

BOTULISM ASSOCIATED WITH BACTERIAL PNEUMONIA IN AN ADULT PATIENT

BOTULISM ASOCIAT CU PNEUMONIA BACTERIANĂ LA UN PACIENT ADULT

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ABSTRACT | REZUMAT

Botulism is a severe foodborne which if not correctly treated and monitoring can lead to the development of fatal complications. This study aims to present a case of type B botulism associated with medial basal left pneumonia with *Hafnia alvei*.

The authors present the case of a patient of a 64 years old male, coming from the rural area, admitted in the Clinic of Infectious Diseases in Timișoara on 30.12.2015 with suspected botulism. A positive diagnosis was confirmed by epidemiological factors, clinical presentations and specific bioassay results for identification of botulinum neurotoxin. The obtained data was statistically processed with Epi Info 6 program. WBC=11780/ μ L, PMN=91.5%, RBC=3.77 mil/ μ L, Hb=12.9 g/dl, ESR=75 mm/1h, CRP=301.74 mg/dL; the seric neutralization reaction in vivo in mice revealed the presence of type B botulinic toxin. The chest radiography describes infiltrative opacities in the left medio-basal pulmonary lobe. The sputum culture revealed *Hafnia alvei* (80%). The etiological treatment was instituted using A, B, E antitoxin serum (5500 IU) with prior desensitisation, associated with intravenous antibiotics (Ceftriaxone 2x2 g/day and Ciprofloxacin 2x200 mg/day), antiinflammatories, laxatives, antispastic medication, intravenous solutions for nutrition and electrolytic rebalance with a favorable evolution of the patient. Clinical and biological correctly monitoring, along with the etiological and pathological optimal therapy may contribute to the favorable evolution of type B botulism diagnosed in adults patients.

Keywords: botulism, *Hafnia alvei*, antitoxin serum

Botulismul este o boală alimentară gravă care dacă nu este tratată și monitorizată corect poate să conducă la complicații fatale. Acest studiu are drept scop prezentarea unui caz de botulism tip B asociat cu pneumonie bazală medială stânga produsă de *Hafnia alvei*.

Autorii prezintă cazul unui pacient de sex masculin, în vârstă de 64 de ani, provenit din zona rurală, internat la Clinica de Boli Infecțioase din Timișoara în data de 30.12.2015, suspect de botulism. Diagnosticul pozitiv a fost confirmat de ancheta epidemiologică, semnele clinice și rezultate specifice pentru identificarea neurotoxinei botulinice. Datele obținute au fost procesate cu programul Epi Info 6. WBC=11780/ μ L, PMN=91.5%, RBC=3.77 million/ μ L, Hb=12.9 g/dl, ESR=75 mm/1h, CRP=301.74 mg/dL; reacția de neutralizare cu ser in vivo la cobai a arătat prezența toxinei botulinice de tip B. Radiografia pulmonară descrie opacități infiltrative la lobul pulmonar mediu bazal. Cultura salivei a relevat prezența *Hafnia alvei* (80%). S-a instituit tratament etiologic folosind serul antitoxin (5500 IU) A, B, E asociat cu antibiotice intravenoase (Ceftriaxone 2x2 g/zi Ciprofloxacin 2x200 mg/zi), antiinflamatoare, laxative, medicație antispastică, soluții intravenoase pentru nutriție și reechilibrare electrolitică, cu evoluție favorabilă pentru pacient.

Monitorizarea corectă clinică și biologică, împreună cu terapia optimă etiologică și patologică, pot contribui la evoluția favorabilă a botulismului de tip B diagnosticat la pacienții adulți.

Cuvinte cheie: botulism, *Hafnia alvei*, ser antitoxin

Botulism is a neuroparalytic illness caused by accidental or intentional exposure to neurotoxins of *Clostridium botulinum*, and rare strains of *Clostridium*. Dormant *C. botulinum* spores germinate and produce toxins (types A–G) under the rare confluence of anaerobic conditions, low acidity (pH>4.5), low salt and sugar concentrations and a temperature >10°C. These toxins irreversibly bind to presynaptic nerve endings and inhibit acetylcholine release, resulting in cranial neuropathy and symmetric descending flaccid paralysis, which may progress to potential respiratory failure and sometimes to death (1).

In the United States, the median number of foodborne botulism cases per year between 1990 and 2000 was 23 (range, 17–43 cases). Most cases were sporadic.

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dic and outbreaks typically involved only 2–3 people; however, larger outbreaks caused by commercially prepared foods have also been reported. Home-canned foods have long represented a major source of foodborne botulism cases in the United States (2, 3).

The median annual incidence rate of botulism in Romania was 0.05 per 100 000 persons during 1980–1989, which increased to 0.06 per 100 000 during 1990–1998 and to 0.09 per 100 000 during 1999–2006 (4). Thus, botulism is becoming a life-threatening disease and a rapid diagnosis is essential.

Here we report a case of type B botulism associated with medial basal left pneumonia with *Hafnia alvei* in an adult patient from Romania.

MATERIALS AND METHODS

We present the case of a patient of a 64 years old male, coming from the rural area, admitted in the Clinic of Infectious Diseases in Timișoara on 30.12.2015 with suspected botulism. According to the patient, he ate food from domestic production in 12.19.2015. and in 12.21.2015 accuses numerous vomiting, abdominal cramping. Two days later, the patient accuses nausea, vomiting, abdominal pain. One day later, the digestive symptoms disappear, but the patient presents progressively loss of appetite, dry mouth, double vision, constipation. One week later, the patient is taken to the emergency room in the County Hospital Timișoara where he is consulted by a hepatologist and a neurologist. After wards the patient is referred to the Clinic of Infectious diseases with suspected botulism.

The physical examination at admission showed: fever, dry mucous membranes, dysphagia, mild mydriasis; muco-purulent coughing, without pulmonary rales; painless abdomen, recently installed constipation. A positive diagnosis was confirmed by epidemiological factors, clinical presentations and specific bioassay results for identification of botulinum neurotoxin. To identify the botulinum neurotoxin, serum samples were obtained from patient on the first day of hospitalization, before initiation of therapy with polyvalent anti-botulinic serum. The case were reported to the Public Health Department of Timiș County, Romania, as foodborne botulism within the first 48 hours of patient hospitalization.

Clinical diagnosis was confirmed by the intraperitoneal inoculation test performed using mice for detection of botulinum toxin in the serum, and the seroneutralization test was performed with types A, B, E anti-botulinum sera (bioMérieux, Marcy l'Etoile, France) to

determine the exact type of botulinum toxin.

The tests were performed at the National Institute of Research and Development for Microbiology and Immunology „I. Cantacuzino” Bucharest, Romania.

The obtained data was statistically processed using the Microsoft Excel and Epi Info 6 program.

RESULTS AND DISCUSSIONS

The results of bioassay performed dynamically in the Departments of Biochemistry, Haematology and Bacteriology in the „Victor Babeș” Clinic of Infectious Diseases and Pneumology in Timișoara are shown in **Table 1**. When he was admitted in the clinic, the patient presented a mild leukocytosis ($L=11780/\mu L$), neutrophilia ($PMN=91,5\%$), mild anemia ($Hb=12,9\text{ g/dL}$, $h=3770000/\mu L$) and a significant increase of reactive protein C ($CRP=301,74\text{ mg/dL}$), a higher speed of red blood cell sedimentation ($ERS=75\text{ mm/1h}$).

The chest radiography on 30/12/2015: infiltrative opacities in the left basal pulmonary lobe.

Antibiogram for *Hafnia alvei*: sensitive to ceftriaxone, ceftazidime, cefoxitine, amikacin, gentamicin, tobramycin, amoxycilin and clavulanic acid, piperacillin + tazobactam, ertapenem and levofloxacin.

The seric neutralization reaction in vivo in mice made with the help from the National Research Institute Cantacuzino Bucharest revealed the presence of type B botulinic toxin.

The positive diagnosis, type B botulism, associated with acute left medio-basal pneumonia with *Hafnia alvei* was established according to anamnestic data, clinical data and biological sample results and paraclinical investigations.

To identify potential allergic reactions to treatment, 1 vial (10 mL) of trivalent equine anti-toxin (A, B, E) containing 5500–8500 IU of antibodies of each type was diluted (1:10) with 0.9% saline solution, and 0.1 mL of the dilution was intradermally administered to the patients. After 20 min, a second 0.1-mL dose was administered subcutaneously. Because neither woman showed an allergic response, the remaining trivalent anti-botulinic serum was administered intramuscularly. The patients were clinically observed during therapy and arterial blood pressure, heart rate, body temperature, electrocardiogram readings and blood oxygen saturation were recorded in the patient charts. Of note, no local or systemic secondary reactions occurred in either patient after administration of the trivalent anti-botulinic serum. Furthermore, the patient was cooperative, apyretic and conscious.

Table 1

Biological tests	Normal range	30.12.2015	05.01.2016	13.01.2016
WBC (μL)	4.000-10.000	11780	3940	5840
Leucocyte formula	N= 2.200-6.600 E= 0-400 L= 1.200-4.400 B= 0-100 M= 220-1.000	N= 91,5 % E= 0 % L= 5,1% B= 0 % M= 3,4%	N=58,5 % E= 3 % L= 22,3% B= 0,5 % M= 15,7%	N= 65,2 % E=2,1% L= 19,5% B=0,5% M= 12,7%
RBC (μL)	4.700.000-5.500.000	3770000	3570000	3430000
Hb (g/dL)	14-17.2	12,9	12	11,6
Ht (%)	35-45	36,1	34,6	33,9
PT (μL)	150.000-400.000	210.000	307.000	446.000
ESR (mm/1h)	3-10	75	90	115
Fibrinogen (g%)	1.8-3.5	-	-	-
CRP mg/dl	0-5	301,74	66,97	53,09
ALT (U/L)	0-41	16,9	20,2	18,9
AST (U/L)	0-40	30,6	26,2	
Creatinine (mg/dL)	0.7-1.2	0,8	0,44	0,49
Pharyngeal exudate	-	negativ	-	-
Lingual exudate	-	negativ	-	-
Nasal exudate	-	negativ	-	-
Blood culture	-	negativ	-	-
Urine culture	-	negativ	-	-
Saliva culture	-	10% streptococcus viridans, 10% neisserii, 80% Hafnia alvei	-	-

After previous desensitisation, which consisted of antibiotic treatment with ceftriaxone (Cefort 1g fl - 2x2g /day, i.v., for 14 days) and Ciprofloxacin (2x200 mg/day i.v., for 10 days), an etiological treatment with botulinic ABE antitoxin was established.

For hydro-electrolytic rebalancing, perfusable solutions such as NaCl 0.9% (500ml/i.v.), Ringer solution (500 ml/i.v.), 5% Glucose (500 ml/i.v.) were administered. Symptomatic treatment included: antipyretics and painkillers (Paracetamol fl, Algocalmin f), NSAIDs (Diclofenac sp), expectorants (Bromhexim cp), antiallergics (Dasselta cp.) and gastroprotectors (Arnetin f). The clinical evolution was favorable, due to the remission of fever since the 5th day of antibiotics, continuing with a gradual remission and ending with the disappearance of any coughing, dysphagia, dysphoria and partial remission of dryness of the buccal mucosa. The checking radiography made on 13.01.2016 showed the remission of the lesions which appeared on the radiography taken on 30.12.2015.

Foodborne botulism is a severe paralytic illness caused by consumption of food that contains botulinum toxin, a product of *C. botulinum*. The acute illness is well-described and involves cranial nerve dysfunction, symmetric descending flaccid paralysis which may progress to respiratory distress and death (5,1).

C. botulinum is ubiquitously found in soil and aquatic sediments and produces 7 immunologically distinct toxins (A-G) that cause clinically similar, highly recognizable syndromes. Human cases are mostly caused by toxin types A, B, E and F (1).

C. botulinum infections occur worldwide, but botulism is difficult to diagnose because botulism is a rare disease with which most clinicians are unfamiliar. The botulinum toxin is not always detectable in food samples. The type A botulinum toxin occurs more frequently in soils in the United States, whereas type B is more frequently found in Europe.

Baltic countries display the highest level of type E contamination in the world (9). From 2006 to 2008,

there were 477 confirmed cases in the European Union, which equates to an average of 119 (range, 104–132) cases per year, but no discernible trend has been identified (11). According to the European Centre for Disease Prevention and Control's (ECDC) Annual Epidemiological Report 2011, major causes of botulism in industrialised countries are contaminated and inadequately cooked foods, and ingestion by infants of spores in the environment (12). To our knowledge, association with acute botulism type B and bacterial pneumonia with *Hafnia alvei* it has not been described in the literature. *Hafnia alvei* is a gram-negative bacterium that is rarely isolated from human specimens and is rarely considered to be pathogenic. It has been associated with gastroenteritis, meningitis, bacteraemia, pneumonia, nosocomial wound infections, endophthalmitis, and a buttock abscess. Treatment of *H. alvei* infection on the basis of antimicrobial susceptibility testing results is effective.

From 2003 to 2008, in Romania, botulinum toxin was detected in 80 serum samples (79 type B and 1 type E): 27 cases (13%) in 2003, 18 (9%) in 2004, 21 (10%) in 2005, 23 (11%) in 2006, 110 (52%) in 2007 and 11 (5%) in 2008 (9). This case of type B foodborne botulism presented in this article confirms the predominance of type B botulism in Romania.

Most food borne botulism outbreaks in Romania are attributable to improperly home-canned foods, including liver, fish, vegetables, chicken products, pork and other marine species. The type B botulinum toxin is most often isolated from food, although type E is most often isolated from fish products (10).

It is necessary to educate the population regarding compliance with food storage standards, especially during the summer months, and to reiterate the importance of reading the expiration date printed on food containers before consuming the contents. Moreover, preparation of canned goods in the household (compotes, pickles, ham, sausage, drum, etc.) must be performed in strict compliance with the rules of hygiene in all stages of preparation of the product.

The standard test for laboratory diagnosis and confirmation is a bioassay involving intraperitoneal injection of toxin into mice and observation of the development of botulism-specific symptoms. Toxin type is determined by injecting a panel of mice with mixtures of the test sample and a specific monoclonal anti-toxin (e.g. anti-A or anti-B) and by observing which anti-toxin confers protection to the mice (1).

Because botulism is a life-threatening condition, rapid diagnosis is essential and relies on clinical obser-

vation and laboratory analysis to subsequently confirm or refute the clinical diagnosis (6). The advanced administration of anti-botulinum serum was very likely to have influenced the positive evolution of this case. Anti-toxin should be administered early in the course of illness, ideally <24 h after the onset of symptoms (7,8), because anti-toxin neutralizes only toxin molecules that are yet unbound to nerve endings (9).

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