

Review Article

Impaired Neuronal Communication Syndrome (INCS) as Novel Neurological Side Effect to Botulinum Toxin Type A Therapy with 16 Case Reports

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Abstract

Based on the data of the 16 cases we report here, prior experience with side effects to botulinum, and the published literature, we define a novel syndrome as an adverse reaction to the toxin therapy which we named: Impaired Neuronal Communication Syndrome (INCS). We define INCS as a disease of toxin-induced impairment of neuronal connection and impaired mediators balance in the central, peripheral and/or autonomic nervous system with, most of the time, no identifiable anatomical cell damage. This syndrome differs from the reported in the literature autoimmune adverse reactions to the toxin, affecting the central (encephalitis) or the peripheral nervous system (inflammatory polyneuropathies, brachial plexitis etc). The major difference is that, in toxin-induced autoimmune syndromes, it is more likely to have positive findings in laboratory tests and imaging or on examination while, in INCS the majority of the patients have normal laboratory and imaging tests and neurological examination. This suggests that, in such cases, the clinical features and the proximity of the symptoms' occurrence to the toxin therapy are the main and often the only tools to achieve a correct diagnosis. We identify that the side effects from botulinum toxin therapy can be severe, persistent, and disabling. We suggest an algorithm for safer toxin use.

Keywords: Botulinum toxin; Side effects; Adverse events; Central nervous system; Autonomic nervous system; Impaired Neuronal Communication Syndrome (INCS)

Abbreviations

3-4 DAP: 3-4 Diaminopyridine; Ach: Acetylcholine; BoNT/A: Botulinum Neurotoxin Type A; CNS: Central Nervous System; EEG: Electroencephalogram; ELISA: Enzyme Linked Immunosorbent Assay; INCS: Impaired Neuronal Communication Syndrome; LD50: Lethal Dose of the toxin that kills 50% of the mice population; SNAP-25: Synaptosomal Associated Protein - 25.

Introduction

Botulinum Neurotoxin Type A (BoNT/A) was purified in 1928. Three forms of BoNT/A were approved for use in USA: onabotulinumtoxinA: for therapeutic in 1989 and for cosmetic in 2002; abobotulinumtoxinA: for therapeutic and cosmetic in 2009 and incobotulinumtoxinA: for therapeutic in 2010 and for cosmetic in 2011. Since then thousands of patients have been injected worldwide, while the toxin sales have been increasing through the decades. Numerous double-blind trials have been conducted. The toxin therapy was regarded as generally safe. In September 2009, after 20 years of use, a black-box warning for the toxin was issued indicating that BoNT/A could cause death and disability. This is not unusual in type B adverse events to drugs as defined in pharmacovigilance research [1], indicating that, double blind studies often cannot establish the long term safety of a drug even after decades of use. Type B adverse events are events displaying phenomenology other than the well known and expected reactions to a drug, known as type A events. Typically, type

B events require a significant amount of adverse events reports before a drug to side effect connection is established [1]. Historically, in such cases, there are abnormal findings in the laboratory, imaging or other tests or in the clinical examination, facilitating the establishment of a relationship. A good example of the above is the chloramphenicol-induced aplastic anemia [2]. Unfortunately, in the case of BoNT/A, we do not have readily available laboratory, imaging or other study abnormalities to establish a relationship between an adverse event and the toxin. Autopsies of people and animals who died from botulism did not display specific changes to identify the toxin as cause of death [3, 4]. Herrero et al. [5] sacrificed monkeys to intra-venous injection with the toxin and found no specific morphological changes. In this article we demonstrate that, at present, the main and often the only tools to establish diagnosis are the symptoms proximity to the toxin injection and the clinical picture which bears great similarity across the patient's population. We established the phenomenology of a novel syndrome, as an adverse event syndrome to BoNT/A and named it an "Impaired Neuronal Communication Syndrome" (INCS). We suggest an algorithm for safer BoNT/A use.

Clinical Cases

All patients were consulted for their condition by the author over a period of 4 years. Available data from other providers, imaging, laboratory and test results have been included in the data summaries (Tables 1a, 1b, 2a, 2b). The symptoms are graded in each case with one

Table 1a: ANA: Anti-Nuclear Antibodies; CRP: C-reactive Protein; HA: Headaches; H: Hour; F: Female; °F: Fahrenheit; M: Male; PET: Positron Emission Tomography; R arm: Right arm; SOB: Shortness of Breath; WBC: White Blood Cells; Toxins: ABO: AbobotulinumtoxinA; INCO: IncobotulinumtoxinA; ONA: OnabotulinumtoxinA; the number after the toxin indicated the number of treatment with the particular type botulinum and the sequence of treatments ; 'Patient was injected with ABO to which she had reaction and later with ONA when the disability occurred; "Patient received filler around the mouth the same day, ""After initial improvement over a year period this patient's symptoms worsened again after scombroid fish poisoning, "" First ONA injection was done together with lidocaine.

Patient number	1	2	3	4	5	6	7	8
Demographics								
Age	54	35	34	48	41	32	53	44
Gender	F	F	F	F	F	F	F	F
Toxin	ONA	ONA	ONA	ONA	ONA	ONA/ABO	ONA	ONA
Cosmetic/dose	117U (injected in neck and face)	ONA1-30U ONA2-30U ONA3-49U	27U	ONA1: unknown ONA2: unknown given < 3 mo perior ONA3: 30 units	V1: 25U V2: 25U V3: 50U V4: 50U	ONA1: 10-24U ABO1: 60U ABO2: 60U ONA11:25U	ONA1-30: (30-35U) ONA31: (30-35U) ONA32: (30-35U) ONA33: 10 units	no
Therapeutic/dose	no	no	no	no	no	no	no	ONA1- 100"" ONA2- 120 ONA3- 200 migraines/neck pain
Years of toxin treatment	1 st injection	3 years	1 st Injection	9 months	2 years	5 years	12 years	3 years
Time to Symptoms onset	10 minutes	ONA1: 24 h ONA2-7: none ONA8: 2 h	1h	ONA1: 24 h ONA2: none ONA3: minutes	ONA1: 2 months ONA2: 2 months ONA3: 1 month ONA4: 5 h	ONA1 to B10:none ABO1: couple of weeks ABO2: couple of days ONA11: 48-72h	ONA1-30: none ONA31: few hours ONA32: 30 minutes ONA33: few hours	ONA1: 48-72h ONA2: none ONA3: 48-72h
Symptoms								
First Symptom	Lightheaded Tingling face Ringing ear	ONA1; headaches ONA2-7-none ONA8- inner trembling	Profound Tiredness	ONA1- SOB ONA2- none ONA3- dizziness R arm tingling	ONA1: generalized rash ONA2: chest pain, fatigue ONA3: SOB, chest pain ONA4: burning head	ONA1-10:none ABO1: myoclonus, insomnia ABO2: insomnia, vision, color changes ONA11: myoclonus, insomnia, vision dystortion	ONA1-30: none ONA31: anxiety, fatigue ONA32: palpitations ONA33: heart racing, feeling of dying	ONA1: droopy eyelid, neck weakness ONA2: no side effects ONA3: fatigue
Fatigue/endurance	+++	+++	+++	+++	++	+++	+++	+++
Central nervous system	+++	++	++	+++	+++	+++	+	++
Peripheral nervous system	+++	++	+	+	+++	+	+	++
Autonomic	+++	+++	+++	+++	+++	++	+++	+++
Psychiatric	++	+	++	+++	+++	+++	+++	++
Hypothalamic syndrome	yes	yes	yes	yes	yes	yes	yes	yes
Muscle weakness	yes	yes	no	yes	no	yes	yes	yes
Immune system activation markers and symptoms	yes: small fiber polyneuropathy, ulcers in mouth	yes: Eosinophils elevated and temp 99.9° F	no	no	yes: rash; CRP, globulines and ANA elevated	yes: blotchy, itchy hands and chest ANA elevated	yes: ANA elevated	yes: soar throat 3 days
Trophic changes	yes	yes	no	yes	no	yes	no	no
Specific Features								
Sensitivity to food and meds	yes	yes	no	no	yes	no	yes	yes
Crises and relapses	yes	yes	no	yes	yes	yes	yes	yes
Wax/ Wane of Symptoms	yes	yes	no	yes	yes	yes	yes	yes
Increase rate of Infections	yes	yes	yes	no	no	no	no	no

<i>Reaction to prior injections</i>	n/a	yes	n/a	yes	yes	yes	yes	yes
Quality of life								
<i>Functionality before toxin</i>	100%	100%	100%	100%	100%	100%	100%	100%
<i>Disability after the toxin</i>	yes	yes	yes	yes	yes	yes	yes for 5 years	yes
<i>Recovered 100%</i>	no	no	yes in 4 months	no	no	no	yes in 5 years	no

Table 1b:

Patient number	9	10	11	12	13	14	15	16
Demographics								
<i>Age</i>	38	34	43	41	32	53	36	27
<i>Gender</i>	F	F	F	F	F	F	F	M
<i>Toxin</i>	ONA [™]	ONA	ONA	ONA	ONA	ABO	ONA	ONA/INCO
<i>Cosmetic/dose</i>	20U	28U	16U	25U	26U	100U	ONA1-6: 25U (<3 mo apart)	no
<i>Therapeutic/dose</i>	no	no	no	no	no	no	ONA7: 50 U (injected for armpit sweating in 20 spots)	ONA1-5: 175U ONA6: 190U ONA7: 190U INCO1: 190U (injected for cervical dystonia)
<i>Years of toxin treatment</i>	1 st injection	1 st injection	1 st injection	1 st injection	1 injection	1 year	2 years	4 years
<i>Time to Symptoms onset</i>	4h	24h	minutes	72h	4h	ABO1: none ABO2: 72h	ONA 1-3: none ONA 4-5: seven days ONA 6: hours ONA 7: 24-48h	ONA1-5: several days ONA6: several days ONA7: several days INCO1: 72h
Symptoms								
<i>First Symptom</i>	Burning in brain, diarrhea, vomiting chest pain	Head pressure Palpitations	Numbness head, nausea, vomiting, breathing, swallowing	Heaviness Forehead, headaches	Numbness of face SOB	ABO1: none ABO2: Difficulty breathing	ONA 1-3: none ONA 4-5: SOB, urination difficulty ONA 6: SOB, loss of appetite, malaise ONA 7: Flu- like, feeling of suffocation	ONA1-5: anxiety, HA, neck weakness ONA6: SOB, HA (for 3 days) ONA7: SOB, HA (for 12 days) INCO1: SOB, swallow, anxiety, speech, symptoms persisted
<i>Fatigue/endurance</i>	+++	+++	+++	+++	+	+++	+++	+++
<i>Central nervous system</i>	++	++	+++	+++	++	+++	++	+
<i>Peripheral nervous system</i>	+++	-	+	+++	++	+	++	++
<i>Autonomic</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>Psychiatric</i>	+++	+++	+++	+++	+++	+++	+++	+++
<i>Hypothalamic syndrome</i>	yes	yes	yes	yes	yes	yes	yes	yes
<i>Muscle weakness</i>	yes	yes	yes	yes	no	yes	no	yes
<i>Autoimmune symptoms or markers</i>	yes: small fiber polyneuropathy hives face and hands	yes: anticardiolipin antibody	no	no	Yes, rash forehead, C3 complement	yes: botulinum antibody	yes: elevated ANA, WBC and platelets	yes: asthma like symptoms and rash, PET scan increase intake is thymus, tongue ulcers
<i>Trophic changes</i>	no	no	no	no	yes	yes	yes	yes
Specific Features								
<i>Sensitivity to food and meds</i>	yes	yes	no	no	yes	yes	yes	no
<i>Crises and relapses</i>	yes	yes	yes	yes	yes	yes ^{***}	yes	yes
<i>Wax/ Wane of Symptoms</i>	yes	yes	yes	yes	yes	yes	yes	yes
<i>Increase rate of Infections</i>	no	yes	yes	no	no	no	yes	no

Reaction to prior injections	n/a	n/a	n/a	n/a	n/a	no	yes	yes
Quality of life								
Functionality before toxin	100%	100%	100%	100%	100%	100%	100%	100%
Disability after the toxin	yes	yes	yes	yes	yes	yes	yes	yes
Recovered	no	no	no	no	yes in 4 mo	no	no	no

to three pluses in order of their severity and prevalence. All patients experienced multiple symptoms from each group with 12-25 range of total number of symptoms per patient. All patients had clinical effects from their injections. The demographics show significant female predominance due to the cosmetic use of the toxin in the majority of the presented cases. In addition, most of the patients were injected with onabotulinumtoxinA, likely because the drug has the longest market presence. The relevant data from past and family history and the abnormal findings from the general and the neurological examinations, done at time of presence of significant complains, are presented in Table 3a and 3b. The examination is often normal or minimally affected. The subtle positive signs can often be missed if fast or only a gross neurological examination is performed. We found that clock drawing, pictures and animals naming and at times muscle fatigability testing were useful tools in assessing patients' status.

We composed and analyzed 9 groups of clinical symptoms as phenomenology expression of INCS:

Central Nervous System (CNS)

Patients often use the following expressions: "I am in a fog... ..I am in a bubble...I feel detached...My body is not listening to my brain...I am disconnected...Somebody else is operating my body". The most common symptoms are impaired concentration, word finding difficulties, impairment of memory, significant head pressure, phono- and photophobia and inability to multitask. Processing speed is decreased. Events happening at a faster speed are not perceived well, such as: retrieving all information from a person speaking quickly or from a group of people speaking at the same time reading and looking at fast moving objects. Most of all, there is a mental fatigue which often leaves the patients debilitated and with the ability to only do a few tasks before exhaustion sets in. Other reported symptoms in order of frequency are: internal shaking, electric feeling in the head or along the spine, headaches, episodes of speech arrest with duration of 10-15 minutes, loss of smell, myoclonus, abnormal movements, distortion of the visual fields, gait ataxia and tremors.

Peripheral nervous system

The most common symptoms are pain and/or paresthesia in limbs, face and/or torso and a "buzzing" feeling in the nerves. Many times the pain is severe and is described as, "crushed glass under my skin" or "acid dripping", leaving the patient suffering 24/7. Feelings of pins and needles, itching and numbness are less frequent symptoms, but can be, as well, pronounced. Cranial nerves, such as the acoustic nerve, can be affected with tinnitus, and the optic nerve with tunnel vision. Maximum pain at the area of the injection is one sign which can be used to establish connection to the toxin. Sensation disturbances may occur in the vaginal area, without presence of infection.

Autonomic nervous system

Most common and disabling are the following symptoms: shortness of breath, arrhythmia, heart racing, palpitations, chest pain, dizziness, dry mouth, dry eyes and stuffy ears, nose and head. Sometimes the intensity is severe, especially of the shortness of breath. The patents describe it as "not being able to take a deep breath", "involuntary arrest of breathing for several seconds" and "need to constantly remind the self to breathe". When present and intense, the later symptom can linger for years deeply affecting the quality of life of the patient. Other reported symptoms are: urine incontinence, frequent urination, straining on initiation of urination, nausea, vomiting, constipation, diarrhea, blurred vision, eye strain, icy cold limbs, night sweats, abdominal pain, runny nose, hoarseness and chest pressure. Constipation can be very severe.

Psychiatric

Psychiatric symptoms are very common and debilitating such as severe anxiety, panic attacks, feeling desperate, hopeless, negative and fixated on the symptoms. There is a deep feeling that something is wrong and the patients feel terrified or fearful for a long time. The patient's whole world is absorbed by the disease. It is common for the patient to wake up at night with agonizing fear that death is imminent. Usually, depression sets in as the patient is unable to receive help or acknowledgment of his/her symptoms from others or, at times, from the medical providers. Psychosis is less common but no less crippling. One patient had constant visions of killing others, seeing aliens and other people in her room.

Hypothalamic syndrome

Weight loss, loss of appetite, insomnia, reduction of libido, change in menses or sperm, change in tolerance of cold and warm, hot flashes and increased thirst are frequent symptoms. Increased hunger is rare. Weight loss (6-35 pounds), though most of the time transient, could be extreme at times. Insomnia is often disabling and prolonged. Amenorrhea can last for months. The one male patient reported in this series said that his sperm looked like water for one year after the occurrence of the side effects. Patient #10 had initial normal thyroid tests and then pattern suggesting "central" hypothyroidism.

Fatigue, lack of endurance, muscle twitches and muscle stiffness

Fatigue is usually profound and present in all patients. Sometimes even the slightest effort tires the patients, such as holding a book for several minutes when reading or rigorously stirring tea. Muscle stiffness and tightness are very common and disturbing to the patients. Myoclonic jerks and other twitches occur, but are rare.

Muscle weakness

It is very important to note, that although the patients experience and report lack of muscle power and complain of weakness, the muscle power on manual testing is almost always normal suggesting

Table 2a: Ab: antibody; ABG: Arterial Blood Gas; Ach: acetylcholine; AchR: Acetylcholine Receptors; ALT: Alanine Aminotransferase; ANA: Anti-Nuclear Antibody; AP: Alkaline Phosphatase, AST: Aspartate Aminotransferase; BNP: B type Natriuretic Peptide; C-ANCA: Cytoplasmic Anti-Neutrophil Cytoplasmic Antibody; CD57+NK: CD+57 Natural Killers Lymphocytes; C and L spine: cervical and lumbar spine; C1Q: anti-ganglioside C1Q antibody; CPK: Creatine Phosphokinase; CRF: Corticotrophin Release Factor; CRH: Corticotrophin Release Hormone; CRMP Ab: collapsin response mediator protein antibody; CRP: C-reactive Protein; CT: Computer Tomography; CXR: Chest X-ray; DHEA: Didehydroepiandrosterone; ds-DNA: Anti-Double Stranded DNA; ECHO: Echocardiography; ECG: Electrocardiogram; EEG: Electroencephalogram; EMG: Electromyography; ENT: Ear Nose Throat; ESR: Erythrocyte Sedimentation Rate; FSH: Follicle Stimulating Hormone; FVC: Forced Vital Capacity; G-6PD: Glucose-6-Phosphate Dehydrogenase; GAD-65: Glutamic Acid Decarboxylase; GGT: Gamma Glutamyl Transpeptidase; GM1a: anti-ganglioside GM1a antibody; GM1: anti-ganglioside GM1 antibody; GM2: anti-ganglioside GM2 antibody; GQ1B: anti-ganglioside GQ1B antibody; Hb: hemoglobin. HbA1C: hemoglobin A1C; HGC: Human Gonadotropic Hormone; HIV: Human Immunodeficiency Virus; HSV1: Herpes Simplex Virus type 1; HSV2: Herpes Simplex Virus type 2; Ht: Hematocrit; HbA1C: Hemoglobin A1C; IEF: Immuno-Electrophoresis; IgM: Immunoglobulin M; INR: International Normalized Ratio; LH: Luteinizing Hormone; LDL: Low Density Lipoprotein; LP: Lumbar Puncture; Mg: Magnesium, MMA: Methylmalonic Acid; MRI: Magnetic Resonance Imaging; MTHFR: Methyl-Enetetrahydrofolate Reductase; MUSK: Anti-Muscle Specific Kinase Antibody; Na: Sodium; NIF: Negative Inspiratory Force; O2 Sat: Oxygen saturation; P-ANCA: Perinuclear Anti-Neutrophil Cytoplasmic Antibody; PCA: Anti-Parietal Cell Antibody; PET: Positron Emission Tomography; PFT: Pulmonary Function Test; PT: Prothrombin Time; PTH: Parathyroid Hormone; RBC: Red Blood Cells; RF: Rheumatoid Factor; RNP: Anti-Ribonucleoprotein Antibody; RPR: Rapid Plasma Reagins; SCL70: Scleroderma 70 antibody; SPEP: Serum Protein Electrophoresis; SSA: Anti-Sjogren Syndrome A Antibody; SSB: Anti-Sjogren Syndrome B Antibody; TRH: Thyroid Releasing Hormone; TSH: Thyroid Stimulating Hormone; US abdo: Ultrasound Abdomen; vit B12: vitamin B12; vit B6: vitamin B6; vit D3: vitamin D3; WBC: White Blood Cell Count; *CD57+NK have been reported to be lower in chronic diseases such as Lyme compared to normal controls.

Patient #	1	2	3	4	5	6	7	8
Positive test results	Skin biopsy: positive for small fiber polyneuropathy, ENT (atrophy of vocal cords) Cholesterol 209 (<200)	ECG: ST and T abnormal, possible posterior ischemia, anion gap 18 (7-16) platelets 134 (150-350) prolactin 4.1 (4.8-23.3), estrogen 119 (156-350) RBC 5.44 (4-5.2), Hb 15.7 (12-15), Ht 47.1 (36-46), Eosinophils: 3.3 (<2) 2 days after the injection and 4.6 (<2) during a crisis, FVC 1.61L (3-5), NIF: 40 (>60)	Urine blood 2+	Folate 201 (>280) supplemented	WBC: 10.67 (4.5-10), neutrophils 0.74 (<0.65), lymphocytes 0.19 (>20), CRP 25 (<1), globulin 33 (<30), ANA 1:60 (<1:40), (ECG holter: random episodes of tachycardia), heart rate variability 58 (60-80), thermo paraspinal scan: inflammation C1-C3 level	ANA 1:160 MRI head: 6 mm white matter lesion in the right centrum semiovale cortisol 27.74H (2.3-11.9)	Vital Capacity 2.1L (2.9L) ANA 1:80 speckled, HCO3 30.2 high (23-27), Indirect bilirubin 0.9 (0.2-0.7) CD 57+NK: 75 (60-350)*	Ionized calcium actual 1.31(1.17-1.29)
Negative test results	Electrolytes, calcium, glucose, albumin, globulin, WBC, liver enzymes, ds DNA bilirubin, ESR, CRP, LDL triglycerides, TSH, T3, T4 free, ANA, triglycerides, MRI of brain	Electrolytes, glucose, creatinine, liver enzymes, urine, CPK, copper, vit B12, TSH, Mg, HGC, antibodies: MUSK, dsDNA, ANA, AchR blocking and modulating vit D3, phosphate, osmolality, magnesium, CRP, ESR, C1 esterase inhibitor, ANA, TRH, CRF, CRH, GH-RH, antidiuretic hormone, plasma Catecholamines (norepinephrin, epinephrine, dopamine), testosterone, aldosterone, estriol	Electrolytes, glucose, creatinine, liver enzymes, WBC	Electrolytes creatinine, glucose, iron, ferritin, calcium, TSH, free T4, Mg, vit D3, vit B12, Gliandin Ab, ANA,SSA, SSB, Ht	Electrolytes, liver enzymes, bilirubin, acetone, transferrin, amylase, lipase, CPK, lipid profile, albumin, RF, anti ds DNA, anti-Smith Ab, TSH, T3 and T4, vit D3, iron, pulmonary function test, cardiac stress test, MRI brain, EEG, ECG	Electrolytes, WBC, creatinine, liver enzymes,CRP, ESR, vit D3, vit B12, MMA, Vit B1ferritin, Hb A1C, glucose, thyroid profile, DHEA,FSH,L H,progesterone,testosterone, glucose, Lyme, gliandin Ab, lead, mercury, copper, complement, Raji cells, Immuno-globulins, food allergies, ova and parasites G-6PD, MTHFR, SPEP, antibodies:SSA, SSB,Anti-Smith, anti-Jo, Scl 70, RNP, Ach R Ab, P-ANCA, C-ANCA,GQ1B Ab, GM1a, GM1, GM2, paraneoplastic panel, rheumatoid factor, EMG, EEG, tilt table, bone density	Electrolytes, creatinine, glucose, WBC, Hb, AST, ALT, ESR, CRP, TSH, globulin, aldolase, lyme, copper, venous ABG, SPEP, TSH, CPK complement C3 and C4, RF, RPR, BMP, vit B12, folate, INR, PT, liver enzymes, Lyme	Electrolytes, glucose, WBC, calcium, Mg, lactate, pH, urea, creatinine, CPK, CRP, TSH, MRI c spine: minor disk bulging in mid cervical level, normal cervical cord

more central than peripheral nervous system origin of the symptom. If present, the weakness is usually mild, transient and easily can be missed if more gross or quick neurological examination is carried out, especially because most of the times the patients look “generally well”. The most common features are fatigability of the neck and proximal hip flexor muscles, ptosis and swallowing deficit. The swallowing deficit may persist for months though rarely were swallowing studies performed.

Atrophic syndrome

Patients complain of skin atrophy, subcutaneous tissue loss, lumping of the subcutaneous tissue, hair loss and nail impairment.

Loss of muscle mass, at times significant, usually occurs in the first year of the syndrome, though it can occur later too.

Autoimmune syndromes

Seventy-five percent of the patients have some autoimmune marker or clinical symptom suggestive of immune activation at some time during the course of their disease. However, those markers and symptoms are usually non-specific and many times only transiently present. It is important to note that, at times, the markers can reappear shortly during subsequent bouts of the disease. In these case-series, the only persistent and visible damage over the nervous system had been the presence of small fiber polyneuropathy with skin

Table 2b:

Patient #	9	10	11	12	13	14	15	16
Positive test results	Skin biopsy: small fiber polyneuropathy	ECG depression with rate 120, H pylori and Blastocystis homini positive - 1.5 years after the injection. anti-cardiolipin Ab (one pregnancy before Botox, and frozen embryo. After Botox: Miscariage at first trimester when the positive anti-cardiolipin Ab were discovered. Thyroid initially normal later low TSH-0.87 U/ml (1.8-3), T3-82 ng/dL (100-180) and T4-4.7 ng/dL (6-12)	ECG: sinus arrhythmia ST suppression, Partial block V3, 02 saturation 89-90% (pre-Botox ECG normal)	Holter ECG extrasystoli with sinus tachycardia of 90.	C3 complement79 (90-180)	Botulinum blocking Ab acetylcholine esterase 50.4 (36.7-49.2) Whole body thermography: multiple foci of inflammation ECG: sinus tachycardia	Cardiac monitor: sinus tachycardia episodes up to 140 later normal ECG, ANA 1:160 (<1:40) 2 mo after injection, 3 mo later 1:320 homogeneous, 1 month later normal, low iron sat (supplemented) HSV1 IgG 1.13 (>1.10), IgM 1.14 (<1.10), LDL 134 (<130), cholesterol 213 (200), CRP 1.3 (<1), D3 24 (>30) corrected with supplements, WBC 11 (3.8-10.8), Lyme 23.7 (24-44), platelets 448 (130-400), Na 134 (135-146) only once, US bladder: slightly elevated residual urine 72 ml, polysomnography: no dissaturation, one central apnea episode and 13 arousals per h.	ALT 61 (7-55), PET scan increase intake of radioactive glucose in thymus which was not enlarged, diaphragmatic weakness as per pulmology possible phrenic nerve dysfunction, in hospital record suggests vagus dysfunction, ECG: sinus tachycardia 107 and numerous examinations with pulse recorded between 103 and 114, sweat test abnormal
Negative test results	Electrolytes, glucose, WBC, vit B12, MMA, SPEP, IEF, Hb A1C, vit B6, Antibodies: ANA, AchR, paraneoplastic glandin, tissue trans-glucatminase, Ca P/Q, Purkinje, amphiphysine, CRMP, SSA, SSB, Smith, Scl70, RNP, MRI brain and spine, EMG	Electrolytes, calcium, glucose, creatinine, WBC, bilirubine, CRP, CPK, troponins, BNP, drug screen, urine analysis, HIV 1 and 2, RPR, Hepatitis A, B, C, prolactin, hemoglobin, liver enzymes, ESR, antibodies: ANA, dsDNA, SSA, SSB, Scl70, anti-Smith, anti-centomere, CXR, X-ray abdomen, CT head	Creatinine, electrolytes, glucose, WBC, ECHO	WBC, Hb, glucose, creatinine, potassium, electrolytes, vit D, vit B12, CPK, Calcium, Homocysteine, liver enzymes, troponins, TSH, T3, T4 cardiac enzymes, Lyme test, CT head with contrast	Electrolytes, creatinine, glucose, WBC, CRP, ANA, C4 and total complement, ESR, WBC, ANA	Electrolytes, glucose, WBC, Raji cells, C1Q complement: C3, C4, antibodies: Lyme", anti-ds DNA, CXR, MRI head,	Electrolytes, glucose, protein, Hb A1C, iron, bilirubin, 02 Sat, liver enzymes, HIV, folic acid, protein, RPR, RF, TSH, T3 uptake, free T4, hepatitis A,B,C, ESR, D-dimer, HSV2, complement C3, C4, CH50, vit B12, vit D3, triglycerides, PTH antibodies: thyroglobuline chlamydia, ds DNA, SSA, SSB, anti-Smith, SCL70, Jo-1, peroxidase, centromere, histone, chromatin, Ach binding, MUSK; catecholamines urine, allergy test, MRI brain with contrast, PFT's x3, allergy tests, ultrasound abdomen, CXR, hearing test, Ishihara color test, EEG, cardiac stress test, US abdo, ENT: unknown etiology of sinus swelling	WBC, electrolytes, glucose, D-dimer, calcium, copper, troponin, bilirubin, creatinine, GGT, ALT, AP, TSH eosinophils, antibodies: AchR ganglionic, binding, amphiphisin, GAD 65, neuronal K channels, P/Q calcium, PCA-1,2,3, striatal muscle, LP normal with no oligoclonal bands, CXR, MRI L spine, CT head, video swallowing, EEG 48 h, PFT without methacholine challenge, stress ECHO, EMG/ NCS, tilt table test. MRI c spine after the first injection: minimal degenerative changes.

Table 3a:

	Past Medical History	Family History	General and Neurological Examination
Case 1	Chronic obstructive pulmonary disease for 15 years: occasional inhaler use, rare premature ventricular contractions with normal angiography, allergies to dogs and melons, occasional headaches, smoker	Son: asthma Sister, father and grand mother: cancer	Dehydration: skin fold: 2 seconds; word recall 1/3 on 1 st attempt, 3/3 on second, verbal paraphasias, able to draw clock with significant contemplation time, names 13 animals in 60 seconds, though 12 is the norm, people of her age and education usually generate 20, difficulty naming rare objects, hoarse voice, right eye vision: 20/30, left eye vision: 20/25, bilateral spasm and pain on palpation of trapezius, levator scapulae, scalene; reflexes 3+ with questionable Babinski on the left and tonic big toe extension on the right.
Case 2	Asymptomatic cervical disk, benign neuromas of skull, allergy to dog fur	Father and grand father: cancer	Pin thin, significant muscle wasting proximal>distal, during conversation slow retrieving of some words, dry eye requiring frequent artificial tears, 10 degree lateral head tilt to the right, 4+/5 proximal leg weakness with some fatigability
Case 3	Cushing like syndrome after significant stress in life with normal magnetic resonance imaging head, the condition resolved 2-3 years before the toxin therapy	Cancer in father and mother	difficulty in retrieving words but able to execute the mental tasks correctly
Case 4	Postural orthostatic tachycardia syndrome with dizziness when standing up and some fatigue, able to work 30 or more hours before the toxin injection, melanoma left arm: cured surgically, allergies: seasonal, nuts, sea food and mushrooms	Brother: ulcerative colitis, father: cancer	Pulse increases from 87 to 95 after standing up, hair and nails very dry, some skin thinning and atrophy, minimal tiredness of the proximal legs, significant lack of endurance: the patient became profoundly exhausted after 10 minutes examination
Case 5	None	Grand mother with cancer	Normal
Case 6	Anorexia and bulimia in college, at present some bulimia binges but fully functional, alcoholism in college (sober for one year before the toxin therapy)	Multiple family members with cancer	When speaking some trouble finding words, pupils sluggish and not fully contracting to strong light
Case 7	Post-traumatic splenectomy decades before the toxin therapy, 3 miscarriages in the 1 st trimester with 6 living children birth after, breast implants	Mother with autoimmune disease, father with cancer	In wheelchair, cannot sit more than 10-15 minutes due to fatigue and pain. Visible pain and discomfort with any movement. 4+ reflexes, with bilateral positive Babinski, one clonic bit on the right, tingling in the legs on Lassegue test, L'Hermitte sign positive on neck flexion, can sit only if the back of the head is supported, needs two people's support to transfer to bed.
Case 8	Endometriosis proven with laparoscopy, glucose intolerance, 90% hearing loss on the right from birth, esophageal dysmotility, neck pain with some headaches for which the patient was injected with the toxin	Grand mother with leukemia, father with cancer	Pupils reactive and unequal: 3 mm on the left, 5 mm on the right, difficulty elevating the left eyebrow, hyperreflexia in all four limbs with normal jaw jerk, presence of cross abductors and foot clonus bilaterally; hearing decreased on the right(old)

biopsy performed when the patient had significant peripheral nervous system symptoms. Unfortunately, this was done only in 13.3% of the patients who had clinical symptoms of peripheral nervous system involvement. Flu-like symptoms are rare.

Other prominent features

Increased frequency of infections: One-third of the patients have increased rate of infections. The most common are upper respiratory tract infections, pneumonia, urinary tract infections and sinusitis. Activation of dormant diseases such as herpes may occur.

Sensitivity to food and medications: This peculiar symptom is present in 62.5% of the patients. The sensitivity is especially pronounced to serotonin uptake inhibitors, coffee, sugar and alcohol which almost uniformly worsen the symptoms of the patients. Steroids either worsen the symptoms or are ineffective. Patients have adverse reactions to most of the CNS modulating drugs with the exception of benzodiazepines (alprazolam, lorazepam, clonazepam) in a low dose, which helped some of the psychiatric symptoms in some patients. Here is the list of the drugs which the reported patients were not able to tolerate: paroxetine, fluoxetine, sertraline, pregabalin, escitalopram, duloxetine, desvenlafaxine, trihexyphenidyl, lamotrigine and quetiapine. Usually, with time and attempted treatments, the patients become afraid of trying any new drugs or therapies.

Crises and waxing and waning of the symptoms: This is a uniform feature throughout the population. The patients have bad and

good days with fluctuation of their symptoms. Different symptoms can be more pronounced at different times. In addition, there are sudden crises where some or all symptoms worsen. We identified common triggers such as medications, food, pushing through fatigue and stress. Most patients become very fragile and afraid of activity, food, and medications since the reaction is often unpredictable and can be severe. Crises continue to occur many years after the injection, with new symptoms developing over time, though the majority of the new symptoms develop during the first year.

Symptoms onset: The symptoms onset ranges from minutes to weeks with most common frame of time onset 24-72 hours.

Years of toxin treatment and reaction to prior injections: Our data demonstrate that a generalized spread of the toxin can occur even after years of uneventful toxin therapy. Patient #7 had 30 successful treatments over a decade before the adverse events occurred. Prior uneventful injections cannot be a predictor for toxin safety, because in our experience, generalized spread can occur at any time and with any injection. Forty-four percent of the patients develop a reaction after their first time injection. From those who had more than one injection, 50% had an adverse event to prior injections which remained unrecognized and lead to devastating effects with next injections. Our data suggests that with each subsequent toxin reaction the time of symptoms onset may shorten, as the patient's immune system sensitizes further to the toxin. Some of the injections were done together with Lidocaine which increases the injected volume and, with this, the possibility of generalized spread. Some patients

Table 3b:

	Past Medical History	Family History	General and Neurological Examination
Case 9	Hypothyroidism: thyroid hormones before the toxin therapy: thyroid stimulating hormone: normal, T3 1.87 ng/dl (2-4.90), T4: 0.71 ng/dl (0.76-1.6), vagal syncope since teenage, 24 000 premature ventricular contractions on 24 hours heart holter not responding to ablation but treated successfully with beta blockers, mild essential tremor	Father with cancer	Word registration 2/3 on first attempt, and 3/3 on second, 29 animals in the first 30 seconds, no animals generated despite significant mental strain in the next 20 seconds and a few more animals named in the last 10 seconds, 5-/5 tiring weakness of neck flexion and in the proximal legs, mild right>left and postural>action hands tremor, draws spirals with mild bilateral tremor (due to presence of old and mild essential tremor)
Case 10	one successful in vitro fertilization and natural birth before toxin therapy, one miscarriage after toxin therapy when anti-cardiolipin antibodies were found positive. Initial 90% recovery from the toxin syndrome but then relapsed after the second in vitro fertilization, mild asthma at age 8	Father: cancer	2/3 words recall at 5 minutes, literal paraphasias, visual-spatial abnormalities on clock drawing, able to draw it right only on 5 th attempt, names less frequent objects with significant delay, reflexes 2+ in the upper and 1+ in the lower limbs.
Case 11	Controlled hypothyroidism, left elbow reconstruction after trauma with normal muscle strength, right breast lumpectomy	Cancer in the family	Left eye afferent like papillary reaction with normal vision, hearing decreased on the right to fingers rub, minimal facial weakness: cannot hold air in mouth with cheek pressure, 4/5 neck and hip flexion weakness, 3+ reflexes, decreased speed of foot tapping bilaterally
Case 12	Cancer of uterine cervix 8 years before the toxin therapy: surgically healed with conization, at age 5 left leg osteoid osteoma: cured surgically	Non-relevant	Normal
Case 13	3 months post-partum depression, generalized anxiety and compulsive cleaning, one week before the toxin therapy had a swollen lymph node in the neck which cleared spontaneously	Father: cancer	Pulse 90, normal neurological examination
Case 14	None	Father and sister: major depression, 3 family members with cancer	Slight fatigability of neck flexion, minimal dysmetria on finger-to-nose test, slight reduction of vibration sense in legs
Case 15	None	Father: depression Grand mother: cancer	Normal
Case 16	At age 12 and 18 months episodes of neck and head fixation with 2 days duration, hands fixation in claw position for seconds or minutes (suggesting elements of generalized dystonia), social anxiety: treated, Helicobacter pilory ulcer: treated and cured, Hepatitis B (active carrier state as per gastro-enterology consult: positive e and surface antigene, vial load 116 UI/ml. Patient asymptomatic before the toxin therapy. The hepatitis virus discovered after the toxin injection. Cervical dystonia after one dose of metoclopramide. Cholecystectomy after the toxin therapy to relieve toxin related symptoms with only minor relief from the procedure. Smoker. Magnetic resonance imaging head before toxin therapy: non-specific T2 lesion in right external capsule most likely due to peri-natal trauma, which remained unchanged after the toxin therapy (on second imaging).	Essential tremor and epilepsy in family, Father and sister with hepatitis	Severe rash: red pimples blanching to some extent, on the back and upper chest (persistent for months) frequent throat clearing and coughing during the examination, some visual-spatial distortion on clock drawing, named a pretzel a snake, 3+ reflexes and tandem difficulties.

were injected more frequently than the recommended 3-month inter-injection interval, which may have created a cumulative effect and an unnecessary stimulation of the patient's immune system. Some patients were injected as "models" during BoNT/A conferences with no prior establishment of doctor to patient report and without documentation for the event. Others were given BoNT/A because it was offered for free to the staff at injector's clinics during drug representatives visits.

Functionality, disability, course of disease and prognosis: Before the BoNT/A therapy, all patients were 100% functional and highly active individuals, apart from patient #4 who had mild pre-morbid impairment due to postural orthostatic tachycardia syndrome. In all patients, disability occurred after BoNT/A therapy with duration from months to years (at the present moment of observation it is 8 years or more). The disability is often significant: patient #7 was wheelchair bound for 1.5 years. The maximum disability duration is unknown at present. Many patients loose their jobs and relationships. Apart from the 3 patients who recovered completely (19%), all others continued to have symptoms for years after the injection. Though, in our experience, mild and slow yearly improvement takes place as

the toxin is cleared from the system, the majority of the patients do not reach his/her pre-morbid state of health at 5-6 years time mark after the last injection. The only BoNT/A condition that seems to persist over the years, with no or very little improvement, is the small fiber polyneuropathy, since it is an auto-immune disease. In our experience, if the patients have no yearly (even slow) improvement of their symptoms in the first 3-5 years, the prognosis for recovery is less favorable. Since the syndrome is not yet well recognized, it is not surprising that when a physician encounters normal neurological examination, normal or minimally positive laboratory results and normal imaging studies in the face of multiple patient's complains, the symptoms are assumed to be due to anxiety or somatization. This, often, leads to referral to psychiatry or to the start of psychiatric drugs which then usually leads to worsening of the symptoms. The patients often go from doctor to doctor seeking help. Thousands of dollars worth of investigations are conducted, with little or no help or relief from their symptoms and/or establishment of the true diagnosis. The majority of the cases reported here were admitted to a hospital or ER at least once during the course of their disease. Many times the affected patients end up alone, depressed, broken and terrified, prisoners to their homes with restricted social contacts and very

few people who believe them. The impact of INCS on the patients' quality of life is enormous. From the three patients who recovered, patient #3 and #13 came to see me within 1-2 months from the onset of the adverse event. I advised them not to inject the toxin again, not to vaccinate, I put them on activated charcoal, echinacea and diet therapy and supported them emotionally. Patient number #7 did daily enemas with activated charcoal and coffee, in slow dose increments reaching a maximum of two table spoons of charcoal in 3 cups of coffee. The treatment persisted for 1.5 years and the patient did not stop the therapy when temporary worsening of her symptoms occurred after each enema. However, there were patients who did not respond to charcoal/echinacea therapy suggesting that there is likely a subpopulation in this patients' group. For example, charcoal may worsen patient's symptoms if severe constipation is present, since no elimination can occur and toxin re-absorption from the gastrointestinal tract and secondary hematogenic spread may occur.

Therapy: All therapies are in a research stage of development. Here is a list of modalities which helped some of the patients reported here: Diet: gluten free, organic, vegan; chlorella; celery juicing; abstinence from sugar, alcohol and caffeine; avoidance of CNS modulating drugs, with the exception of benzodiazepines, which can be used in small doses for anxiety and panic attacks therapy; bananas (likely because of the high potassium content); benadryl (the symptoms in many patients suggest high histamine state); aspirin (for the small fiber polyneuropathy); valerian root (for the internal shaking); magnesium gluconate (for the muscle tightness and spasms); hyperbaric oxygenation (each treatment may induce several hours of healing crisis with temporary worsening before improvement occurs); activated charcoal; and echinacea.

Discussion, Hypotheses and Algorithm

Discussion and hypotheses of the phenomenology of INCS

Central nervous system: Research has established that botulinum can block many mediators in CNS [6- 9], though its preference is upon the cholinergic pathways. On central level, the peripheral acetylcholine (Ach) specificity is lost suggesting that the target of botulinum is common to many, and may be all central neurons of mammals as Bigalke et al. concluded [10]. Ach plays a role in neural plasticity, word finding, concentration, attention, learning, abstract thinking, creativity, brain speed processing, noise/signal ratio improvement and intra-cortical transfer of information. It is not a surprise, that the patients presented here frequently display cognitive deficiency. Luvisetto et al. [11] injected the brain ventricles of live mice with botulinum and demonstrated that the animals produced dementia-like features. Some of the important principles of CNS functions are mediators' balance and neuronal nets interactions. When one mediator pathway is impaired the brain adapts by altering the remaining mediators balance. Most of the patients presented here exhibit combined features of depletion of some mediators and increment of others, such as: *Ach depletion* with impaired concentration, memory, focus, learning, thinking clearly and coherently, recall and ability to process information; *dopamine depletion* with affection of memory, attention and concentration and problem-solving, slowness of movement and processing, low libido, nausea, vomiting, lack of appetite and depression; *hyper-adrenergic*

state with rapid heartbeat, insomnia, fatigue, headaches, anxiety, panic attacks, sweating, frequent or impaired onset of urination, thirst, weight loss and constipation; and *histaminergic state* with skin rash, runny nose, diarrhea, nausea, vomiting, cough, broncho-constriction and vasodilation. In our experience, it is impossible to predict which mediator systems will be affected and to what extent in a particular patient, but the clinical symptoms may guide us to which are the main mediator systems involved in each case. Russell et al. [12] demonstrate that rats with 9 months diet- induced hypocholinergic state (via a choline intake restriction) do not recover after cessation of the diet restriction and continue to display memory impairment and hyperalgesia despite the fact that the biochemical parameters recovered. The authors suggested the presence of adaptive CNS changes as an etiology of the condition. This data sheds some light on why cognitive impairment and other symptoms may persist for so long in many of the cases presented here.

Peripheral nervous system: The symptoms of peripheral nervous system involvement suggest autoimmune process affecting the small fibers confirmed with skin biopsy in two patients. Increased pain perception due to prolonged hypocholinergic state [12] may be in play as well.

Autonomic nervous system: It is well established that botulinum affects the motor and the autonomic fibers to equal extent. The toxin can spread via blood and retrograde axonal transport through both motor and sensory nerves [13-15]. Vagus nerve is the major autonomic nerve in the body, therefore blocking it may create hyper-sympathetic tone in the affected organs with symptoms of tachycardia, nausea, diarrhea, chest pain, weight loss, stomach pain and spasms, poor digestion, and difficult urine control which were frequently experienced by the presented cases. In addition, the toxin acts on the potassium channels [10] on which the heart is rich. Cardiac deaths after botulinum therapy have been reported in the post-marketing period (Botox® insert). Blocking the vagus nerve can also, affect the related central pathways such as brainstem, hypothalamus and limbic system. The normal breathing studies, performed during time of significant breathing problems in some of the presented patients, suggested a central breathing pacemaker impairment and central hypoventilation problem similar but not equal to Ondine's curse syndrome. Only one patient had a polysomnography demonstrating a central apnea episode without desaturation. The vital capacity, the forced vital capacity and the negative inspiratory pressure are rarely measured, while they may be a helpful tool when significant shortness of breath is present. Stone et al. demonstrated that locally applied toxin leads to long lasting arteriolar dilatation which may explain the common complaint of nose, head, ears or sinuses congestion, without presence of infection. It is well established that Ach plays a role in the autoregulation of the cerebral blood flow.

Psychiatric: Deficiency in acetylcholinergic pathways has been related to anxiety, bipolar behavior and delirium. We speculate that retrograde toxin spread to the limbic system or shift in the global brain mediator balance are possible causes of psychiatric symptoms experienced in these patients' series. Hyper-histaminic state has been considered in schizophrenia patients and there is a report of a patient who's mental illness responded well to treatment with the histamine receptor antagonist famotidine [18].

Hypothalamic syndrome: Theoretically, the toxin can reach hypothalamus via retrograde vagus or cranial nerves' transport, or via blood spread. Though the blood brain barrier protects the brain, it is thinned at the hypothalamus in order for monitoring of body homeostasis to occur. Boroff et al. [16] demonstrated that radio-labeled botulinum crosses the blood brain barrier. Thirunavukkarasu et al. [17] showed that, once injected, the toxin alters the expression of 227 genes including those responsible for transmigration of monocytes and T-cells across the blood brain barrier. In respect to the alteration of the mediator balance, the highest concentration of histamine in the brain is in the hypothalamus. Histamine is involved in the regulation of cognition, circadian rhythms (including sleep) and neuroendocrine balance [18]. Increased concentration of histamine is reported in serum and spinal fluid of Alzheimer dementia patients [18].

Fatigue, lack of endurance, muscle twitches and muscle stiffness: The pathophysiology of fatigue has always been a challenging and complex question. The symptom has been reported to persist from 6 to 13.5 years after botulinum food poisoning [19,20]. It is likely mediated by cholinergic receptors in the ventromedial hypothalamus [21]. Alteration of brain circulation may be involved too [22]. Studies on chronic fatigue syndrome suggest involvement of hyper-serotonergic mechanisms, hypoactivity of the hypothalamus-pituitary-adrenal axes [23] and the presence of cytokines inflammatory mediators [24]. Profound fatigue has been reported in chronic and advanced cases of multiple sclerosis, human immunodeficiency syndrome and neurolyme, suggesting central etiology of the syndrome. Mazzoni et al. [25] studied Parkinson's disease patients and demonstrated that patients with Parkinson's move slower than controls when moving naturally but, if encouraged, they can execute movements at a normal speed. We contemplate if the INCS may have a "central motor drive deficiency", as well. Muscle spasms and tightness are frequently reported in our patient population and in post-foodborne-botulism [20]. This is most likely due to affection of the Renshaw neurons in the spinal cord, which have been shown to be targeted by the toxin [26].

Muscle weakness: Is related to the direct actions of the toxin on the neuromuscular junction. Since the junctions effects persist for 3-6 months, a true muscle weakness has a relatively short duration in these patients with the exception of diaphragmatic and swallowing deficits when the duration is unknown. It is unclear, at present, if swallowing and breathing problems are due to true weakness (some patients had abnormalities in pulmonary function test) or to discoordination of the peripheral and central neuronal pathways, or to both.

Atrophic syndrome: Weight loss associated with BoNT/A has been demonstrated in animal studies [27]. When trophic syndromes are present we need to consider the enormous effect of non-neuronal Ach on the body systems. Research has demonstrated that extraneuronal Ach is crucial for the survival and the function of the cells: blockage of Ach receptors on non-innervated cells results in cell dysfunction and death; adding Ach antagonists to cell cultures induces almost immediate cell shape change with shrinkage and cell separation; Ach regulates skin regeneration with keratocytes containing 100 times higher Ach levels than airway cells; Ach is

crucial for almost any cell to cell contact [28]. Ach nicotinic receptors are involved in appetite and food seeking behavior.

Autoimmune syndromes: The presence of BoNT/A blocking antibodies in one of the cases presented here did not prohibit the presence of good wrinkle abolishing effect suggesting that, autoimmune mechanisms other than just the production of toxin blocking antibodies are at play. Oshima et al. [29,30] have shown that human lymphocytes are activated by the toxin irrespective if the patients' blood had or did not have blocking antibodies. This suggests that assays of toxin blocking antibodies may not be an efficient marker for the presence or the absence of autoimmune reaction to the toxin. Singh et al. [31] showed that the toxin-associated proteins, injected in the body together with BoNT/A and consisting of 2/3 of the complex, are 5 times more immunogenic than the toxin itself. Reports of rash or eosinophils elevation, as in the presented cases, suggest toxin connection. The published reports of botulinum induced autoimmune encephalitis [32] and Guillain-Barre syndrome [33-35] indicate that BoNT/A is capable of inducing an auto-immune reaction in humans.

Discussion of other features of INCS and hypotheses

Increased frequency of infections: Since the acetylcholinergic pathways are involved in regulation of immunity, it is not a surprise that this symptom is common in the patients' population presented here. Pneumonia was documented in animals sacrificed to the toxin [5].

Sensitivity to food and medications: Serotonin given to animals after BoNT/A injection increased the toxin's activity up to 1000 times [36]. This may explain why, almost uniformly, the cases presented here had worsening with serotonin-reuptake inhibitors therapy. Large amounts of sugar intake has been related to suppression of Ach release. Only one patient was vaccinated (flu shot) after the toxin exposure and had worsening of her symptoms, likely due to stimulation of an already upset immune system. Note that while steroid therapy is likely not indicated for treatment of INCS, steroids may be useful in botulinum induced autoimmune encephalitis [32].

Crises and waxing and waning of the symptoms: The latest research of Bomba-Warczac et al. [37] demonstrated unequivocally that the toxin travels via retrograde axonal transport and transfers between neurons while remaining enzymatically active. This suggests that the occurrence of new symptoms or worsening of old symptoms may co-occur with the time of BoNT/A transfer and engagement of new neuronal nets. Polley et al. [38] demonstrated in animals, that 5 minutes after intravenous injection of BoNT/A there was cessation or significant decrement of EEG recorded cortical activity associated with loss of consciousness. The animals recovered in 30-60 minutes to have another 3-4 similar episodes of EEG depression in the next 24 hours. It is difficult to explain the repetitive appearance of the cortical depression, after initial recovery, unless we accept the theory that the time of appearance of cortical depression is a marker of the time of intoxication of new neuronal nets by botulinum. Herrero et al. [5] gave lethal dose BoNT/A to monkey, and though most of the monkeys died shortly after the toxin exposure, some recovered and died 40 days later, again suggesting an existence of transneuronal passage of the toxin, or later absorption of the toxin from peripheral

peri-neural depots. Pharmacokinetic studies in animals suggest that the clearance of the toxin is a significant issue. Each biological system has parameters of detection and operation below or above which the data may be regarded as noise. Due to the minimal substrate cleavage and nano-dose concentrations, the toxin seems to stay under the radar of the elimination mechanisms of the body such as liver and other metabolic cells [39]. It seems that BoNT/A just squats on the inner layer of the pre-synaptic membrane of the neuron, locked into its substrate: the synaptosomal associated protein – 25 (SNAP-25) [40], and jamming the transmission until the neuronal membrane is recycled, which occurs at a very slow rate. Once injected in the muscle, only a small amount of the total toxin load is absorbed in the neuronal terminals [39] making the rest of perineuronal-pool toxin available for further absorption over time. Re-absorption from the micro-compartments could be responsible for the patients' crises and can explain why some patients report worsening after having massage or after rigorous detoxification procedures. Research has shown that once in the neurons, BoNT/A can persist more than one year [39], suggesting that the elimination of the toxin may take years, as it is our experience with the cases presented here. Keller et al. [41] showed that in spinal neuronal cultures the enzymatic activity of BoNT/A decreased only with 50% in 78 days. This is significant because the toxin needs to preserve only 6% of its enzymatic activity in order to block a synaptic transmission [40]. Since blood does not alter the toxin at all, it has been regarded by some researchers as a toxin holding compartment [39]. Unless methods of toxin detection become very sensitive, readily available and frequently used, the true duration of toxin presence in humans cannot be established. A mouse assay was reported positive in the blood of a patient, 2 years after onset of her symptoms (author's correspondence). None of the cases cited here had mouse bioassays or other direct assays for detection of the toxin.

Symptoms onset: It is possible that the symptoms occurring within the first minutes to a few hours after the injection are due to hematogenic spread and autonomic nerve endings blockage, while those occurring later are due to retrograde axonal transport, which is slower. It is unclear, at present, of how many hours are needed for the toxin to generate axonal spread related symptoms. Moreno-Lopez et al. showed that reduction of field potential of cranial nerve IV occurred 3 hours after toxin injection in the lateral rectus muscle of cats and that the effects were dose dependent [42].

Dose and toxin brand: The general belief in the medical community has been that generalized BoNT/A spread may occur only above certain toxin doses. However, we need to keep in mind that we do not have any studies on safety of hematogenic or retrograde axonal transport BoNT/toxicity in humans. Our data shows that spread can occur with a dose as low as 10 units (0.08 units/kg) signifying that likely no dose is safe when a generalized spread occurs. Matak et al. [43] demonstrated that 3.5U/kg of BoNT/A, injected in the whisker pad of rats, reached CNS in 72 hours and remained enzymatically active. It is important to note that BoNT/A organ toxicity dose, which is a dose that affects the function of the organs, is much lower than the lethal dose (LD50) (the one that kills 50% of the injected animals). Therefore LD50 cannot be used as a safety measure for organ toxicity and disease occurrence but can be used only to assess risk of death. Young et al. [44] reported generalized spread symptoms in patient

case series after a toxin dose as low as 6.8 units. Switching a patient from one brand drug to another, as it occurred in patient #16, does not increase safety since most drug brands contain BoNT/A. As well, botulinum toxin types A and B carry cross reactivity, due to their similar molecular structure.

Examination and past medical history: Almost all patients, presented here, had a weakened immune background with presence of past personal or family history of cancer, immune, autonomic or allergic diseases. It is possible, that such patients' population may be more prone to toxin reaction, but this is to be established in the future in larger population. A significant finding is that only two patients had pupillary reaction impairment (minimal one). This fact together with the usually normal muscle power examination indicates that the INCS differs in symptomatology from that of foodborne botulism. Less frequent pupillary involvement has been demonstrated in animals injected with the toxin [5].

Diagnosis and clinical markers: At present, the diagnosis is a real challenge since there is not a high alertness in the medical society about this significant problem, there are no guidelines for diagnosing iatrogenic botulinum side effects and there are no easily available methods to prove the toxin-symptoms connection. In foodborne botulism, the diagnosis is usually established by testing for clostridium bacteria and toxin in food and/or stool samples. The gold standard for testing is lethal mouse bioassay which detects the biologically active toxin in 1-4 days. Its sensitivity of detection is 20-30 pg/ml [45]. ELISA (Enzyme Linked Immunosorbent Assay) uses polyclonal antibodies. The method is faster (8 hours) and its sensitivity is 0.2 ng/ml [45]. However, it usually uses amplification methods, which require culturing live bacteria (not present in iatrogenic cases) to increase and detect toxin production. The same applies to the live time PCR method which detects 5 pg/ml and uses monoclonal antibodies [45,46]. Since it is very likely that the serum toxin levels in INCS are much lower than that in foodborne botulism, where bacteria are producing toxin continuously, it is possible, that we may need more sensitive methods such as mass spectroscopy where not only the toxin, but as well parts of the toxin can be detected. It seems that detection of the entire toxin or the entire light chain in serum is preferable. There is a laboratory in Poland (National Veterinary Research Institute, Pulawy) which is conducting research on mass spectroscopy methods for detecting the toxin and its particles in human serum. The same laboratory offers lethal mouse bioassay to the public. In the USA, the mouse assay is not available to the public and requires approval of the state or the federal health authorities when the diagnosis of botulism is suspected. As the Center for Disease Control reported, in the USA, due to the nature of the toxin, even foodborne botulism has been substantially under-diagnosed or misdiagnosed with whole disease clusters and deaths related to the toxin not being detected [47].

Possible, future therapies

Activated charcoal: Charcoal has been studied to have no toxicity, even in high doses. In research, charcoal, when given through the mouth, enhances the elimination of multiple intravenously administered drugs [48]. When BoNT/A was injected intraperitoneally in increasing concentrations in two groups of mice, none of the mice in the treated with activated charcoal group developed botulism or died, while all the mice in the untreated group developed botulism and died in

progressive numbers with each increment of the toxin potency. The highest potency group, which was not exposed to charcoal, had 100% mortality [49]. Our observation with charcoal therapy is that almost all patients who took it had initial good effects and some alleviation of their symptoms. After that the group divided in two considering the effectiveness of the therapy: the first group kept getting better and improving, though at times mild crises may have occurred; the second group had more pronounced crises of temporary worsening and the patients stopped the therapy. However, there were two patients who pushed through the therapy despite the temporary worsening and had beneficial results at the end. One of them, as we mentioned above, achieved full recovery, despite that she had the worst clinical picture from all the cases, which is an encouraging finding. This unexpected split response prompted us to look deeper into the mechanism of the effect of activated charcoal upon the elimination of BoNT/A from the body. It looked like the crashes occurred more often in patients who took a higher dose of charcoal on a daily basis or if they had constipation. The pharmacokinetics in animals clearly suggest that the liver is about the only organ via which the toxin can be eliminated, since blood leaves the toxin unchanged and the BoNT/A molecule is too big to pass the 50 kDa size of molecule elimination-kidney threshold [39]. This has been supported by autopsies of animals who died from botulism where the highest accumulation of botulinum has been found in the liver and then in the spleen [50]. We speculated that if charcoal pools the toxin out of the tissue micro-compartment and blood into the bowel lumen, via liver elimination while bowel motility is slower than normal, a partial re-absorption of the toxin may occur. This can induce secondary BoNT/A re-introduction in the blood stream, which may present as worsening of the clinical picture. As well, it is possible that there is a liver threshold of accumulation of toxin before elimination. If the threshold is surpassed by pooling more toxin from the depots to the blood than the liver can handle at a given moment of time, the unfiltered amount of toxin may re-lodge into the nerve terminals. We found that 1 capsule of 280mg charcoal through the mouth taken every other day or twice a week was generally safe (no crises, or very mild ones) unless the patient had severe constipation. In our opinion, charcoal should not be used until the constipation is resolved. The fact that the two cases whose symptoms resolved 100% within the first 4 months of the initial insult after taking charcoal early into the disease course is encouraging too.

Research has shown that **Echinacea** plant has some similarity to botulinum toxin, and may compete with it [51]. Some of the reported here patients improved while taking 400mg of Echinacea daily.

Yuliang et al. [52] suggested that the Chinese herb **toosendanin** can inhibit the translocation of the light chain of the toxin. Zhou et al. [53] stated that toosendanin has been an effective cure of experimental botulism. The substance made synaptosomes of a rat brain completely resistant to BoNT/A. Even after toxin entrance into the cells, toosendanin was still able to partially antagonize BoNT/A substrate cleavage. The researchers concluded that the effect is likely due to blocking the toxin approach to the substrate. Mice, which developed botulism, cured completely in seven days after being treated with toosendanin. Since toosendanin has a low toxicity profile, further research and clinical trials are highly desirable.

3-4 diamino pyridine (3-4 DAP): Is a reversible inhibitor of

voltage gated potassium channels and cholinergic agonist. Zakhari et al. [54] demonstrated that the 3-4 DAP facilitates recovery of post-botulinum intoxication by blocking the potassium channels but there were some toxic effects. A less toxic cousin of 3-4 DAP, 4-aminopyridine (dalfampridine) has been available on the market for treatment of multiple sclerosis but has never been used or studied in BoNT/A affected patients.

Young et al. [44] reported improvement of botulinum induced constipation with **pyridostigmine**.

Benadryl, which is an anti-histamine agent, has been beneficial to some of the cases presented here, likely due to effect of tuning down the discussed above hyper histaminic state.

Boroff et al. [36] showed in animals that BoNT/A activity can be blocked by **reserpine**.

Tryptophan is critical for toxin activity [36], rising the question if a tryptophan restricted diet may be beneficial for some patients.

Antibodies to the toxin: (anti-toxin). Frequently asked question by patients and physicians is if anti-toxin should be given? The present practice guidelines allow the toxin to be given only in the first 72 hours after exposure, since once BoNT/A reaches the nerve terminals the anti-toxin becomes ineffective. However, the possibility of longer lasting local toxin micro-depots presence has not been explored yet.

Suggested algorithm for safer toxin use:

1. Inform yourself, your colleagues and your patients about the existence of the INCS and its consequences.
2. Advise the patients who develop a reaction that it is likely not safe to continue injecting.
3. If possible, restrain from vaccinating during the period of symptoms activity. Be aware that tetanus toxoid carries 35% amino-acid similarity to botulinum [55].
4. Avoid giving serotonin re-uptake inhibitors and other mediator modulating drugs or steroids since worsening may occur.
5. Advise not to push through fatigue, do massages or use rigorous detoxifying methods, since crises may occur.
6. Consider skin biopsy to diagnose small fiber polyneuropathy if persistent pain and tingling are present.
7. Consider that injecting botulinum for cosmetic reasons, most likely, does not weight against the side effect profile of the drug.
8. Report all reactions to the toxin to The Food and Drug Administration or its equivalents in other countries and publish the results in peer review literature to increase awareness.
9. Try to send serum for lethal mouse bioassay when possible at early or later stages of the disease to establish the assay sensitivity in such cases.
10. Know the difference between INCS and botulinum induced autoimmune encephalitis and polyneuropathy since there is a difference in the therapy.

11. We suggest establishment of a separate reporting and monitoring system for botulinum related side effects to increase safety.

Conclusion

We conclude that Impaired Neuronal Communication Syndrome (INCS) is a serious and long lasting complication to BoNT/A therapy. The syndrome has specific features which distinguish it from the foodborne botulism by the rare involvement of the pupillary reaction and the rare presence of true weakness. The syndrome, as well, differs from the autoimmune side effects to BoNT/A described in the literature, such as autoimmune encephalitis, Guillain-Barre syndrome and others, by having, most of the time normal examination, laboratory results and imaging. There is no current wide-spread awareness of the syndrome among the medical society. There are no readily available markers to establish connection to the toxin making the clinical knowledge highly important and often the only tool for correct diagnosis. Further research is urgently needed.

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