

Respiratory support
- Non-invasive [40,104–106]

1. Crib should maintain the patient's body in one plane
2. Head elevated at a 30° angle to the feet
3. Placing a small cloth roll behind the neck
4. Cloth harness that passes through the legs of the infant or a cloth bumper placed below the buttocks
5. Oxygen

- Invasive [40,104–107]

1. Intubation (50% of cases)[a]
2. Tracheostomy (rarely)

Nutritional support [40,104–106]
- Guarantee enteral feeding (consider naso-gastric or naso-jejunal feeding tubes)
- Breast-milk is the optimal nutrition
- Electrolytes monitoring
- Consider Thiamine supplementation [107]

Management of constipation [104–106]
- Guarantee enteral feeding
- Cathartics are not recommended
- Stool softeners (e.g. lactulose) may be beneficial
- Sporadic rectal stimulation or glycerine suppository may be beneficial
- Monitoring for *C. difficile* infection

Management of urinary retention [104–106]
- Credé method: gentle manual pressure on the bladder
- Urinary catheterisation (indwelling or intermittent) usually not recommended
- Monitor for urinary tract infection

[a]Approximately one-half of all patients with IB will require mechanical ventilatory support due to the inability to protect their airway and/or the respiratory insufficiency. Patients with suspected IB should be carefully monitored and observed for signs of airway obstruction, and a prompt endotracheal intubation may be necessary.

occurs as a result of regeneration of motor neurons over weeks to months [40]. Accordingly, infants with IB often required hospital stays of months for complete recovery, with high human and economic costs [108]. Over the last 80 years, botulinum antitoxins have been developed, first an equine botulinum antitoxin (EqBA) in 1940 [109], and subsequently, a human-derived antitoxin (BIG-IV) [40,108,110,111]. Although BIG-IV has significantly changed the course of IB, supportive care remains critically essential for reducing immediate complications and long-term sequelae of infection [3,104,106].

Intravenous botulism immune globulin

From 1990, the California Department of Health Services along with the IB Treatment and Prevention Program began efforts to produce and test a specific drug for IB. The California Department of Health Services tested a single booster dose of an investigational pentavalent botulinum toxoid on adult volunteers previously immunised against BoNT for occupational protection purposes [110]. Plasma from these subjects was fractionated and treated to obtain a purified IgG human-derived antitoxin with a high-titer of neutralising antibody against BoNT type A or type B, called Botulism Immune Globulin (human) Intravenous (BIG-IV). BIG-IV was tested in a randomised, double blind placebo-controlled trial from 1992 to 1997 and, in an open-label study, from 1997 until 2003 [110].

In the first study, infants with an acute-onset flaccid paralysis consistent with botulism were randomised within 3 days of hospitalisation. Although not mandatory before initiation of treatment, confirmatory testing was performed in all patients [110]. This study showed that the administration of BIG-IV was safe and effective. Average hospital stay was 2.6 weeks in BIG-IV treated *versus* 5.7 weeks in non-BIG-IV-treated infants ($p < .001$). Moreover, in the BIG-IV treated group of infants, the duration of intensive care, mechanical ventilation, and intravenous or tube feeding were significantly decreased. These benefits were observed for both serotypes A and B. The data analysis showed that treatment initiated earlier was associated with better recovery. No significant adverse effects were reported except for a transient, blush-like rash. Economic analysis showed a reduction in total charges for hospital stays from $163,400 to $73,800 (USD), demonstrating the cost-effectiveness of this therapy. The subsequent 6-year follow-up open-label study of BIG-IV confirmed previous data, demonstrating reductions in mean hospital length of stay to 2.2 weeks when given within 7 days of admission [110].

Finally, on 23 October 2003, BIG-IV was licenced in the USA by the FDA to the California Department of Health Services under its proprietary name BabyBIG®. Since 2005, BIG-IV has been available outside of the US, even if the high cost and limited availability remain a critical issue in many countries [37,110,112].

A 12-year follow-up study post BIG-IV licence (2003–2015) showed that about 93% of US laboratory-confirmed IB cases were treated with BIG-IV, sparing 66.9 years of length of stay and $86.2 million in hospital charges, in total. The mean reduction in length of stay was dependent on the day of treatment after admission (DoTaA): 3.7 weeks if DoTaA = 0–3; 2.6 weeks if DoTaA = 4–7; 1 week if DoTaA = 8–10 [108]. Thus, BIG-IV should be administered promptly, as soon as there is a clinical suspicion of IB (within 7 days if possible), even if the diagnosis has not been confirmed. A practical guide for BIG-IV treatment of IB is summarised in Table 3 [113–116].

Table 3. A practical guide for BIG-IV treatment of infant botulism.

Formulation	Lyophilized powder containing about 5% human immunoglobulin [113,116] with at least: - 15 IU/ml of neutralising antibodies against toxin type A - 4 IU/ml of neutralising antibodies against toxin type B [40,108,110]
Mechanism of action	Human IgG neutralise circulating BoNT type A and B
Half-life and duration of action	- About 28 days - Blood concentration remains sufficient to bind all free botulinum toxin that an infant may absorb for at least six months [102,114]
Dosage	50 mg/kg [1 ml/kg] intravenous dose, in one-time
Mode of Administration	Stabilize with 5% sucrose and 1% albumin Administration must be started within 2 h and completed within 4 h after reconstitution. The infusion rate should be slow at the beginning with a rate of 0.5 ml/kg/h (25 mg/kg/h) If no adverse effects after 15 min, the rate may be increased to 1.0 ml/kg/h (50 mg/kg/h) until the end (approximately one hour) [113,116]
To obtain BIG-IV	Documenting a clinical suspicion of IB Contacting an IB Treatment and Prevention Program physician to discuss patient (tel. number: +1-510-231-7600) Filling out necessary paperwork Receive consignment of BabyBIG®from IB Treatment and Prevention Program [55,110,115]

Equine botulinum antitoxin (EqBA)

EqBA is derived from horses hyperimmunized with BoNT. Since the 1960s, different formulations of EqBA have been available in the US. A newer heptavalent botulinum antitoxin formulation was tested in 2010 for its ability to neutralise BoNT serotypes (A, B, C, D, E, F, G), and, in 2013, it officially replaced previous formulations, and was approved by the US FDA to be licenced as BAT [109]. Other EqBA preparations contain antibodies to fewer BoNT serotypes (e.g. trivalent antitoxin ABE, bivalent antitoxin AB, monovalent antitoxin E) [117]. In Europe, heptavalent botulinum antitoxin and the trivalent antitoxin ABE are the most commonly used [52]. Heptavalent botulinum antitoxin may neutralise BoNT by targeting either its heavy chain C-terminal receptor binding or its light chain N-terminal Zn2+-metalloprotease components [118].

Currently, EqBA is the first-line therapy for botulism forms other than IB, because of its availability, relative safety and efficacy. However, EqBA use is controversial in IB due to the peculiar features of this form. Concerning aspects are mainly three: (1) the risk of life-threatening reactions such as serum sickness, anaphylaxis, or cardiovascular collapse; (2) the potential lifelong sensitisation to equine proteins**;** (3) the short product half-life. In order to decrease hypersensitivity reaction, heptavalent botulinum antitoxin formulation contains only traces of intact equine IgG (<2%) and mainly consists of Fab or F(ab')2 Ig fragments (≥90%): this reduces drug half-life to about 7 days [119–121]. Such a short half-life gives a potential risk of relapse in cases with a longer course of disease [122,123]. Accordingly, since BIG-IV commercialisation, EqBA should not be used as a first-line treatment in IB [40], although it could be considered as an alternative specific treatment when BabyBIG is not available [109]. A practical guide for EqBA treatment of botulism is summarised in Table 4 [124].

Antibiotics

Currently, clostridiocidal antibiotics are not indicated for IB treatment, because of a theoretical concern about increasing the amount of free toxin in the large bowel and worsen the patient's clinical status. Secondary bacterial infections (e.g. pneumonia, otitis media, urinary infections) are the only indication for the use of antibiotics. In particular, in non-BIG-IV-treated patients, non-clostridiocidal antibiotic treatment (e.g. trimethoprim/sulfamethoxazole) may be prudent. On the other hand, if BIG-IV has been administered, any antibiotic can be used for the treatment of secondary infections, with the exception of aminoglycosides. These agents should always be avoided in IB because they might potentiate the toxin paralytic effects, precipitating acute respiratory arrest [3,105].

Discharge criteria and follow up

Standard criteria in order to discharge an infant affected by IB are: (1) no need for inpatient care, such as mechanical ventilation or oxygen supplementation for at least three days; (2) no worsening in the preceding three days of a palsy, and confirmed motor and bulbar function improvement; (3) gag, suck, and swallow reflexes must be sufficiently strong to ensure the adequacy of oral intake (trophic feeding should be started as soon as possible) and to avoid accidental food aspiration [21]. The assessment of these reflexes is essential, particularly in the evening, when muscular fatigability is more pronounced [36].

Table 4. A practical guide for EqBA treatment of botulism.

Formulation	Each 20 ml vial contains 7 antitoxins: - 4500 UI serotype A - 3300 UI serotype B - 3000 UI serotype C - 600 UI serotype D - 5100 UI serotype E - 3000 UI serotype F - 600 UI serotype G
Mechanism of action	Fab or F(ab')2 fragments of equine IgG neutralise circulating BoNT from type A to type G.
Half-life	About 7 days [119–121]
Dosage	Age dependent: 1. Adult: the recommended dose is 1 vial (*). 2. Infant: 10% of the adult dose (1/10 of a single vial) regardless of weight. 3. Children from 1 to 16 years of age (*): a. 20–100% of recommended adult dose, based on the Salisbury Rule: i. $\leq$30 kg: 2 $\times$ child's weight (kg) ii. $>$30 kg: child's weight (kg) + 30 b. Minimum dosage is 20% of the recommended adult dosage[124]
Mode of Administration	1. Should administer within 5 days from the admission [109] 2. Check drug hypersensitivity by an intradermal injection of 0.2 ml of EqBA diluted 1:1000 (0.9% saline solution) into the volar surface of patient's forearm; after 15–20 min, compare to histamine control [109,124] 3. If preliminary skin test is negative: a 1:10 dilution in normal saline solution is given at the infusion rate of 0.01 ml/kg/h. 4. If no reaction: infusion rate is increased of 0.01 ml/kg/h per time, until reaching the maximum infusion rate of 0.03 ml/kg/h [124]

(*) Use for indications other than infant botulism.

There are no standardised follow-up protocols for IB. *C. botulinum* and BoNT may be present in patient faeces for months, despite resolution of neurological symptomatology. Nevertheless, follow-up stool tests are usually not performed.

Complications and prognosis

In the absence of serious hospital-acquired complications, the prognosis for IB patients is excellent, with anticipated full and complete recovery. In the United States, the mortality rate for IB is less than 1% [40,104]. The course of recovery from IB usually proceeds with a gradual improvement in muscle function, usually without relapses. The worsening of clinical symptoms during the recovery of the patient should let the physician suspect a complication or inadequate respiratory or nutritional support. Infection is the most common complication and can affect the middle ear (otitis media), lungs (aspiration pneumonia) and urinary and intestinal tracts. Regarding intestinal involvement, patients should be carefully monitored for signs of secondary *C. difficile* infection (diarrhea, change in stool colour, abdominal tenseness, or distention), which can result from colonic stasis due to botulism. Bacteraemia and sepsis may also develop from indwelling venous lines [40,104,106]. Among complications, concomitant intestinal viral infections (mostly caused by enteroviruses) have been also described [125]. Infections and other potential causes of clinical deterioration are listed in Table 5 [72]. Sedatives or other drugs potentially resulting in CNS depression are relatively contraindicated [40].

Recent advances

The two major limitations about the current antitoxins, namely the limited stocks of BIG-IV and the potential serious adverse effects to the equine serum (HBAT) [119,121], prompt the search for new strategies of intervention.

Several studies have reported on the use of other BoNT neutralising antibodies, but only a few of them were humanised and, because BoNT amino acid sequences of different serotypes differ more than 70%, the antibodies are able to neutralise only one BoNT subtype [99,126–128]. Therefore, currently we still are searching for new human-like antibody preparations that are both highly effective and well tolerated. With this aim, the European AntiBotABE project has been able to create a mixture of oligoclonal antibodies that are highly protective and, in contrast to BabyBIG® or heptavalent botulinum antitoxin, can be produced in large quantities without the use of animals [118].

The modular structure of native BoNTs, which include a light chain and a heavy chain, is suitable to a multitude of novel fusions and mutations by using molecular biology methods. The novel recombinant BoNTs have

Table 5. Major complications of infant botulism.

Respiratory complications	Complications of intubation	Infectious complications	Miscellaneous
ARDS	Misplaced or plugged endotracheal tube	Bacteraemia and sepsis	Anemia
Aspiration	Subglottic stenosis	*C. difficile* colitis (toxic megacolon)	Blood pressure instability
Recurrent atelectasis	Pneumothorax (tension pneumothorax)	Otitis media (Eustachian tube dysfunction and/or NGT)	SIADH and hyponatremia
Respiratory arrest	Tracheal stenosis and granuloma	Urinary tract infection	
	Tracheitis	Pneumonia	
	Tracheomalacia		

ARDS: Acute respiratory distress syndrome; NGT: naso-gastric-tube; SIADH: syndrome of inappropriate antidiuretic hormone secretion.
Data source: State of California – Health and Human Services Agency [72].

been utilised or are being developed to better characterise the biology of toxins, to help with vaccine production, to act as delivery vehicles to neurons, and to be used as novel therapeutics for both neuronal and non-neuronal cells [129,130].

Regarding vaccination, the investigational formalin-inactivated pentavalent (ABCDE) botulinum toxoid vaccine, which was administered to at-risk individuals for nearly 50 years, was discontinued due to declining immunogenicity and an increasing rate of adverse events. Accordingly, research efforts have been made to develop next-generation vaccines including DNA-based, viral vector-based (adenovirus, influenza virus, rabies virus, etc.) and recombinant protein-based vaccines [131]. Large-scale vaccination against BoNT could be an effective strategy but, according to Rasetti-Escargueil et al. [132], it remains undesirable because of the growing expectation about therapeutic use of BoNTs in a wide range of medical disorders.

Concluding remarks

The limited reporting of IB worldwide suggests that this disease may be under-recognised, under-reported, or both, likely due to diagnostic difficulties and nonspecific presentation. IB should be always suspected in any infant with an acute onset of constipation followed by signs of bulbar palsies and then generalised hypotonia, with or without a suggestive history. In these infants, *C. botulinum* and BoNT research on stool should be performed and, in the case of positive test results, a prompt treatment including support of vital functions and specific therapy with botulinum antitoxin is of crucial importance. Regarding specific therapy, BIG-IV is the first choice, but EqBA can be infused when BIG-IV is unavailable.

Clostridium eradication therapy is not currently indicated. In the case of secondary infections, antibiotics with anti-clostridium activity should always be avoided before the administration of the antitoxin. The patient can be discharged once no further hospital treatment is needed and a valid sucking and swallowing are restored. Despite clinical improvement, the elimination of spores and toxin continues for many months so that a follow-up of at least one year is required. It is important to reassure the parents/carers that, in the absence of complications, full and complete recovery is the expected outcome.

Ongoing research for future treatment of IB involves the search for new molecular targets to produce a new generation of laboratory-produced antitoxins, and the development of new vaccines with safety and efficacy profiles that can be scaled up for clinical use.

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ORCID

Cristian Locci http://orcid.org/0000-0002-5556-5813
Maria Grazia Clemente http://orcid.org/0000-0002-4586-3868
Roberto Antonucci http://orcid.org/0000-0001-9767-7904

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