MINISTRY OF HEALTH OF UKRAINE
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MINISTRY OF HEALTH OF UKRAINE

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Master's Thesis

UDC:

THE USE OF BOTULINUM TOXINS IN MEDICINE AND MEDICAL COSMETOLOGY

Nursing

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Ternopil - 2021

ABREVIATIONS

BS	Blepharospasm
BTA	Botulinum toxin type A
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration is responsible for protecting the public health by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation.
GAIS	Global Aesthetic Improvement Scale
HFS	Hemifacial Spasm
SNAP-25	Synaptosomal-Associated Protein
SNARE complex	S (soluble) N SF A (attachment) protein RE (receptor) complex

INTRODUCTION

Relevance of the research topic

Therapy with botulinum toxin type A (BTA) is one of the most popular and quite demanded, in US and around the world, method of correction of mimic wrinkles and hyperhidrosis in aesthetic medicine in over the past 20 years. This therapy is widely used to control and correct facial muscle dystonia as well as facial pain and facial asymmetry due to muscle spasm.

Pioneering in the use of botulinum toxin type A to correct wrinkles on the face belongs to Alastair Carruthers and Jean Carruthers and belongs to the period 90s of the last century [Carruthers J., Carruthers A., 1991, 1992; Carruthers A., Carruthers J., 1998; Becker-Wegerich P. et al., 2001]. First publications on cosmetic use BTA appeared in early 1990, after 10 years BTA has been approved by the FDA (Food and Drug Administration) in the United States. BTA therapy quickly began to attract attention of specialists from different countries [Becker-Wegerich P. et al., 2001].

Botulinum toxin type A (BTA) drugs are widely used in many branches of medicine - in ophthalmology, neurology, surgery, aesthetic medicine as an effective and safe muscle relaxant. According to the American Society of Plastic surgeons, in 2012, 4,125,179 BTA injections were performed, which made BTA injections the leader among non-surgical aesthetic procedures [URL:http://www.surgery.org/sites/default/files/2011-top5.pdf].

However, in practice, there are cases of ineffectiveness of the therapy. Some authors analyzing mild side effects or complications after the introduction of BTA, found that there is insufficient therapeutic and cosmetic effect in patients [Cote T.R., 2005]. Among the possible reasons the ineffectiveness of treatment with BTA drugs is considered by

factors associated with drug (violation of transportation and storage conditions, rare cases decrease in the serial activity of the drug), with a doctor (lack of knowledge and skills, and as a result - incorrectly selected target muscles, inadequate dosage of the drug, violations in the injection technique), with the patient (non-compliance with the recommendations after the procedure, individual resistance) [Jankovic J., Schwartz K., 1995; Cote T. R. 2005; Orlova O.R., 2006; Timerbaeva S.L., 2012].

BTA is known to be an immunogenic protein capable of stimulate the production of neutralizing anti-botulinum toxin antibodies, leading to a decrease in the patient's sensitivity to the drug, which contributes to the weakening or complete absence of the clinical effect [Goschel H. et al., 1997; Dressier D., Bigalke H. 2002; Ravichandran E. et al., 2006; Naumann M., Carruthers A., Carruthers J., et al., 2010]. Patients are described, in which antibodies were the reason for the lack of clinical effect on the injection BTA. [Doellgast G.J., Brown J.E., Koufman J.A., 1997; Dressier D., Munchau A., 2002; Dressier D. et al., 2010].

However, not all authors give a significant role to antibodies to BTA in the formation of such resistance [Dressier D., Munchau A., 2002; Timerbaeva S.L., 2012]. Therefore, a scientific search for factors is popular, in addition to the formation of antibodies, or in conjunction with anti telogenesis, have an effect on the severity and duration of the effect of correction of mimic wrinkles and facial muscle movements with the help of BTA. These factors may be the main regulators of the immune answer - cytokines [Ketlinsky SA, Simbirtsev AS, 2008] Search for new diagnostic algorithms for predicting the effectiveness of the application BTA drugs are becoming even more relevant in connection with the Directive of the European Parliament of 22.09.2010 (2010/63 / EU) "On the protection of animals,used for scientific purposes ", which limits the conduct of acute experiments on animal(URL:http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:IT:PDF)

Purpose of the study

The thesis is based on research in the field of application BTA drugs of different scientists all over the world: O. R. Orlova, S. L. Timerbaeva, A. Carruthers, J. Carruthers, D. Dressler, M. Naumann, I. Lawrence, R. Moy, J.C. Lee, Department of Neurology, Keimyung University school of Medicine, Taegu, Korea for the prevention and correction facial movements disorders and of external signs of aging facial skin.

The works of the listed scientists describe the mechanism of action of BTA, indications for the use of BTA in clinical practice are listed, described methods of using specific drugs, cases of clinical observations. Single sources describe cases of complete or partial secondary ineffectiveness of using BTA for the treatment of facial wrinkles, and facial muscle spasms confirmed by biological studies (test for the protection of mice and mouse diaphragmatic test), allowing to reliably determine the presence of neutralizing antibodies.

Study of factors that can influence the severity and the duration of the effect of BTA treatment, would make it possible to predict effective use of BTA drugs in patients with facial movements disorders and mimic wrinkles.

Research aims

Scientific and practical substantiation of the therapeutic and cosmetic effects of botulinum therapy patients with facial muscles disorders and mimic wrinkles.

Objectives

1. Assess the dynamics of objective indicators of clinical status in patients with facial movements disorders and mimic wrinkles under the influence of botulinum toxin type A.
2. Assess the dynamics of subjective indicators of clinical status in patients with movements disorders mimic wrinkles under the influence of botulinum toxin type A.

3. Determine the structure of the reasons for the absence of the expected treatment effect with botulinum toxin type A.
4. To study the dependence of the effect of treatment with drugs of botulinum toxin type A from the levels of antibodies to botulinum toxin type A.

Research methodology and methods

The methodological basis of the thesis research was consistent application of methods of scientific knowledge. The work was done in design of a prospective and retrospective cohort study with using clinical, laboratory, analytical, sociological, statistical research methods.

Role the author in the research

The author substantiates the goal, objectives and design of the study, formulated conclusions. The candidate personally organized and carried out clinical trials, developed a formalized map examination of the patient, a consent form and history card of cosmetologists, independently completed courses of treatment with botulinum toxin type A, formed a primary database and statistically processed it.

Structure of the thesis

The thesis is presented on 72 pages and consists of an introduction, 4 chapters (literature review, description of material and research methods, discussion of the results), conclusions, practical recommendations, bibliography, list illustrative material and applications.
The thesis is illustrated with 7 tables and 7 figures.

Chapter 1. USE OF BOTULOTOXIN TYPE A IN MEDICINE AND MEDICAL COSMETOLOGY (Literature review)

More than twenty years in medicine of different countries the method of botulinum therapy is used. According to the American Society In 2012, plastic surgeons performed 4,125,179 BTA injections, which has made them a leader among non-surgical aesthetic procedures [URL: http://www.surgery.org/sites/default/files/2011-top5.pdfj. This method of therapy has established itself as the method of choice for the correction of mimic wrinkles and facial movements disorders. However, every time health medical professionals are not entirely sure about the expected result of the treatment, especially in its duration. In order to get closer to the answer to this question, we need to familiarize yourself with the materials related to botulinum toxins in various fields of medicine.

1.1 History of Botulism and prevention measures

Botulism is a rare but serious paralytic illness caused by a nerve toxin produced by the bacterium *Clostridium botulinum.* Human botulism is mainly caused by four of the seven (A, B, C, D, E, F, G) recognized types of *Clostridium botulinum* bacteria: types A, B, rarely F and possibly E.

Food-borne botulism probably has accompanied mankind since its beginning. However, we have only few historical sources and documents on food poisoning before the 19th century. Some ancient dietary laws and taboos may reflect some knowledge about the life-threatening consumption of poisoned food. One example of such a dietary taboo is the 10th century edict of Emperor Leo VI of Byzantium in which manufacturing of blood sausages was forbidden. At the end of the 18th century, some well-documented

outbreaks of "sausage poisoning" in Southern Germany, especially in Württemberg, prompted early systematic botulinum toxin research.

Jastinus Kerner (1786-1862), a poet and German physician, first identified the strong effects of botulinum toxin during the Napoleonic Wars, after which he noticed that the number of deaths from food poisoning was increasing among people who ate blood sausages. After a series of experiments on animals and on himself, he hypothesized that the toxin under study was produced under anaerobic conditions, acted on the autonomic and motor nervous system, and was fatal in small doses [Erb et al. R., 1998].

Kerner did not succeed in defining the suspected "biological poison" which he called "sausage poison" or "fatty poison." However, he developed the idea of a possible therapeutic use of the toxin. Eighty years after Kerner's work, in 1895, a botulism outbreak after a funeral dinner with smoked ham in the small Belgian village of Ellezelles led to the discovery of the pathogen Clostridium botulinum by Emile Pierre van Ermengem, Professor of bacteriology at the University of Ghent. The bacterium was so called because of its pathological association with the sausages (Latin word for sausage = "botulus") and not-as it was suggested-because of its shape. Modern botulinum toxin treatment was pioneered by Alan B. Scott and Edward J. Schantz.

The disease is caused by neurotoxic proteins so poisonous that one-millionth of a gram of them can kill a man and one pint would be enough to kill everyone on earth.

Botulism is a serious disease that develops when eating meat products, fish, canned vegetables, infected with the causative agent of botulism. Very often, the cause of poisoning is homemade products (canned, pickled) [Timakov V.D. et al., 1983; MacDonald K. L. et al., 1986; Simpson L. L., 1986; Hatheway C. L. 1995; Sobel J., 2005].

Among the seven serotypes, types A, B and E most often cause botulism in humans, type C toxin usually causes botulism in domestic animals [Sakaguchi G., 1982; MacDonald K. L. et al., 1986; Simpson L. L., 1986; Simpson L. L. et al. 1999;

Skripchenko H.B., 2007; Lobzin Yu.V., Zhdanov K.B., 2011]. Of all cases of foodborne botulism, serotype A is responsible for 60%, type B - for 30%, in 10% of cases, botulism caused by serotype E develops [MacDonald K. L. et al., 1986]. The bacterium C. botulinum and the botulinum toxin produced by it enter the body most often through the gastrointestinal tract.

The Centers for Disease Control and Prevention and the US National Institute of Allergy and Infectious Diseases classify botulinum toxin as an extremely high risk agent potentially used by terrorists [Herrerous J. et al., 1999; Arnon S.S. et al., 2001; Kobayashi R. et al., 2005; Dembek Z.F. et al., 2007].

Several nations produced botulism toxins in the World War II as a potential bacteriological weapon, and they were said to have been test sprayed over a section of Canadian wilderness (reportedly killing all animals within six hours), but they were never used in combat.

Since the main way of eliminating botulinum toxin is to bind it specific antibodies, means of immunoprophylaxis of botulism - vaccines and serum - have been developed.

Currently, a pentavalent botulinum vaccine (toxoid) against serotypes A-E is used to prevent botulism. This toxoid is used to vaccinate groups at higher risk for botulism: researchers working with the toxin, doctors working with botulism patients, and the military. This vaccine is administered parenterally and causes prolonged pain and swelling at the injection site [Byrne MR, Smith L.A., 2000; Dembek Z.F. et al., 2007]. Tetravalent toxoid (A, B, E, F) has also been developed against the main clinically significant serotypes [Torii, Y. et al., 2002] and trivalent equine toxoid of types A, B, E [Hatheway, S.N. et al., 1984].

Passive immunoprophylaxis of botulism is also developing. Drugs carrying specific antibodies against botulinum toxin not only able to prevent the development of botulism, but also to treat. So, the use of anti-botulinum immunoglobulin for intravenous administration significantly reduces the time of artificial ventilation, reduces the length

of stay in the intensive care unit, thereby reducing the overall cost of treating patients [Arnon S.S. et al., 2006]. Attempts are being made to clone human IgM antibodies that neutralize botulinum toxin [Adekar S.P. et al., 2008].

In the 1960s, A.B. Scott et al. (1973, 1988) first investigated the therapeutic use of a toxin drug in monkeys.

1.2. Structure and pharmacodynamics of botulinum toxin

Botulinum toxin is an exotoxin, the most powerful of all biological poisons [Lamanna S., 1959; Gill D. M., 1982]. The lethal dose for humans is only 0.3 mcg. It has a neurotoxic and hemagglutinating effect, while being highly resistant to heat (it remains viable for 10-15 about minutes at 100 C), acidic environment, high concentrations of sodium chloride, freezing, digestive enzymes [Aleksandrov VN, Emelyanov VI, 1990; Herreros J. et al. 1999].

Botulinum toxin is an exotoxin produced by the spore-forming bacterium Clostridium botulinum. Currently, 7 serotypes of this toxin are known: A, B, C (C1, C2), D, E, F, G. The human nervous system is sensitive only to five of them - A, B, E, F and G and is insensitive to -C and D. The most potent for humans is type A [Akhtyamov SN, Golodenko H.A., 2004].

All serotypes of the toxin block the release of acetylcholine from the presynaptic terminal of the neuromuscular synapse [Niemann H, 1991; Coffield J.A. et al., 1994]. Also, toxins are able to bind to the terminals of the ganglia of the autonomic nervous system, causing a corresponding reaction, but in very large doses. Therefore, the likelihood of developing adverse reactions from the autonomic nervous system when using botulinum toxin in therapeutic doses is very small [Tsui J.K., 1996].

There are three main stages of botulinum toxin action [Simpson L.L., 1986; Tsui J.K. 1996].

The first step is linking. Botulinum toxin irreversibly binds to presynaptic cholinergic receptors at the carboxyl end of its heavy chain [Black J.D., Dolly J.O., 1986; Schiavo G. et al., 1992]. It is not completely clear which molecule acts as a binding site. Presumably it is the synaptic vesicle protein synaptotagmin capable of binding serotypes A, B and E [Li L., Singh B.R., 1998].

The second stage is internalization. The toxin enters the cell through the mechanism of receptor-mediated endocytosis [Brin MR, 1997]. After the endosome containing the toxin enters the cell, the disulfide bridge is cleaved [Simpson L.L. et al, 2004]. Half of the 50 kDa heavy chain carrying the amine end is involved in the formation of the channel and the transfer of the light chain from the endosome into the cytoplasm of the neuron [Montecucco C. et al, 1996; Schiavo G. et al., 2000]. This process does not depend on the concentration of calcium and partially depends on the stimulation of the nerve [Hughes R., Whaler B.C., 1962; Nathan P. et al, 1985].

The third stage is neuromuscular blockade. In the presynaptic terminal there is a protein complex that ensures the docking and fusion of the acetylcholine vesicle with the presynaptic membrane, followed by the release of the transmitter into the synaptic cleft. This complex of proteins is formed by synaptobrevin (or VAMP), syntaxin and SNAP-25 protein (synaptosomal protein with a molecular weight of 25 kDa) [Sollner T., 1993; Huttner W.B., 1993]. The light chain of botulinum toxin at its amine end contains a highly specific zinc endopeptidase, due to the proteolytic activity of which the above-described protein complex is cleaved [Tsui J.K., 1996]. Serotypes A and E cleave SNAP-25 [Sellin L.C., 1981], serotypes B, D, F, G-synaptobrevin-2, and serotype C - syntaxin and SNAP-25 [Methodical recommendations ..., 2004]. Muscle contraction causes acetylcholine, molecules of which are found inside synaptic vesicles. Normally, the membrane of the vesicle merges with the membrane of the nerve ending, the vesicle opens and acetylcholine enters the space between the nerve ending and the muscle cell. Acetylcholine binds to muscle receptors and causes the muscle to contract. The process

of fusion of the vesicle with the outer membrane occurs with the help of a special set of proteins - the SNARE complex. Botulinum toxin cuts the SNAP-25 protein included in this complex. As a result, acetylcholine cannot leave the bubble and transmit a signal to the muscle to contract [Methodical recommendations No. 2002/149. Botulism toxin type A in the correction of involutionally altered skin, 2004].

The process of presynaptic cleavage of transport proteins by botulinum toxin is irreversible and takes on average 30-60 minutes; therefore, specific botulinum antitoxin is effective only within an hour after the toxin reaches the target organs [Orlova O.P., 2006].

Botulinum toxin type A are widely used in many branches of medicine - in ophthalmology, neurology, surgery, aesthetic medicine as an effective and safe muscle relaxant.

The use of botulinum toxin for therapeutic purposes was first developed by A. and G. Carruthers in the late 1980s for the treatment of certain types of strabismus and blepharospasm [Carruthers A., Carruthers J., 1998]. FDA (Food and Drug Administration) in 1989 approved the use of botulism toxin type A in these therapeutic diseases [Bogs G.E., Ferrante R., 1992; Carruthers A., Carruthers J. 1994; 1997; Cather J. C. et al., 2002]. In 2000, the FDA expanded its current indications to include cervical dystonia [American Society, 2005; Cote T.R. et al., 2005]. Further, botulinum toxin A was used to treat nystagmus, facial hemispasm, spastic torticollis and blepharospasm by injecting small doses of the toxin into the corresponding muscles as well as in cosmetic to prevent and facial mimic wrinkles [Scott A.B., 1980; 1981; 1989; 1994; Schantz E. J., Johnson E. A., 1997]. Thus, the beginning of the application of botulinum toxin in neurology was laid.

When administered locally in therapeutic doses, botulinum toxin does not penetrate the blood-brain barrier and does not cause significant systemic effects [Brin M.F., 1997].

With intramuscular injection of botulinum toxin, 2 effects develop: direct inhibition of α-motor neurons at the level of the neuromuscular synapse and inhibition of γ-motor neuron cholinergic synapse on intrafusal fiber. Clinically, this manifests itself in a pronounced relaxation of the injected muscles and a significant decrease in pain in them. When intradermal injection develops a blockade of postganglionic sympathetic nerves for 6-8 months and stops sweating [Orlova O.P., 2006].

The clinical scope of application of botulinum toxin can include 4 groups of different syndromes:

- hyperactivity of striated muscles (strabismus, hemifacial spasm, blepharospasm, spastic torticollis and other focal dystonia, spasticity, cerebral palsy, rigidity, tremor, tics, etc.) [Savino P.J. et al., 1985; Dutton J. J., Buckley E. G., 1988; Gelb D.J. et al. 1989; Poewe W. et al., 1992; Tsui J. K., 1995; Edelstein C. et al. 1998; Kostrzewa R. M., Segura-Aguilar J. 2007; Khatkova C.E., 2008];
- hyperactivity of the sphincter muscles (achalasia of the cardia, urinary disorders - detrusor-sphincter dyssynergia, spastic constipation, hemorrhoids and rectal fissures, vaginismus) [Pasricha P.J. et al. 1995; Trzciński R. et al., 2002; Kostrzewa R. M., Segura-Aguilar J., 2007];
- hyperfunction of exocrine glands (hyperhidrosis, hypersalivation, lacrimation) [Huang W. et al., 2000; Eisenach J.H. et al., 2005; Kostrzewa R. M., Segura-Aguilar J., 2007];
- pain syndromes (myofascial and muscle-tonic syndromes, tension headache, migraine, facial pain) [Orlova O.P., 2006; Kostrzewa R. M., Segura-Aguilar J. 2007; Ranoux D. et al., 2008].

Many of the listed indications are officially registered in most countries of the world. For others, clinical trials are underway or have already been successfully completed in accordance with all the rules of evidence-based medicine [Orlova O.P., 2006; Kostrzewa R. M., Segura-Aguilar J., 2007]. The pharmaceutical prospects for botulinum toxin can be endless [Kostrzewa R.M., Segura-Aguilar J., 2007].

1.3 Kinetics and immunogenicity of botulinum toxin

The usual, "traditional" route of entry of the toxin, typical for all forms of botulism, is its absorption through the intestine or the epithelium of the respiratory tract. The toxin binds to the apical surface of cells, undergoes endocytosis and transcytosis, and is released on the basal cell surface. Then the toxin is distributed through the bloodstream along the periphery [Maksymowych AV, Simpson L.L., 1998; Park J. P., Simpson L. L. 2003; Ahsan C.R. et al., 2005]. Blood flow is the link between the natural site of absorption of the toxin (for example, the intestinal and respiratory epithelium) and its main site of action (neuromuscular synapse). Despite this, the systemic pharmacokinetics of botulinum toxin remains unexplored [Ravichandran E. et al., 2006]. One of the areas of pharmacokinetic research may be to study the spread of botulinum toxin after injection into areas containing nerve endings, for example, during its therapeutic use [Lange D.J. et al., 1991; Tang-Liu D.D-S. et al., 2003].

Pharmacokinetic studies carried out by Ravichandran E. et al. (2006) showed that blood, with its main cells and proteases, does not bind or metabolize botulinum toxin. The blood does not contain components that in any way sequester the toxin. The authors showed that the values of the amount and molecular weight of type A botulinum toxin incubated in the blood of rats did not differ from those in the control samples. At the same time, incubation in blood did not affect the enzymatic properties of the toxin and its ability to block neuromuscular synapses. Also, when administered intravenously, lethal doses in mice and rats have been shown to have a half-life of 230-240 minutes in both blood and serum. This proves that the shaped elements are not able to bind and accumulate botulinum toxin. These observations prove that the toxin in the blood is practically available to target organs. Therefore, the most obvious way to eliminate this proteinaceous neurotoxin is its binding with specific antibodies [Ravichandran E. et al., 2006].

The botulinum toxin molecule is a large protein with a sufficient number of linear and conformational epitopes. Many, and possibly all of these epitopes are potential binding sites for antibodies that help remove the toxin from the bloodstream [Chen F. et al., 1997; Oshima M. et al., 1997; Atassi M.Z., Oshima M., 1999]. However, things are different in the nervous system. The binding domain of the toxin molecule is rather small; accordingly, the epitopes located in it or near it are also small. This has significant clinical implications. The prospect of achieving neutralization of the toxin by active (use of a vaccine) or passive (administration of therapeutic antibodies) measures will increase both due to the elimination of the toxin from the bloodstream and due to the blockade of its receptor binding [Ravichandran E. et al., 2006]. research is underway to identify these epitopes. So, Atassi M.Z. et al (2008) found that serum antibodies isolated from patients resistant to botulinum toxin B are able to bind at least 11 epitopes, while antibodies against botulinum toxin type A bind 5 regions of the toxin heavy chain. It should be noted that antibody-dependent neutralization of botulinum toxin can be carried out by both homologous immunoglobulins and heterologous antisera, for example, horse. These drugs are available for human use [Arnon S.S. et al., 2006].

However, there is evidence that the light chain of botulinum toxin can also be immunogenic and cause the production of specific antibodies that can neutralize the effect of the toxin [Kiyatkin N. et al., 1997]. As shown by Takahashi T. et al (2009), antibodies against toxin light chains provide three levels of protection. At the first level, they block the absorption of the toxin through the intestinal and respiratory epithelium. At the second level, antibodies ensure the elimination of the toxin from the systemic circulation. Finally, antibodies protect cholinergic synapses from the action of the toxin, which was shown by the authors in neuromuscular preparations of the mouse diaphragm. It is assumed promising to create a vaccine that promotes the production of antibodies against light chains of botulinum toxin [Takahashi T. et al., 2009].

Circulating antibodies in patients can be detected after suffering botulism, after being vaccinated (for example, in military) and cause primary resistance. This condition is extremely rare, less than 0.1% after the first botulinum therapy procedure. If, after a two-month break after the first procedure, repeated administration of the drug into the same muscles and at the same dose as in the first does not lead to a clinical effect determined by objective and subjective methods, it can be assumed that the patient has primary resistance [Orlova O. R., Yakhno N.N., 2001].

Secondary resistance develops with repeated injections of the toxin and indicates the presence of neutralizing anti-botulinum toxin antibodies.

Although the functional significance of various titers of neutralizing BTA antibodies is still not well understood, it is generally accepted that a high titer of antibodies can completely block the action of a neurotoxin, causing immunoresistance, complete secondary treatment failure and depriving patients of the possibility of using one of the most promising treatment methods [Dressier D., Miinchau A., 2002].

The immunogenic properties of the toxin can stimulate antibody production, potentially leading to further treatment failure. The minimum doses and injection regimen that promote the development of antibody production are not fully understood. Immunogenicity may depend on a single dose, accumulated dose, frequency of injections, drug quality, individual characteristics of the patient's immune system [Huang W. et al., 2000; Dressier D., 2008].

The assumption that a lower protein load of the BTA drugs corresponds to a reduced immunogenicity was expressed for a long time [Borodic G., Johnson E., 1996; Jankovic J., Vuong K.D. 2003]. An increase in the immunogenicity of BTA in the presence of complexing proteins can be explained by the properties of some hemagglutinins, in particular HA1 and HAZb, in the complex [Lee J.C., Yokota K., 2005; Aoki K.R., Ranoux D., 2006;]. Innovations in the field of botulinum therapy include the drug

Xeomin, free of complexing proteins [Frevert J., 2009; Panova O.S., Sanchez E.A., 2011].

According to A. Carruthers et al. (1996) in patients with cervical dystonia who received from 100 to 1200 IU of botulinum toxin per session, the frequency of antibody formation was 3-5%. The resulting antibodies, regardless of titer, could contribute to resistance. In such situations, according to the authors, it is possible to use another type of botulinum toxin, because antibodies of one serotype do not cross-react with other serotypes. Patients who were seropositive to botulinum toxin type A were injected with botulinum toxin type F. Minimal interactions were noted between serotypes C and D, as well as serotypes E and F.

In another study, antibodies were detected by the "mouse" test in six women aged 41-80 years, suffering from dystonia, with a duration of the disease from 84 to 360 months, receiving an average dose of 207 units per injection. The average time between the first session of botulinum toxin type A and the development of antibodies was 27 months. The median duration between antibody detection and subsequent negativity was 30 months (range 10–78 months). With repeated administration of botulinum toxin type A, all six had an effect similar to the first one. Three patients in subsequent sessions had no clinical effect, and again became resistant. Two of them subsequently received botulinum toxin type F, one received botulinum toxin type B. Patients who were seropositive and subsequently became seronegative, as a rule, responded positively to repeated injections of botulinum toxin type A, but some had no clinical effect. It is assumed that the immune response to botulinum toxin type A may decrease but may be revived by repeated injections of the toxin. The presence of antibodies to botulinum toxin type A did not interfere with the clinical effect on the administration of botulinum toxins types F and B, thereby confirming the allergenic specificity of various serotypes of the toxin [Sankhla C, Jankovic J., Duane D., 1998].

Biglan A.W. and co-authors (1986) revealed the absence of antibody production in patients who received less than 50 U of botulinum toxin type A per injection. Gonnering R.S. (1988) noted the appearance of antibodies in patients with mimic muscle spasticity syndrome who received doses ranging from 150 to 300 U, but antibodies did not appear in those who received up to 52.5 U per course for 163 weeks. Studying the production of antibodies in patients with oromandibular and cervical pathology, Jankovic J. and Schwartz K. (1995) observed statistically significant differences between patients with average cumulative doses of 1709 IU and 1066 IU in patients with a higher dose: antibodies were detected in patients with a higher dose.

Zuber M. et al (1993) noted the presence of antibodies in 3% of patients with focal dystonia who received a cumulative dose greater than 50 ng (~ 1162 U). A study of patients with spastic torticollis who received doses in the range of 150 - 300 IU showed the presence of neutralizing antibodies in 4.3%. In addition, it was noted that patients resistant to botulinum toxin received injections with a greater frequency, 2-3 weeks after the first injection [Greene P. et al., 1994].

Lange O. et al (2009) examined 504 neurological patients for the presence of neutralizing antibodies using a "mouse" test, who developed secondary resistance to botulinum toxin preparations during therapy. Antibodies to botulinum toxin were detected in 44.5% of patients, from which the authors conclude that antibodies play a significant but not leading role in the development of secondary resistance. At the same time, it was noted that the role of antibodies in resistance to therapy increases with the use of higher doses of botulinum toxin preparations. At the same time, there was no dependence on which commercial preparation of botulinum toxin was used [Lange O. et al., 2009]

Research by Brin M.F. et al (2008) showed a very low immunogenicity of botulinum toxin A. Thus, out of 326 patients with spastic torticollis, long-term treated with Botox at doses of 148-213 IU per session, only 4 had antibodies, of which three patients had

resistance to treatment ... It is assumed that antibodies are little able to reduce the effectiveness of therapy due to the fact that the toxin has a sufficiently large area for receptor interactions [Earep B.R., 2008]. Nevertheless, some authors point to the development of seroresistance as a significant drawback of botulinum therapy, which reduces its effectiveness and forces one to look for other therapeutic approaches [Schrader S. et al., 2009].

Studying the production of antibotulinic antibodies in patients with spasticity, Muller K. et al (2009) found that antibodies are formed in 12% of patients, of which 6% had tolerance to botulinum toxin.

With long-term use (within 5-10 years) of Dysport diluted in 0.1% albumin solution, out of 106 patients, neutralizing antibodies appeared in only one. At the same time, a decrease in the frequency and severity of side effects of therapy was noted [Mohammadi V. et al., 2009]. The detection rate of clinically significant toxin-blocking antibodies in the course of BTA treatment, according to different authors, is 2% in spastic curve [Kessler K.R., 1999] and 1.2% [Brin M.F., 2008]; with spasticity in adults - 6% [Muller K., 2009]; with infantile cerebral palsy - 6% [Koman L.A., 2001].

To minimize the resistance caused by antibodies, it is necessary to use the minimum effective dose, interruptions in treatment should be at least 3 months, it is also necessary to avoid booster injections (within one month after the initial injection) [Greene P. et al., 1994; Inagi K. et al., 1997]. In some cases of ineffectiveness of treatment due to the formation of antibodies, botulinum therapy may be discontinued for a long period [Dressier D., Benecke R., 2007].

A clinical experimental study using pentavalent botulinum toxoid showed that after a year, antibodies disappear in about half of seropositive patients (regardless of age, sex, initiation of treatment, number of injections, average interval between injections, average single and cumulative doses of BTA, duration of treatment) , giving them the opportunity to resume treatment [Dressier D., BigalkeH., 2002].

The role of antibodies is less than 10% of the total number of all causes of therapeutic failure. There are opinions that most often the reason for the unsatisfactory result of the injection is an insufficient dose of the drug or an inaccurate choice of muscles for injection [Jankovic J., Schwartz K., 1995; Orlova O.R., 2006].

Several groups of diagnostic methods are identified that allow detecting the presence of immunoresistance to botulinum toxin, among which clinical, immunochemical and biological tests are distinguished. The sensitivity and specificity of these techniques are significantly different, which means that their clinical value is not the same with respect to confirmation or refutation of immunological mechanisms for the development of therapy ineffectiveness [Dolgov VV, 2012; Timerbaeva S.L., 2012].

Clinical tests include a frontal test or an eyebrow frown test (unilateral BTA administration, clinical assessment of the result) [Brin M.F. 2008]; test m. extensor digitorum brevis assumes electrical stimulation of the item peroneus and a change in the amplitude of the action potential of the short extensor of the big toe before and 2-4 weeks after the administration of botulinum toxin (20U Botox) [Cordivari C, 2006]; Sudomotor test is based on a qualitative assessment of the function of sweating before and after intradermal injection of BTA according to Minor's iodine-starch test and a quantitative (hygrometry) assessment of thermally induced sweating [Birklein F., 2000].

Immunochemical tests include ELISA with a fixed antigen (ELISA), which determines the level of antibodies, without distinguishing between neutralizing and non-neutralizing antibodies [Dressier D., 2004]; immunoprecipitation - determines a quantitative assessment of the level of antibodies, but has low specificity: the clinical significance of a negative response is 100%, positive - only 24% [Lawrence I., 2009]. All types of in vitro immunodetection, including immunoblotting, are sensitive, but total antibodies are determined [Naumann M., Carruthers A, 2010].

Biological tests include the test for protection of mice / lethality of mice, which is considered the "gold standard" of the qualitative and quantitative level of 30 toxin-

neutralizing antibodies [Nappa RA, 1999]; murine diaphragmatic / hemidiaphragmatic test (the time of 50% reduction in the amplitude of the contractile response of the diaphragmatic muscle is used as the determined parameter) [Dressier D., 2005].

Currently, the most widely used test for the determination of antibodies is the Mouse neutralization assay (Northview Pacific Labs, Berkeley, Calif), "mouse test" [Guyuron B., Huddleston S.W., 1994]. However, the "mouse" test is time consuming and expensive [Tsui J.K., 1996]. Also used and faster in the performance of immunochemical tests, but their disadvantages are low specificity [Nappa P.A., Jankovic J., 1998; Egorov A.M., et al, 1991] and the lack of correlation between detectable antibodies and clinical resistance [Doellgast G.J. et al., 1993].

Patients with resistance to botulinum toxin type A may be shown injections of botulinum toxin type B (Athena Neurosciences, San Francisco) [Tsui J.K. et al., 1995], botulinum toxin type C (Speywood Pharmaceuticals) [Eleopra R. et al., 1997] or botulinum toxin type F (Speywood Pharmaceuticals) [Sheean G.L., Lees A.J., 1995]. Due to the fact that other serotypes have different properties, the duration of their effect may vary [Brin M.F., 1997]. The use of botulinum toxin type B in patients with no clinical effect on botulinum toxin type A was also safe and effective [Truong D.D. et al., 1997]. Type B, C and F toxins are currently in clinical trials, with botulinum toxin type A being the only commercially available drug for routine clinical use.

1.4 Facial Movement Disorders and Mimic wrinkles

1.4.1. Benign Essential Blepharospasm

Blepharospasm is a cranial dystonia involves the eyelid and forehead muscles. It manifests as involuntary orbicularis muscle contraction resulting in increased frequency of blinking. In severe cases, blinking is repetitive and forceful, resulting in functional

blindness. The etiology remains unknown but may involve dysfunction of the central coordination of visual sensory input and motor output to the eyelids. Patients have increased sensitivity to visual stimuli and an exaggerated motor response manifested as excessive blinking and forced eyelid closure.

The development of Botulinum Toxin, a muscle paralyzing agent from the bacteria, Clostridium botulinum, has produced major advances in the treatment of dystonia (such as blepharospasm or torticollis), hemifacial spasm, tremor, tic disorders, and a number of other conditions - including those annoying crow's feet! In 2000, because of the wide variety of injection techniques in treating blepharospasm, a group of ophthalmologists and neurologists recognized as "experts" were surveyed for injection locations and amount of toxin injected on initial and average visits. Each of the respondents indicated dosages, dilutions, and number of injections for a typical patient. Although there were some differences in the sites for injection between injectors with ophthalmology and neurology training, these differences have declined. The five sites per eye identified in the figure are the most common locations for toxin injection. Other sites will vary with the presentation, and the experience of the physician. The purpose of this handout is to assist patients and physicians with discussion points to tailor future toxin therapy.

Toxin dosage varies from patient to patient, and units vary from toxin to toxin. Currently, there are several commercially available toxins. The three BTA include Botox®, Dysport® and Xeomin®; Myobloc®/Neurobloc® is the only available a B-type toxin. While the injection techniques will not change with these toxins, dosages will differ. It is therefore important to know the brand name, not just A or B, of toxin used at each injection session.

Initial dosage range for each toxin is listed in the Table 1.

Table 1

Relative dosages for Botulinum Toxin[a]

Toxin	Frontalis		Upper Lid		Lower Lid	Other	Recommended Dosage/eye[b]
	Site	Total	Site	Total	Site	Site	
BoNT - Type A							
Botox®	5	10	5	10	8	5	30-50
Dysport®	15	30	15	30	25	15	100-120
Xeomin®	5	10	5	10	10	5	35
BoNT - Type B							
Myobloc®	150	300	150	300	200	200	1,000-2,500

[a] This table is adapted from Stacy M, Handbook of Dystonia. Informa Press 2006.

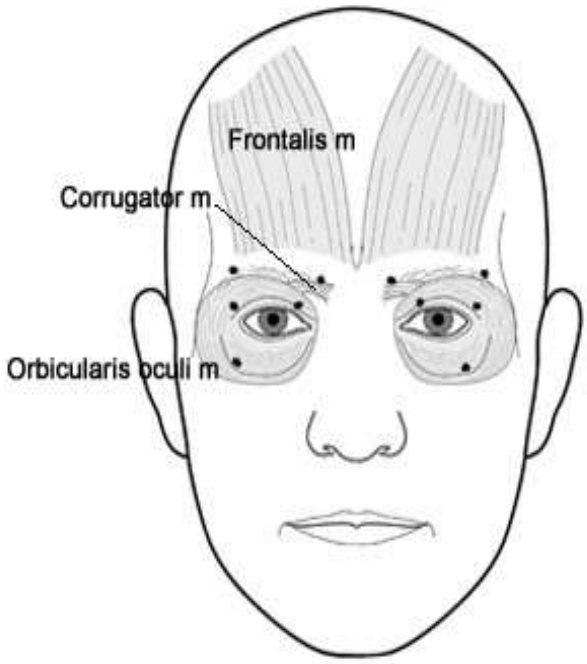
[b] Recommended dosages are obtained from the Package Insert from countries that have approved the toxin. They are listed as a per eye dosage. Product approvals vary from country to country.

Further dosage adjustment may be made for size of the patient, severity of the symptoms, and prior response to therapy. Ideal dosing should produce a benefit within one week of the session, and a total duration of benefit of approximately twelve weeks. In instances where side effects, such as lid drooping (ptosis), double vision (diplopia), or tearing occurs, injection with less toxin or in a slightly different location may allow more effective response.

Each of the A-type toxins requires reconstitution with preservative free, normal saline. Most experienced injectors reconstitute with 1 cc of solution, thus with Botox® and Xeomin®, 0.01 cc = 1 unit, and Dysport®, 0.01 cc = 5 units. Myobloc®/Neurobloc® may offer some convenience of use, because this preparation does not require reconstitution and may be stored in the refrigerator. The dilution for this toxin is 0.01 cc = 50 units. It is difficult to convert from one toxin to another on the basis of dosage ratio, and each compound should be treated as a unique toxin.

The 10 most recommended injections sites are marked on the diagram (Table 2).

Table 2



Injection of the corrugator supercilii m is often helpful in patients with furrowing around the eyebrows. Injection under the skin of the upper lids is more effective than injections higher in the orbit (off the lid margin). Injections to the lower lids may be off the lower lid margin, and usually just to the lateral site to avoid tearing.

Other injections sites include the lateral obicularis oculi m. - particularly if deep furrows in the skin indicate spasm in this area. With midline brow furrowing, injection of the procerus may be helpful. Nasal movements may respond to levator labii superioris nasi m., but this injection may cause tearing and lip weakness.

1.4.2. Hemifacial Spasm

Hemifacial spasm is a neuromuscular disorder characterized by unilateral recurrent twitches of the facial muscles innervated by the facial nerve. Unlike blepharospasm, these spasms persist during sleep and are not related to hyper sensory input. The anatomic basis for the spasms is usually a mechanical irritation of the facial nerve at its exit root by a sagging arterial branch. Although surgical treatment is available, most patients are treated medically with botulinum toxin, and the results are highly successful.

Although surgical treatment is available and some proposed medications have been helpful (Alexander and Moses 1982; Herzberg, 1985), most drugs (including herbs) and acupuncture are generally not effective in HFS and BS. Microsurgical decompression of the facial nerve can be offered to patients with HFS and has an 81-87,5% success rate, however serious complications occurred in 19-35% (Loesert and Chen, 1983; Piatt and Wilkins, 1984, Auger et al, 1986).

1.5. Botulinum Toxin A in Medical Cosmetology

In 2002, the FDA approved the use of type A botulinum toxin for use in cosmetology (Botox, Allergan) "for the temporary improvement of moderate to severe wrinkles appearing in the glabella region associated with the activity of m. corrugator and / or m.procerus in adult patients. " In recent years, botulinum therapy has been increasingly used in aesthetic medicine [American Society, 2005; Cote TR et al., 2005; Zatler G., 2012]. The cosmetic use of botulinum toxin type A is based on the use of low doses of the toxin to correct mimic wrinkles and hyperhidrosis.

Upper face injections for dynamic wrinkles can have a positive effect on emotional status by reducing the ability to frown and create negative facial expressions. BTA injections can improve the emotional state of patients [Alam M, Barrett KS, et al., 2008]. Other authors believe that patients whose occupations depend on communication, due to the limitation of the ability to express their emotions, should avoid the introduction of botulinum toxin, suggesting to choose an alternative cosmetic therapy [Lackey J.N., Norton S.A. 2006].

The discovery of the effect of botulism toxin type A on mimic wrinkles was a significant progress in aesthetic medicine [Jankovic J., Brin M., 1991; Brin M. F., 1997]. Easily performed by experienced doctors, botulinum toxin injections are one of the most widespread procedures in modern cosmetology. Their popularity and success among doctors and patients can be associated with good clinical effect, safety, minimal

invasiveness and short duration of the procedure itself [Hexsel D., 2005]. The anatomy of facial muscles, their functions and intermuscular interactions are well known, this knowledge is extremely important in the correct implementation of botulinum therapy applications. Indications for use are: the presence of mimic wrinkles in the forehead, glabellar and paraorbital zones, the back of the nose, chin, lips, corners of the mouth, neck, décolleté [Foster J.A. et al. 1996; Heckmann M., Schon-Hupka G. 2001; Collection of articles on Dysport®, 2002; Methodical recommendations ..., 2004, 2006, 2008; Monheit G, et al., 2007; Carraders A., Carraders D., 2010].

The cosmetic procedure itself is quite simple, the drugs are injected into the target muscles in the projection area of m.frontalis, m.corrugator supercilii, m.orbicularis oculi, m.procerus, m.nasalis, m.depressor anguli oris, m.platisma, m.mentalis.

However, even though the anatomy of the facial muscles is well described, there are still individual differences in its structure in men and women, there are peculiarities in different population, ethnic groups, etc. The individual clinical features of each patient are no less important than the anatomy of the muscles. factors that must be considered before the introduction of botulinum toxin [Hexsel D., 2005]. The choice of optimal dosages of type A botulinum toxin depends on the patient's age, gender and area of administration, as well as the severity of wrinkles [Shaari CM., 1993; Moore J. 1995; Garcia A., Fulton J.E. Jr., 1996]. The smoothing effect of facial wrinkles after the administration of botulinum toxin type A depends not only on muscle paralysis, but also on certain skin qualities, such as turgor and elasticity, which decrease with age. Thus, when selecting patients for the procedure, it should be borne in mind that the best effect is observed in younger patients, and in older patients, only with a small amount of damage to the elastic fibers of the skin [Carruthers A., Carruthers J., 1998]. Botulinum toxin is most effective in the fight against mimic wrinkles in young patients with good activity of the mimic muscles of the face [Manturova N.Ye. et al., 2008], the results of treatment have an inverse correlation with age [Heckmann M., Schon-Hupka G., 2001].

There are certain requirements for the position of the patient during the procedure and for his behavior after the administration of botulinum toxin. During the procedure, the patient is in a sitting position. After the injection, the patient must perform active muscle movements for 30-60 minutes, and also observe a strictly vertical position to prevent diffusion of the drug into the deep muscle layers. For the first 2 weeks, the patient should avoid hot compresses on the injection area, do not go to baths and saunas, and do not consume an alcohol. The action of the drug begins in 3-4 days, the maximum effect occurs in 2 weeks. The duration of action of the drug is from 3 to 12 months [Methodical recommendations ..., 2004; The use of the drug Dysport ..., 2006, 2011].

Recently, the high efficiency of BTA in the treatment of plantar hyperhidrosis has been demonstrated [Swartling C, Naver H, 2001; Naumann M, at al., 2003; Swartling C, et al., 2004; Lowe N.J., et al., 2007.

For medical purposes, in particular in cosmetology, only highly purified toxins with a large dilution are used, causing temporary paralysis of the striated muscles in the area of administration and not leading to death [Akhtyamov SN, Golodenko NA, 2004; Baumann L., 2012].

Currently in North America registered and used for professional use several drugs containing botulinum toxin type A [Cote T.R., 2005; Pickett A., Mewies M. 2009; US Food 2009].

- Botox - onabotulinumtoxinA ("Botox"), company Allergan (USA), containing: complex botulinum toxin type A - hemagglutinin 100 U; human plasma albumin 0.5 mg; sodium chloride 0.5 mg. (2)

- Dysport - abobotulinumtoxinA ("Dysport"), manufactured by Beaufour Ipsen (France), containing as an active component - a complex of botulinum toxin type A - hemagglutinin - 500 IU or 300 ED, and excipients - human albumin 125 μg, lactose 2.5 mg.

- Xeomin - incobotulinumtoxinA ("Xeomin"), manufactured by Merz (Germany), which contains - botulinum toxin type A 50 U or 100 U, sucrose 4 , 7 mg, human serum albumin 1.0 mg.

Common to these drugs is: the presence of botulinum toxin type A as an active substance and the presence of a registration certificate that allows the use of drugs in medical practice, in particular in cosmetology. The structural and functional properties of type A botulinum toxin can influence the severity and duration of the clinical effect [Eapen B.R., 2008]. Dosages of drugs are based on their biological activity and expressed in units (U).

Special attention is required to the dosage of the drug, its dissolution, dilution, route of administration, storage and site of administration for an optimal treatment result [Gargland M.G., Hoffman NT, 1993]. Basically, the requirements are reduced to the need to store the unreconstituted drug at a temperature of 2 to 8 ° C, and after reconstitution, the vial should be stored in the refrigerator and used for a time not exceeding 24 hours, provided that the dissolution was carried out under aseptic conditions. In a review of reports on side effects and complications, the authors provide numerous examples of insufficient attention to these prescriptions. First of all, changes in dosage led to the prescription of the drug in quantities greater than indicated in the recommendations. Example in cosmetology, 14 patients received 100 units of BTA and more in one treatment. The authors reported that sometimes, when preparing BTA, doctors preferred not recommended saline solution, but other drug diluents, such as bupivacaine, lidocaine, and water. Possible errors include: use of the prepared preparation later than 4 hours from the moment of dilution; re-freezing the prepared preparation; long-term storage of the diluted drug in the refrigerator for the subsequent use of this patient or other patients as required. Most of the messages mentioned the introduction of BTA into zones not specified in the instructions [Cote T.R., 2005]. However, there is information in the

literature that storage and subsequent reuse of BTA is safe for at least 2 weeks after recovery [81oop R.R. et al., 1997].

Another study by the authors showed that reconstituted BTA can be stored safely in the refrigerator for 1 to 4 months. And during this period, repeatedly take the drug from the vial. Long-term storage and use does not lead to the detection of microbiological contamination [Alam M. et al., 2006]. When the required conditions are met, the safety of drugs based on botulinum toxin type A has been confirmed by many studies [Carruthers A., Carruthers J., 1998; Lowe P., et al., 2006; Rzany B., et al., 2007; Monheit G., et al., 2007;]. To assess the long-term safety of repeated Dysport injections in the treatment of glabella wrinkles, 768 participants received a total of 2,259 BTA treatments over 17 months. The authors of the study did not note any significant violations of vital organs [Monheit G.D., Cohen J.L., 2009]. In 2-5% of cases, when using botulinum toxin preparations, local reactions can be observed: pain at the injection site [Alam M., Dover J.S., 2002]. Micro hematomas (up to 7 days). Complications when using botulinum toxin in cosmetology are: drooping of the eyebrows, edema and swelling of the periorbital region, asymmetry of the effect, headache, articulation disorder, ptosis, diplopia, hypo- or overcorrection. All side effects of botulinum toxin are completely reversible, often do not require additional treatment [Heckmann M., Schon-Hupka G., 2001; Cote T. R., Mohan A. et al., 2005; Orlova O.P., 2006; Naumann M., 2008].

The effectiveness of the treatment of mimic wrinkles is assessed by different methods. Electromyography can provide objective information regarding individual muscle activity. However, the results are semi-quantitative and difficult to standardize because they differ significantly depending on the point of injection into a given muscle. In addition, the introduction of botulinum toxin type A for facial wrinkles on the face usually involves several muscles and reflects the cumulative effect of the drug [Klein A.W., Mantell A., 1998].

Some authors have developed another objective assessment of the results of botulinum therapy [Heckmann M., Schon-Hupka G., 2001]. For example, for each patient, a complete set of facial expressions was recorded using a digital camera before and after treatment, which made it possible to objectively measure the distance between stable landmarks, for example, the pupils and eyebrows, before and after botulinum therapy. After treatment with botulinum toxin type A, the ascending mobility of the eyebrows was reduced by 35% at 2 weeks and by 71% at 12 weeks after injection. In contrast, intrinsic mobility (frowning) was reduced by 7% at 2 weeks and 57% at 12 weeks. The interval "between the brows" at rest increased by 13% after injection) and found a negative correlation with age. Digital image analysis is a valuable tool for clinical documentation and evaluation of the effectiveness of treatment [Heckmann M., Schon-Hupka G., 2001]. However, in recent years, patients are interested in incomplete shutdown of the motor activity of the facial muscles. Most often, the assessment of the effectiveness of treatment of facial wrinkles with botulinum toxin is carried out on the basis of empirical or subjective data. Issues related to effective dosages of drugs are constantly discussed. For example, in the treatment of axillary hyperhidrosis, some authors recommend high doses of drugs (up to 200 U of Botox) [Wollina U. et al., 2002], while others achieve a similar clinical result with the introduction of 50 U and are categorically against increasing the dosage, expressing the opinion that high doses increase the risk of side effects and the formation of neutralizing antibodies [Naumann M., Hamm N., 2004].

Attempts to associate the decrease or absence of the results of botulinum therapy with the presence of specific antibodies have been carried out several times. We examined blood samples from patients with primary hyperhidrosis before treatment with botulinum toxin type A and at the final visit for the presence of neutralizing antibodies using the mouse defense test. Of 332 patients with primary axillary hyperhidrosis, 252 completed the study. Neutralizing antibodies were not detected using the mouse protection test

[Lowe N.J., et al., 2007]. In the treatment of glabella wrinkles with Dysport, more than 700 people did not reveal any evidence of the formation of neutralizing anti-botulinum toxin antibodies [Monheit G.D., Cohen J.L., 2009]. Cases of lack of results of botulinum therapy are found in medical practice. Of the 995 cosmetic cases with mild side effects or complications, an insufficient cosmetic effect from the expected one was observed in 623 (63%) [Cote T.R., 2005].

Distinguish between primary and secondary ineffectiveness of botulinum therapy. Primary ineffectiveness is understood as the lack of results when using botulinum toxin for the first time, which is quite rare [Dressier D., 2004]. Primary ineffectiveness is caused by: unclear definition of the purpose of treatment, errors in the assessment of indications for therapy in a particular patient, the choice of target muscles and other technical errors associated with violations in the injection technique, with the patient's behavior (non-compliance with recommendations after the procedure); the use of an insufficient dose of toxin (it is known that the effect of botulinum therapy is dose-dependent); reduced biological activity of the toxin (if the storage and transportation conditions are not observed, rare cases of a decrease in the serial activity of the drug); clinical subtypes with reduced sensitivity to botulinum toxin (apraxia of eyelid opening, etc.); individual reduced sensitivity to botulinum toxin, including those associated with changes in the properties of membrane toxin acceptors [Cote T.R., 2005; Timerbaeva S.L., 2012].

With long-term successful therapy with BTA drugs, secondary ineffectiveness may develop, which is assessed as objective or subjective, complete or partial, permanent or temporary. This effect is extremely variable among patients, and the same person may differ in different areas of the face [Timerbaeva S.L., 2012]. Clinically, patients with secondary insensitivity are defined as cases with a history of at least two successful injections (improvement rate of at least 20% and / or the presence of relaxation of the injected muscles with denervation potentials according to electromyography) [Naumann

M., Carruthers A., 2010]. Complete secondary failure is usually observed in the first 2–3 years of treatment, followed by a period of partial failure. After 4 years of BTA therapy, secondary failure is a rare condition [Dressier D., 2004]. Among the reasons for the secondary ineffectiveness of botulinum therapy are the influence of psychological factors (depression), exacerbation of the underlying disease, technical errors in drug administration, changes in the properties of BTA membrane acceptors during the treatment period, reduced biological activity of the drug and the presence of neutralizing antibodies [Goschel N., 1997; Dressier D., 2004]. Until 2007, there were no publications on the presence of antibodies neutralizing botulinum toxin in patients who were injected with BTA preparations for aesthetic reasons. The first report concerned the immunologically determined secondary ineffectiveness of botulinum therapy for masticatory muscle hypertrophy in a 20-year-old patient after 4 injections of onabotulinumtoxinA (60 U with an interval of 4-5 months). Secondary insensitivity was first confirmed by a positive frontal test, and then antibodies to BTA were determined using ELISA (enzyme-linked immunosorbent assay) and a biological test for the protection of mice. A feature of this case is the fact that immunoresistance developed during treatment with relatively low doses of the drug known as "new Botox" with a low protein content [Lee S.K., 2007].

According to the studies devoted to the problem of immunoresistance to BTA, it should be noted a meta-analysis of the results of 16 large controlled and prospective open-label studies lasting from four months to two or more years with a final sample of 2240 patients regarding the use of onabotulinumtoxinA [Naumann M., Carruthers A. , 2010]. The aim of the study was to assess the frequency of seroconversion (formation of neutralizing antibodies) during botulinum therapy for various, including cosmetic, indications. The authors also analyzed the relationship between the formation of neutralizing antibodies, clinical effect and adverse events that may be of an immune nature. A single dose of onabotulinumtoxinA varied from 10-20 U to 20-500 U, the number of procedures - from

one to 15 (average 3.8). In general, seroconversion was observed in 11 (together with patients with neurological and cosmetological profile) of 2240 patients (0.49%), however, only in 3 cases they had clinical ineffectiveness of therapy. By the end of the study, 0.2% of patients remained seropositive under conditions of continued effective botulinum therapy. The results of this, the most extensive and evidence-based study, showed that the incidence of antibodies to BTA (onabotulinumtoxinA) is very low and extremely rarely leads to the development of therapy ineffectiveness [Timerbaeva S.L., 2012]. It should also be noted that in patients with antibodies neutralizing botulinum toxin, there were no local or systemic adverse events of an immune nature [Naumann M., Carruthers A., 2010].

In cosmetology practice, low doses of BTA are usually used (when correcting wrinkles) or injections are carried out at long intervals (when treating hyperhidrosis), and therefore seroconversion is extremely rare (0.28-0.46% of patients). It is very important to note that seroconversion, as a rule, is not accompanied by a loss of therapy effectiveness [Temirbaeva SL, 2012; Panova O.S., Sanchez E.A., 2013]. In order to prevent the formation of antibodies blocking botulinum toxin, the established risk factors should be avoided: a high single dose of BTA; short intervals between repeated courses (less than 3 months); repeated injections of the drug with an interval of 2-6 weeks ("booster" injections) [Dressier D., 2008; Dressier D., Saberi F.A., 2009; Lange O., Bigalke H., 2009]. It is necessary to take into account the quality of drugs, not to use unregistered products [Timerbaeva S.L., 2012].

Many years of clinical experience indicate that it is impossible to unequivocally put an equal sign between the presence of antibodies, even blocking botulinum toxin, and the absence of a clinical effect. Observation of patients with various diseases in whom the effectiveness of treatment with BTA drugs remains despite the presence of antibodies neutralizing botulinum toxin, made it possible to suggest that the therapeutic effect of BTA depends not only on the presence or absence of neutralizing antibodies as such, but

also on the balance between the doses of BTA used and the levels blocking antibodies [Dressier D., Munchau A., 2002]. Opportunities for overcoming and preventing immunoresistance to BTA are in increasing the BTA dose. In the case of partial ineffectiveness of treatment or low titers of BT blocking antibodies, increasing the dose allows you to maintain the effectiveness of therapy without a subsequent increase in the level of antibodies and the development of complications [Dressier D., Munchau A., 2002]. In the use of drugs of other types of botulinum toxin or other BTA drugs, for example, type B (Myoblock / Neuroblock), however, after repeated injections, antibodies are also produced to these drugs [Dressier D., Lange M., 2005]. In the possibility of inactivation or removal of neutralizing antibodies using plasmapheresis or immunoadsorption, the use of corticosteroids, immunoglobulins [Dressier D., 2004]. Thus, a review of the available literature revealed a wide range of reasons leading to the ineffectiveness of botulinum therapy in aesthetic medicine. Immunoresistance stands out sharply among them, since it is not a consequence of the low qualifications of the doctor or violations of the recommendations for storage and transportation of the drug. The emergence of immunoresistance is due to objective reasons that require a deep scientific approach and special study, which has not yet been carried out in the field of aesthetic medicine. Timely detection of antibodies that affect the final clinical result of botulinum therapy could serve as a risk factor in the selection of patients for the procedure or a mandatory study for an expert assessment of possible conflict situations in the practical work of a doctor - cosmetologist.

CHAPTER 2. MATERIAL AND RESEARCH METHODS

2.1 Assessed groups of patents

The study involved patients who received BTA drugs, as well as individuals in the control groups who did not receive BTA drugs.

Treatment with BTA drugs was received by 172 patients for facial wrinkles.

Indications for prescribing BTA drugs included the presence of facial wrinkles due to increased activity of the facial muscles . In order to correct the existing deficiencies, patients received treatment with obobotulinumtoxinA (Dysport, Ipsen, France) at a dosage of 60 to 200 U and / or onabotulinumtoxinA (Botox, Allergan, USA) - from 20 to 100 U. The treatment of this contingent was carried out on the basis of the Center for Medical Cosmetology of the St. Petersburg Medical Academy of Postgraduate Education (now the State Budgetary Educational Institution of Higher Professional Education, North-Western State Medical University named after II Mechnikov). In the control groups, 187 people were examined.

Also, doctors were involved in the study, who had to analyze the results of using BTA drugs on the basis of their work experience. The survey involved 155 cosmetologists who use BTA preparations in practice.

When examining patients, clinical and immunological examination methods were carried out.

2.2.1. Evaluation of the clinical effect of the administration of botulinum toxin type A in patients with mimic wrinkles

The clinical effect of BTA injections was evaluated according to the International Aesthetic Improvement Scale GAIS (Global Aesthetic Improvement Scale), developed for an objective and subjective assessment of the results of cosmetic procedures (Table 3):

Table 3

Score	Assessment by a doctor	Assessment by a patient
3	Optimal cosmetic result	Completely satisfied with the result
2	Significant improvement, but not full correction	Satisfied with the result, but wanted to improve a little
1	Improvement, but necessary additional correction	Improvement, but additional correction necessary
0	No change, condition is the same as before the procedure	Without changes
-1	Worse compared to initial state	The condition is worse than before the procedure

2.2.2 Botulinum Toxin for facial spasms and facial movement disorders

Department of Neurology, Keimyung University school of Medicine, Taegu, Korea (Journal of Korean Medical Science Vol.8, No 5, 334-340 October 1993, Young Choon Park, MD., Jeoung Keun Lim, MD., Dong Kuck Lee, MD, Sang Doe Yi, MD) conducted a study and reported the results and complications of BTA treatment in 101 patients with HPS and 11 patients with BS. The age range of the patients was 27 to 73 years (mean, 53.5 years), prevalent age range was 40 to 69 years (81%). The sex distribution included 75 (67%) women and 37 (33.0%) men.

Before the treatment with BTA, an informed consent was obtained after explanation of the experimental nature of the study. A complete medical history and physical examination were recorded, and an eye examination was done to rule out of secondary blepharospasm. Spontaneous facial or orbicular spasm intensity was rated on a subjective scale of 0 to 4 (Table 4.).

Table 4.

Rating scale of intensity of facial and orbicularis muscle spasm
0 = No spasm
I = mild, barely noticeable
II = mild, noticeable fluttering, no functional impairment
III = moderate spasm, moderate functional impairment
IV = severe incapacitating spasm

The durations of symptoms before BTA treatment ranged from 4 months to 35 years (mean 5.1 years). Symptoms duration is longer in HFS (6 months to 35 years) than BS (4 months to 4 years). Of 101 patients with HFS, 86.1% belong to grade III or IV and all of the patients with BS had grade III or IV at the time of treatment.

BTA (Oculinum Inc. lot number 79-11) was stored frozen at -10C until use. It was diluted in normal saline 4ml (2,5 units per 0,1ml) and used on the same day.

At first 8 patients with HFS and 4 patients with BS were randomized to receive, in a double-blind trial, either the toxin or saline as a placebo. Later most of the patients (93 HFS, 7 BS) were included in an open trial.

The initial injection consisted of small doses (2,5 units – 0,5 units) titrating them to the needs of the patients, to determine the range of effective dose. BTA was injected subcutaneously or intramuscularly, into multiple sides of the face, the medial and lateral portions of the upper eyelid and the lateral and central portions of the lower eyelid, where greater spasm occurred. The patients were asked to call 3 to 6 days after injection and were examined. Videotapes were recorded before and at the first and second visits after injection.

A higher dose and multiple additional doses were needed according to the severity of muscles spasm or the degree of involved muscle. Seventy one percent of patients with HFS received 7.5 units to 20 units and 91% of patients with BS received 17.5 units to 30

units initially. The total dose of injections varied from 2.5 units to 40 units in each set of treatments. Mean doses of the toxins injection in each patient were 13.5 units in HFS and 22 unit in BS. Patients with severe facial spasm were given multiple additional injections with intervals of one week. One hundred and twelve patients underwent a total of 212 treatments in 25 months total. Of these, 68 patients had 2 to 4 additional injections for maximum benefits, and 24 patients (24.4%) had two sets of treatments and 8 patients (7.1%) had three sets of treatments for recurrence. Recurrence was fined as reappearance of objective and subjective facial and/or orbicular spasm at least 8 weeks after maximal benefit from initial treatment. Follow up duration was 7 to 20 months.

CHAPTER 3. CLINICAL ANALYSIS OF THE RESULTS OF THE TREATMENTS WITH BOTULOTOXIN TYPE A

3.1 Clinic analyse the results of the treatments of mimic wrinkles

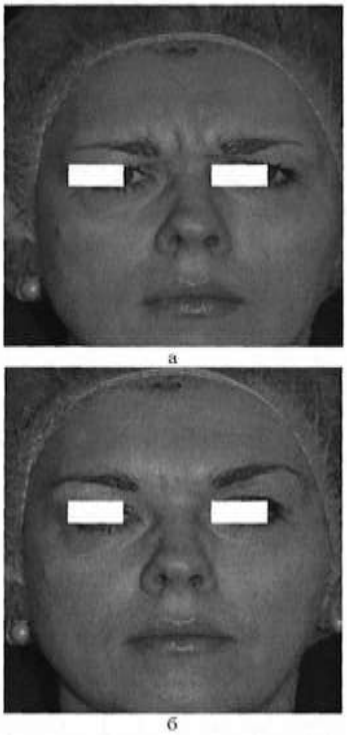
The group of people who received BTA drugs for the correction of facial wrinkles consisted of 172 people, the clinical effect was assessed in 116 people, of which 111 women (96%) and 5 men (4%). The age of all surveyed ranged from 22 to 64 years, the average age was 40.3 ± 9.4 years.

This group was formed randomly, since it consisted of patients who applied for the correction of facial wrinkles. Patients signed informed consent to participate in the study. Criteria for excluding patients from the study: violations of cholinergic transmission (myasthenic, myasthenic-like syndromes), chronic diseases in the acute stage, pregnancy and lactation, hemophilia, high degree of myopia, acute phase of infectious diseases, inflammatory and / or infectious process in the area of the treatment, increased sensitivity to the components of the drug, a burdened allergic history (especially to drugs containing proteins), the patients currently takinh antibiotics of the aminoglycoside group, tetracycline, lincomycin, polymyxins that enhance the muscle relaxant effect of BTA (if not more than 2 weeks have passed after the course of treatment), muscle relaxants (rocuronium bromide, suxamethonium chloride), drugs that increase the intracellular calcium concentration (amidopyrine), tranquilizers (diazepam), anesthetics, anticoagulants, antiplatelet agents, as well as the presence of a pronounced gravitational ptosis of facial tissues in the patient, "hernias" in the upper and lower x century, a tendency to edema.

Within the selected groups, patients had concomitant diseases such as allergic reactions (27%), gynecological diseases (12%), hypothyroidism (5%). These diseases were

compensated by appropriate therapy or previous surgical treatment. Children and respiratory infections (13%) predominated among the transferred diseases.

Thus, the examined patients were practically healthy, more than half of them led an active lifestyle, regularly engaged in physical education (55%). From bad habits: some of the patients smoked (16%), no one use an alcohol. Treatment of mimic wrinkles was carried out in the area of the brow, forehead, outer corners of the eyes (Picture 1, 2, 3).

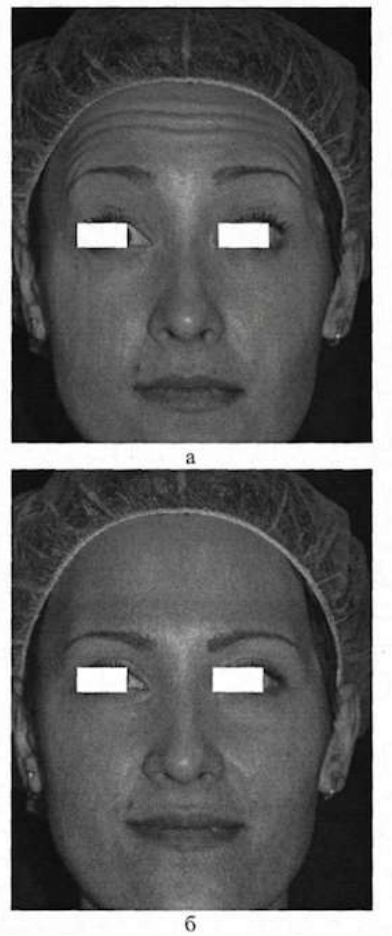


Picture 1 – Patient X with mimic wrinkles in the area between the eyebrows: a – contraction m.m.corrugatores supercilii and m. procerus before BTA administration;
b - blockade of these muscles after BTA administration led to a pronounced smoothing of wrinkles

Picture 1b shows the results of BTA injection into the glabella region.

Hypertonicity of the muscles between the eyebrows is visually manifested by the demonstration of negative emotions - anger, contempt, sadness. Relaxing the muscles gives the face a welcoming expression. The formation of vertical wrinkles involves mm.corrugatores supercilii, horizontal ones - m.procerus. Isolated correction of the zone is performed most often in young patients with mm.corrugatores supercilii and m.proserus hyperactivity in order to prevent the formation of vertical and horizontal wrinkles in the eyebrow area. In older patients, blockade of these muscles and

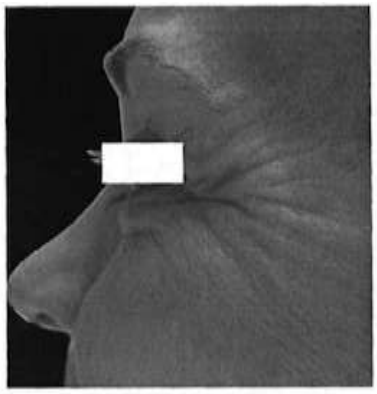
m.depressor supercilii is used to raise the eyebrow head. It has been noticed that with isolated correction of the eyebrow area in young patients, activation of the frontal muscle is often noted, leading to the appearance or strengthening of existing horizontal wrinkles on the forehead.



Picture 2 - Patient X with mimic wrinkles in the forehead:
a - reduction of m.frontalis before the introduction of BTA drug;
b - blockade
of this muscle after administration of BTA led to
complete absence of wrinkles

Picture 2 shows the results of BTA injection into the forehead area. Horizontal wrinkles in the forehead are formed by the fibers of the frontal abdomen m.occipitofrontalis.

Isolated correction of the forehead area is rarely performed, as it provokes the activity of the friendly muscles of the brow region. For young patients with overactive frontal muscle, BTA is prescribed to prevent the formation of horizontal wrinkles on the forehead; and for older patients - for the purpose of raising the eyebrows.



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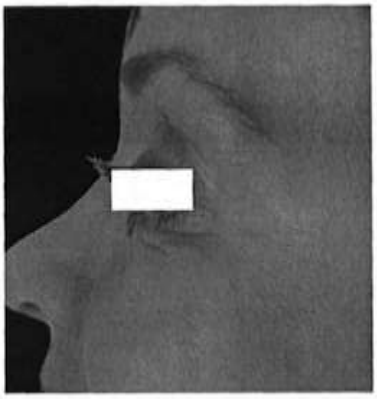

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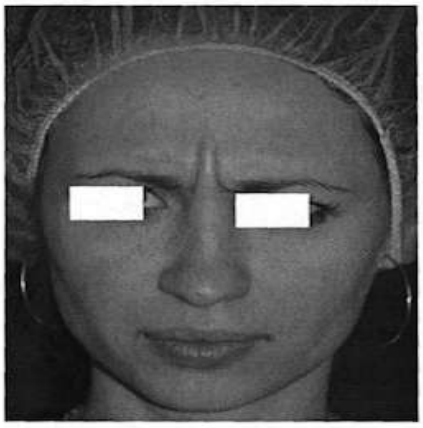
Picture 3 - Patient X with fan-shaped wrinkles at the lateral angle palpebral fissure:
a - contraction of m. orbicularis oculi before BTA administration;
b - blockade of the specified muscle after BTA administration led to significant smoothing of wrinkles

Picture 3 shows the results of BTA injection into the eye area. Performed correction of wrinkles localized at the lateral angle of the palpebral fissure ("Crow's feet"), which are formed when excessive contraction

In rare cases, BTA is used to correct wrinkles that form around the mouth. Vertical wrinkles above the upper lip and below the lower lip are formed as a result of hyperactivity of the m.orbicularis oris. Wrinkles in the corners of the mouth are formed as a result of hyperactivity of m.depressor labii inferioris and m.depressor anguli oris. In order to correct mimic wrinkles, patients underwent injections of BTA preparations into the mimic muscles of the above areas of the face in dosages recommended by the methodological recommendations (Methodical recommendations No. 2002/149. Botulism toxin type A in the correction of involutionally modified skin, 2004; Medical technology. Application of the drug Dysport (botulinum toxin type A) to eliminate excessive activity of mimic wrinkles, 2006; Medical technology. Application of the drug Dysport (botulinum toxin type A) to correct hyperkinetic folds, involutionally changed skin in the facial area, recommendations for the use of the drug Dysport® in cosmetology, 2011) . The amount of the drug administered to each patient was selected individually, depending on the anatomical and physiological characteristics of the facial

muscles, the severity of muscle mass, muscle hyperactivity, as well as the age and sex of the patient. Thus, the dosages of the drugs were: onabotulinumtoxinA - from 20 to 200 units. In 35 people (30%), facial wrinkles were corrected in one of the areas of the face (forehead, between the eyebrows, the corners of the eyes - the zone of the so-called "crow's feet", upper and lower lips, corners of the mouth), the rest (70%) - in several areas ... Control medical examination of patients was carried out on days 14-21 after the procedure. The degree of correction of facial wrinkles due to relaxation of the muscles injected with BTA was assessed using the GAIS scale. According to the results of an objective examination by a doctor, a score of 3 points corresponded to the maximum possible smoothing of mimic wrinkles and the absence of complications in 62 patients. A score of 2 points was assigned with a significant improvement in comparison with the initial state, but not complete relaxation of mimic hyperfunctional wrinkles. A score of 1 point was assigned in the case when mimic wrinkles were smoothed slightly compared to the initial state. A score of 0 points was assigned in the absence of changes after the procedure. A score of 1 point was assigned in case of deterioration compared to the initial state. According to the results of an objective assessment by the doctor, 3 points were assigned to 72.2%) of patients, 2 points - 24.6%, 1 point - 3.2% of patients. The mean GAIS scores for each of the zones practically corresponded to the overall mean score, with only a slight variation. This indicated that the drug was adequately injected into the muscles of various areas of the face.



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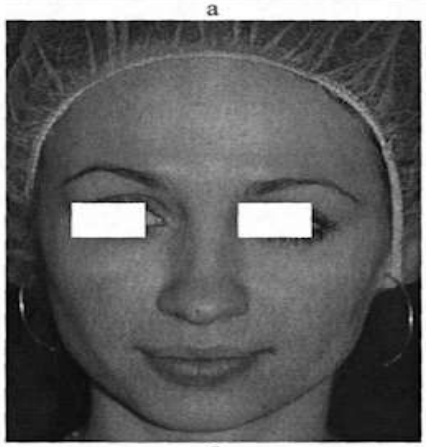

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Picture 4 - Patient X

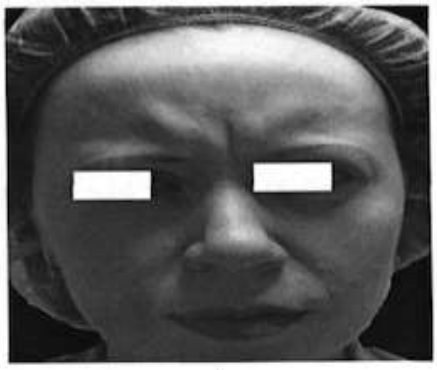
Assessment of the clinical efficacy of treatment with BTA drugs for vertical wrinkles in the eyebrow area:

a - before treatment;

b - after treatment.

There was a complete smoothing of wrinkles, leveling of the skin relief in the area of the eyebrows. The optimal treatment result was obtained for this patient.

Assessment by doctor and patient 3 points



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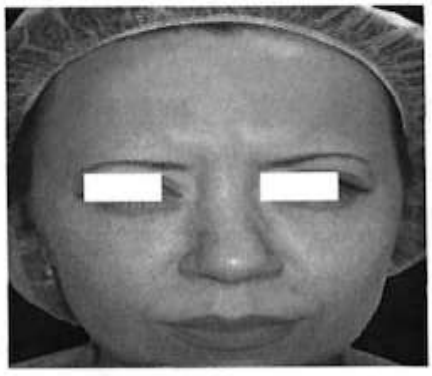

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Picture 5 - Patient X

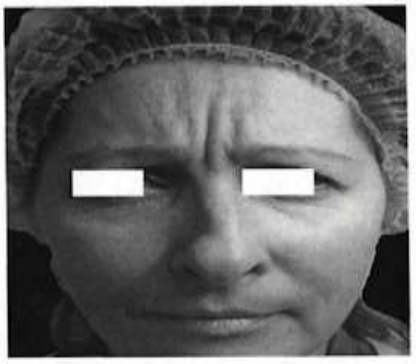
Assessment of the clinical efficacy of treatment with BTA drugs for mimic wrinkles in the eyebrow area:

a - before treatment;

b - after treatment.

Significant reduction in the severity of wrinkles in the area of the brow in comparison with the initial condition.

Assessment by doctor and patient - 2 points



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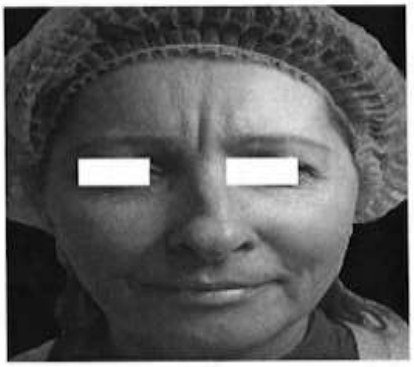

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Picture 6 - Patient X

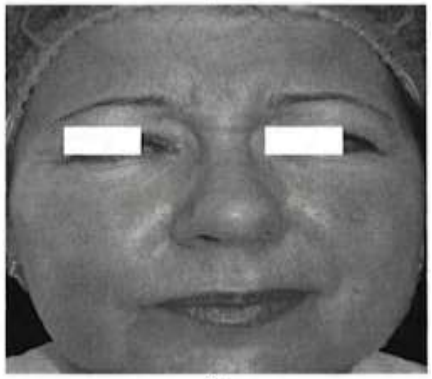
Evaluation of the clinical efficacy of treatment with BTA drugs for mimic wrinkles in the eyebrow area:

a - before treatment;

b - after treatment.

A slight decrease in the severity of wrinkles after treatment.

Assessment by doctor and patient - 1 point



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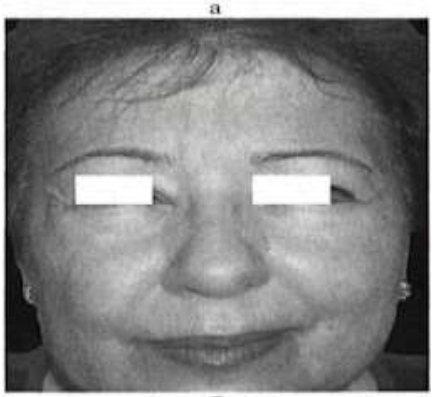

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Picture 7 - Patient X

Evaluation of the clinical efficacy of treatment with BTA drugs for mimic wrinkles in the eyebrow area:

a - before treatment;

b - after treatment.

Incomplete leveling of the skin relief. Doctor's assessment - 2 points. The patient is completely satisfied with the treatment result, the patient's assessment is 3 points

Routine examination of patients was carried out once a month, thus assessing the duration of the muscle relaxant effect of BTA (in months). If muscle relaxation persisted for 6 months, the patient continued to be monitored until the effect was significantly weakened. The distribution of the duration of muscle relaxation after treatment of wrinkles of different localization with BTA injections is shown in Table 5.

Table 5. Duration of muscle relaxation after treatment of facial wrinkles injections of BTA drugs

Site of injections	Duration of the BTA effect, number of patients n=116							Total by zones
	Less than 1 month	Less than 3 months	Less than 4 months	Less than 5 months	Less than 6 months	Less than 7 months	More than 7 months	
Frontal Horizontal	0	3	14	18	26	19	3	83
Between eyebrows	0	2	12	26	29	22	3	94
“Crow-feet”	0	3	10	13	15	19	1	61
Corner of mouth, upper lip, neck	0	1	2	2	3	2	0	10
Result	0	9	38	59	73	62	7	248

Observation of the patients showed that in most cases the effect of muscle relaxation lasted from 4 to 8 months, most often about 6 months. According to the results of the clinical assessment of the results of the treatment of wrinkles, the group of patients was distributed as follows: - good effect of BTA treatment: 79 people (68.1%) (score 3 points); - satisfactory: 32 people (27.6%) (score 2 points); - weak or no effect of BTA treatment: 5 people (4.3%) (score 1 point).

An analysis of the clinical results of the correction of mimic wrinkles with BTA showed that the majority of patients (96%) achieved a positive effect of botulinum therapy. The duration of muscle relaxation in most patients in the group with a good

effect of treatment was 4-8 months, while in the group with a satisfactory effect it was 3-6 months. The effect of muscle relaxation was short-lived only in 4%. Out of 5 people in this group, 3 patients received BTA injections for the first time, one - repeatedly, and one - for the third time. Among the patients who received treatment for the first time, one patient underwent the procedure in the area of the lateral angle of the eye, in the second - in the area of the bridge of the nose and forehead, after the procedure, a decrease in the severity of facial wrinkles was noted, but additional administration of the drug was necessary, which was done, and the third patient - in the upper lip area, and in order to obtain an optimal result in this area, the patient needed a combination therapy. The patients who received botulinum therapy repeatedly, one previously had a positive effect from previous injections, the second was always dissatisfied with something, which did not prevent her from coming for repeated treatment sessions. The rules of storage of the drug, its administration and the adequacy of dosages were observed, the patients did not violate the regimen, however, the expected effect of correction of facial wrinkles was not achieved. Therefore, it was suggested that the reason for the low efficiency of BTA injections was the state of the body of the patients themselves. Thus, the analysis of the clinical results of the correction of mimic wrinkles with BTA preparations in a random sample of patients showed a small number of ineffective cases (4%), which is consistent with the literature data.

3.2. Clinic analyse the results of the treatments of facial movements disorders

One hundred and twelve patients (me 37 and women 75) were enrolled in this study. All patients in the open trial and 6 patients in the double-blind trial improved with the first injection of BTA. Six patients with placebo did not improve.

Forty-five patients (44.5%) with HFS had no maximal effect to initial doses of injection, and required 2nd, 3rd (19.8%) and 4th (3%) additional smaller dose of injection (Table 6.).

Table 6.

Botulinum Toxin (Units)	Hemifacial Spasm						Blepharospasm		
		Additional Injection			Injection for Recurrence		Injection for Recurrence		
	1st	2nd	3rd	4th	2nd	3rd	1st	2nd	3rd
2.5–5.0	5	18	11	2					
7.5–10.0	24	19	7	1	4	1			
12.5–15.0	48	7	2		7	2	1		
17.5–20.0	20	1			6	3	2		
22.5–25.0	4						6		
27.5–30.0							2	2	
32.5–35.0								3	1
37.5–40.0								2	1
Total	101	45	20	3	17	6	11	7	2

* Mean dose of botulinum injection in each patient; Hemifacial Spasm:13.5 units, Blepharospasm:22 units

Each additional dose of injection was given 7 to 10 days after previous injection. Results of BTA shows that 98.6% of total treated patients had an excellent result. Two patients refused further injections.

The durations of beneficial effect ranged from 11 to 40 weeks (mean 16.5 weeks (in HFS and 9-30 weeks (mean 14.2 weeks) in BS.

Peak effect ranged from one to 6 days after injection, and mean peak effect was 4.0 days in HFS and 3.6 days in BS (Table 3.).

It was found that the duration of the effect of botulinum therapy was longer in patients with less severe dyskinesia, enough therapeutic dose and suitable site of injections, and the duration of effect remained the same through subsequent treatments in the same patient.

Complications were encountered in 63.4% in HFS and 72.7% in BS. The most common side effect was dry eyes which was noted in 20 cases (19.8%) in HFS and 3 cases (27.3%) in BS, and mouth droop (facial weakness) was noted in 20 cases (19.8%) in HFS. The second most frequent side effect was ptosis was noted in 2 cases of HFS. In all cases, ptosis was temporary with a mean duration of 2 weeks (range from 1 week to 4 weeks). Ptosis was related to technical failure (injection near center of upper eyelid) or old aged patients who had loose connection tissue of the upper eyelid, so that injected toxin spread to the central part of the upper eyelid. Diplopia (double vision) was noted in 3 cases (2.7%). In one case from weakness of the lateral rectus muscle, in another from weakness of the inferior oblique muscle and in third the cause the diplopia was unidentified. In all cases the diplopia subsided in 1 to 3 weeks. Other complications included lid edema in 6 cases, fatigability in 4 cases and ecchymosis at the injection site in 2 cases. These side effects resolved spontaneously in 1 to 3 weeks.

BTA is currently recognized as an effective and safe therapy for HFS, BS, spasmotic torticollis, oromandibular and laryngeal dystonia (NIH consensus statement, 1991).

Scott et al. (1985) and Dutton et al (1988) found no tendency towards an increased duration of effect in accordance with increasing toxin dose, study have shown that a longer duration of effects of BTA therapy was in milder degrees of dyskinesia as noted by Engstrom (1987), in enough dose of toxin, and in adequate site of injection. Frueh and Musch (1986) suggested that both the duration of spasm relief and the incidents of ptosis and diplopia are directly related to the BTA dose. When dose was increase from 12.5 to 25 units per eyelid, the duration of the spasm relief increased slightly but incidents of ptosis and diplopia increased drastically. Was concluded that the dose of 25 units per eyelid is too high and should not be used.

3.3. Analysis of complications of the use of botulinum toxin type A drugs for the correction of facial wrinkles and facial movement disorders

In addition to evaluating the clinical results of the use of BTA drugs for the correction of facial wrinkles and facial movement disorders, an analysis of complications during treatment was carried out in 116 patients.

In total, out of 116 patients, 73 complications occurred in 10 people (9%). 6 of them received treatment with botulinum toxin type A for the first time. In 9 patients with complications, the aesthetic effect after botulinum therapy was good or moderate.

The characteristics of the noted complications of treatment with BTA drugs are presented in Table 7.

Table 7. Characteristics of the observed complications after the use of BTA drugs.

Treatments result	Number of treatments	Patients	Complications
Good efficacy	First time	Patient 1	Headaches
		Patient 2	Subjective sensation of ptosis eyebrows
	More than 2 times	Patient 3	Flu-like symptoms
		Patient 4	Nausea Day 2
Moderate efficacy	First time	Patient 5	Swelling of the eyelid
	First time	Patient 6	Swelling of the upper eyelids
	First time	Patient 7	Swelling of the upper eyelids. Subjective feeling of ptosis of the eyebrows
	Repeatedly	Patient 8	Swelling of the eyelid
	More than 2 times	Patient 9	Subjective feeling of ptosis of the eyebrows
Low efficacy	First time	Patient 10	Subjective sensation difficulty chewing food and speeches

It should be noted that the so-called common possible complications, in the form of headache and flu-like condition, were noted by two patients, one receiving treatment for the first time, the other for the second time. Also, among those re-treated with BTA drugs, one patient complained of nausea on the second day, however, during subsequent treatment sessions, this complaint was not noted by the patient. Among the possible local complications, edema in the eyelid area was noted in four patients, which is noted when BTA drugs are injected into the brow and forehead area. At the same time, two patients noted a ptosis of the eyebrows after the injection of botulinum toxin into the forehead. At the same time, there were no objective signs of ptosis of the eyebrows, so this sensation was purely subjective. The patient, who received BTA injections in the upper lip, noted a feeling of difficulty in chewing food and talking. All complications that took place were regarded as mild, were of a temporary nature (up to 1-2 weeks) and did not require additional therapeutic measures.

When using BTA drugs in low dosages, there is a possibility of complications. The development of complications is likely both in those who receive the drug for the first time, and after its repeated injections. Moreover, in most cases, the occurrence of complications was observed in persons who had a good or moderate aesthetic effect of treatment with BTA drugs. Thus, cases of complications, with the exception of a single individual reaction, were not associated with a low objective assessment of BTA treatment.

CHAPTER 4. DISCUSSION OF THE OBTAINED RESULTS

The discovery of the possibility of correcting excessive mobility of facial muscles with botulinum toxin type A drugs was a significant progress in medicine and aesthetic fields [Brin M.F., 1997]. Easily performed by experienced doctors, BTA injections are one of the most widespread procedures in modern cosmetology. Their popularity and success among doctors and patients can be associated with good clinical effect, safety, minimal invasiveness and short duration of the procedure itself [Hexsel D., 2005].

However, despite the widespread success of using BTA injections to correct mimic wrinkles and facial muscles disorders, there is a likelihood of an insufficient, weak clinical effect. A review of the available literature revealed a wide range of reasons leading to the ineffectiveness of treatment with BTA drugs. Among the cases of such ineffectiveness, in addition to the low qualifications of the doctor, the patient's misbehavior during therapy or the violation of recommendations for storage and transportation of the drug, there are also cases associated with the individual characteristics of patients [Cote T.R., 2005]. A study devoted to the analysis of the clinical results of correction of mimic wrinkles and facial muscles disorders with BTA drugs in a random choice of patients showed a small number of ineffective cases (4%). Thus, a positive result of treatment was achieved in the vast majority of patients. The duration of muscle relaxation in most patients in the group with a good effect of treatment was 4-8 months, while in the group with a satisfactory effect it was 3-6 months. When assessing the degree of correction of mimic wrinkles due to relaxation of muscles injected with BTA, not only general objective and subjective results were assessed using the GAIS scale, but also paid attention to the areas of the face in which the correction was performed. It was shown that the mean scores on the GAIS scale for each of the zones practically corresponded to the overall mean score, with only slight variations. This indicated that the result of using BTA for the treatment of wrinkles did not depend on the

injection zone into which it was injected and was practically the same for each zone. It was also shown that physician and patient assessments of clinical effect on the GAIS scale differed very slightly. The discrepancy between the estimates by 1 point was noted in 4% of cases, while the discrepancy between the doctor and the patient concerned the choice between a good and a moderate result of therapy. Low efficiency was interpreted by doctor and patient without discrepancy. Such results testified to the adequate dosage of the drug during the treatment, the correct explanation by the doctor and the patient's understanding of the expected results from the treatment. When using BTA drugs in low dosages, there is a possibility of complications. Of the complications encountered, the most common when using BTA in cosmetology and medicine are drooping of the eyebrows, edema and swelling of the periorbital region, hypo- and hypercorrection, asymmetry of the effect, headache, impaired articulation, ptosis, and diplopia. All of these symptoms are reversible and temporary [Cote T.R., Mohan A. et al., 2005; Orlova O.R., 2006; Naumann M., 2008].

When analyzing the clinical results of the use of BTA drugs in order to correct mimic wrinkles of the face in patients who took part in the study, an analysis was carried out that took place in the treatment of complications. In total, out of 116 patients, complications occurred in 10 people (9%). Of these, 6 people received treatment with BTA drugs for the first time. In 9 patients with complications, the effect of BTA therapy was good or moderate.

In study of the patients with facial muscle movement disorder (112 patients) was noted that 98,6% had an excellent result, with maximum duration of the treatment of 11 to 40 weeks. Complications were observed in 63,4% in HFS and 72.7% in BS.

All complications that took place were regarded as mild, which did not require additional assistance, were temporary (up to 1-2 weeks) and were stopped on their own. Thus, the results obtained are consistent with the literature data and indicate that the complications of treatment with BTA drugs are not associated with the clinical effect and

can be observed both in patients receiving BTA for the first time and with its repeated administrations.

Despite the fact that the number of cases of a weak effect of correction of mimic wrinkles and facial muscles movement with BTA drugs is small, nevertheless, the question of the reasons for such an insufficient effect is relevant due to the high cost of the procedure and the patient's high expectations from therapy. In the available literature there are works devoted to the analysis of complications arising from the correction of facial wrinkles with BTA drugs. These works are based on the analysis of reports of adverse events collected over a certain period of time [Cote T.R., 2005]. However, no works devoted to the study of doctors' reviews on the use of BTA drugs in cosmetology and plastic surgery were found. The questioning of doctors made it possible to reveal the frequency of low efficiency of wrinkle correction with BTA drugs and the presumptive causes of its occurrence, which do not have objective confirmation. Summing up the results of the survey of doctors, we can single out the ratio of various reasons for the ineffectiveness of wrinkle correction and facial muscle movement correction with BTA drugs. The reasons that can be called objective, independent of the doctor's erroneous actions or the patient's misbehavior, in their aggregate accounted for, in the opinion of the respondents, 59% of all sources of ineffectiveness of muscle correction with BTA drugs. The rest accounted for 39%, they included reasons depending on the actions of the doctor or patient, i.e., subjective: frequent and booster injections, poorly collected anamnesis, insufficient doses of BTA drugs. The reasons related to improper transportation and storage of the drug accounted for 2%. Thus, if we exclude the low efficiency of wrinkle correction with BTA preparations, which depends on the actions of the doctor or the patient, there remains a significant proportion, perhaps more than half, of the objective reasons that are a property, a feature of the functioning of the patient's body.

It can be assumed that the remaining objective reasons for the low efficiency of wrinkle correction with BTA drugs are associated with the presence of antibodies to BTA. The patient's low sensitivity to the drug and its individual characteristics can be compared with antitelogenesis, since the formation of neutralizing antibodies is, on the one hand, a natural mechanism for the elimination of a protein toxin, and, on the other hand, the main known objective reason for the low effectiveness of BTA drugs, which is described in the existing literature [Naumann M., Hamm H. 2004]. Some authors associate a decrease in BTA sensitivity with a change in the properties of membrane toxin acceptors [Cote T.R., 2005; Timerbaeva SL, 2012], without explaining the detailed mechanisms of this phenomenon. A number of studies have been carried out to identify antitelogenesis to BTA drugs.

For this purpose, a targeted search was carried out for patients who had a weak effect or no effect from the introduction of low doses of BTA drugs. It has been shown that in 87% of patients receiving botulinum therapy, antibodies against BTA in different titers are detected in the blood. More often, resistance was found in individuals with a higher titer of antibodies and could be overcome by increasing the dose of the drug.

Similar results are described in the literature [Dressier D., 2008; Dressier D., Saberi F.A., 2009; Lange O., Bigalke H., 2009]. However, the role of antitelogenesis in the formation of resistance is still a subject of discussion, not all authors pay significant importance to it [Temirbaeva S.L., 2012]. It is quite possible that the main role in the formation of resistance is played not so much by the presence of the antibodies themselves as by the balance between the doses of BTA used and the levels of blocking antibodies [Dressier D., Miinchau A., 2002]. Nevertheless, in this study, the analysis of antitelogenesis to BTA and its preparations does not provide a final understanding of its role in the formation of resistance to BTA. At the same time, the weakening of the therapeutic effect was not significantly associated with the detection of antibodies.

However, the performed correlation analysis did not show the presence of a relationship between the severity of the clinical effect and the frequency of BTA injections in adult patients in cosmetology and medical practice. But they showed a direct weak correlation between the frequency of procedures and the presence of antibodies to botulinum toxins.

In modern medical practice, a number of BTA drugs are used, produced by different manufacturers. These drugs differ in the processes of their production, the strains of the bacteria used, the degree of purification, the methods of stabilization, the determination of biological activity, and, therefore, the properties of the drugs and their biological activity differ: the size and structure of molecules, diffusion properties. Therefore, it is possible that BTA drugs from different manufacturers will stimulate the production of different antibodies with different specificity to different commercial drugs. Accordingly, the produced antibodies can have different effects on the activity of different BTA drugs [Hefter N. et al., 2009; Frevert J., Dressier D., 2010]. The answer to the question why antibodies specifically to abobotulinumtoxinA, and not to onabotuIinumtoxinA, are more associated with sensitivity to treatment with BTA drugs, is not yet clear.

CONCLUSIONS

1. Under the action of botulinum toxin type A drugs, 96% of patients with mimic wrinkles smooth out wrinkles, smooth the skin relief and improve the appearance according to the GAIS scale.
2. Under the action of botulinum toxin type A drugs, 98.6 % had an excellent effect with facial muscle hypertonic movements reduction.
3. Therapy with botulinum toxin type A preparations leads to a pronounced improvement in subjective indicators of clinical status in 96% of patients with mimic wrinkles, corresponding to the most complete smoothing of wrinkles.

4. The leading reasons for the lack of effect of treatment with botulinum toxin type A drugs in patients with mimic wrinkles and facial muscle tonus are: the quality of the drug (2.6%), doctor's error (23.2%), non-compliance with the recommendations by the patient (36.8%), individual characteristics of the patient (59.4%).
5. A pronounced corrective effect of type A botulinum toxin preparations is formed in 59.8% of patients with mimic wrinkles and facial muscles movement disorders with the presence of antibodies and in 40.2% with their absence.

PRACTICAL RECOMMENDATIONS

1. In the absence of the desired clinical effect after the administration of botulinum toxin type A drugs, attention should be paid to the implementation of the recommendations by the patient, to exclude the doctor's technical error, to check the quality of the products used. If all the above conditions are met, it is advisable to examine the patient for cytokine status and the presence of antibodies to the drug.

2. The main indicators allowing to evaluate the possible effect of treatment of patients with mimic wrinkles with injections of BTA drugs are serum levels of cytokines IL-ip, TNF-a, IL-6. As an auxiliary indicator, the determination of antibodies to abobotulinumtoxin A in ELISA is recommended.

3. The main indicators allowing to evaluate the possible effect of treatment of patients with facial movements disorders with injections of BTA drugs are serum levels of cytokines IL-ip, TNF-a, IL-6. As an auxiliary indicator, the determination of antibodies to abobotulinumtoxin A in ELISA is recommended.

4. In patients with a good and moderate effect of treatment with BTA drugs, the levels of cytokines in the blood are: IL-ip not less than 97.6 pg / ml, IL-6 not less than 16.3 pg / ml, TNF-a not less than 4.8 pg / ml. In an enzyme-linked immunosorbent assay for antibodies to BTA preparations, the positivity coefficient should be less than 2 units.

5. A specific feature of the immunoregulatory status in patients with weak or no effect of treatment of mimic wrinkles with BTA drugs is low levels of cytokines IL-ip less than 18.0 pg / ml, IL-6 less than 10.4 pg / ml and TNF-a less than 1. 2 pg / ml in serum. In an enzyme-linked immunosorbent assay for antibodies to botulinum toxin type A drugs, the positivity coefficient is more than 2.

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APPENDIX 1

Questionnaire for doctors and medical cosmetologists

Therapy with BTA for medical and cosmetic purpose:

1. How long have you been using BTA products in your practice?

- less than 1 year
- 1-2 years
- 3-5 years
- more than 5 years.

2. What drugs do you use in your work?

- Botox;
- Dysport;
- Xeomin;
- others (indicate which) _____

3. What side effects and complications have you observed when using botulinum toxin type A drugs?

- unsatisfactory correction (facial asymmetry);
- allergic reactions;
- inflammatory reactions;
- headaches, weakness, nausea, vomiting (circle one or more)
- disorders of the nervous system;
- a wider area of action of the drug outside the injection site;
- others (indicate) _____

4. What, in your opinion, were such complications connected with?

- insufficient information from the manufacturer or training companies;

- incorrect tactics of using the botulinum toxin preparation;
- non-compliance by the patient with the rules of behavior after the injection of the drug;
- individual characteristics of the patient.
- others (indicate) _____

5. Have you encountered cases of low efficiency or complete lack of effect from botulinum toxin preparations?

A) at the first introduction:

- not;
-Yes;

If yes, indicate the possible reason, in your opinion _____

B) with repeated administrations:

- not;
-Yes;

If yes, indicate the possible reason, in your opinion _____

APPENDIX 2

PATIENT’s EXAMINATION CARD

FULL NAME _____

Date of Birth (DOB)_____

Address, telephone_____

Occupation _____

Primary diagnosis _____

Accompanying diseases _____

Anamnesis:

2.Allergic history (allergy to food, drugs) _____

4. Pregnancy at the present time, breastfeeding _____

5. Vaccinations. _____

6. Bad habits (smoking, drinking) _____

7. Lifestyle (active, passive)

- physical education, sports, how many times a week _____

9. Tendency to form keloid and hypertrophic scars _____

10. Endocrine diseases _____

11. Skin diseases _____

12. Hemophilia _____

13. A history of neurological diseases (myasthenia gravis, Lambert's syndrome - Eaton) _____

14. Current or past use of anticoagulant drugs, hormonal drugs _____

15. The use of drugs that enhance the effect of botulinum toxin:

- antibiotics aminoglycosides (erythromycin) (YES/NO)
- curariform muscleloxants
- tetracycline
- lincomycin

- polymyxin

16. Diabetes mellitus _____

17. Obesity, exhaustion, malnutrition _____

18. Cardiovascular system (Hypertension, hypotension) _____

21. The use of BTA in the past (YES/NO)_____

Date Drug name Number of units _____

22. Clinical effect

Duration (number of weeks) Effectiveness (+ good effect)

(-lack of effect)

(± weak, short-lived)

23. Cosmetology procedures performed during the last

6 months (phototherapy, physiotherapy, mesotherapy, peels, massage) (Circle if any)

Treatment Protocol

1.Date of the last BTA procedure _____

2. Name, series of the drug _____

3. Number of units _____

4. Muscle activity (subjective assessment by the patient on a 5-point GAIS scale) up to procedures _____

(objective assessment by a doctor on a 5-point GAIS scale) before the procedure

_____

5. Condition after the procedure (on what day did the effect appear, assessment of the effectiveness patient and doctor on a 5-point scale after 14 days) _____

6. Duration of effect _____

7. Complications _____

Date of the procedure _____

Doctor _____ Signature _____