Long-Term Suppression of Atrial Fibrillation by Botulinum Toxin Injection into Epicardial Fat Pads in Patients Undergoing Cardiac Surgery: One Year Follow Up of a Randomized Pilot Study

Running title: Pokushalov et al.; Botulinum Toxin Injection into Fat Pads

Evgeny Pokushalov, MD1; Boris Kozlov, MD2; Alexander Romanov, MD1; Artem Strelnikov, MD1; Sevda Bayramova, MD1; David Sergeevichev, PhD1; Alexander Bogachev-Prokophiev, MD1; Sergey Zheleznev, MD1; Vladimir Shipulin, MD2; Vladimir V. Lomivorotov, MD1; Alexander Karaskov, MD1; Sunny S. Po, MD3; Jonathan S. Steinberg, MD4

1State Research Institute of Circulation Pathology, Novosibirsk; 2Institute of Cardiology, Siberian Division of Russian Academy of Medical Sciences, Tomsk, Russia; 3Heart Rhythm Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4University of Rochester School of Medicine and Dentistry & The Valley Health System, New York, NY

Correspondence:
Jonathan S. Steinberg, MD
200 West 57th Street, Suite 610
New York, NY 10019
Tel: 212-432-7837
Fax: 212-432-7830
E-mail: steijo@valleyhealth.com

Journal Subject Terms: Arrhythmias; Catheter Ablation and Implantable Cardioverter-Defibrillator
Abstract:

**Background** - Animal models suggest that the neurotransmitter inhibitor, botulinum toxin, when injected into the epicardial fat pads can suppress atrial fibrillation (AF) inducibility. The aim of this prospective randomized double-blind study was to compare the efficacy and safety of botulinum toxin injection into epicardial fat pads for preventing atrial tachyarrhythmias.

**Methods and Results** - Patients with history of PAF and indication for coronary artery bypass graft (CABG) surgery were randomized to botulinum toxin (Xeomin, Merz, Germany; 50 U/1 mL at each fat pad; n=30) or placebo (0.9% normal saline, 1 mL at each fat pad; n=30) injection into epicardial fat pads during surgery. Patients were followed for 1-year to assess maintenance of sinus rhythm using an implantable loop recorder (ILR). All patients in both groups had successful epicardial fat pad injections without complications. The incidence of early postoperative AF within 30 days after CABG was 2 of 30 patients (7%) in the botulinum toxin group and 9 of 30 patients (30%) in the placebo group (P=0.024). Between 30 days and up to the 12-month follow-up examination, 7 of the 30 patients in the placebo group (27%) and none of the 30 patients in the botulinum toxin group (0%) had recurrent AF (P=0.002). There were no complications observed during the 1-year follow-up.

**Conclusions** - Botulinum toxin injection into epicardial fat pads during CABG provided substantial atrial tachyarrhythmia suppression both early as well as during 1-year of follow-up, without any serious adverse events.

**Clinical Trial Registration** - [http://www.clinicaltrials.gov](http://www.clinicaltrials.gov); Unique identifier: NCT01842529.

**Key words**: arrhythmia (heart rhythm disorders), atrial fibrillation, CABG, botulinum toxin, ganglionated plexi, autonomic nervous system
Introduction

Atrial fibrillation (AF) is a common complication of cardiac surgery,\textsuperscript{1-3} with an incidence of 10\% to 50\% and typical appearance at 2 to 5 days after surgery.\textsuperscript{4-5} Postoperative AF may lead to serious complications such as increased risk of acute kidney injury, hemodynamic instability, cardiac failure, stroke, and death.\textsuperscript{6-8} Postoperative AF substantially increased the cost of coronary artery bypass graft (CABG) as well.\textsuperscript{7-11} Guidelines strongly recommend using beta-blockers to prevent post-op AF,\textsuperscript{12-14} however all therapeutic options have variable efficacy and some may adversely affect hemodynamic stability.\textsuperscript{15-20}

Experimental and clinical data suggest that the autonomic nervous system may play a critical role in the initiation and maintenance of AF.\textsuperscript{21-23} Prior studies on spontaneous initiation of AF in patients and animals indicated that activation of both the sympathetic and parasympathetic nervous systems often preceded the onset of AF.\textsuperscript{24-27} Targeting cardiac autonomic ganglia by endocardial or epicardial fat pad ablation has been shown to successfully suppress AF.\textsuperscript{28-31} Recently, animal studies demonstrated that neurotoxins such as botulinum toxin injected into these fat pads could suppress AF inducibility.\textsuperscript{32-33}

Botulinum toxin blocks the exocytotic release of acetylcholine stored in synaptic vesicles and thus interferes with cholinergic neurotransmission, which is important in postganglionic neurons.\textsuperscript{34} The blocking effects are temporary and recover in 1 to 6 months, depending on the injection site.\textsuperscript{35} For patients with a high short-term risk of postoperative AF after cardiac surgery, temporary suppression of AF without any destruction of the anatomic structures is clinically desirable.

We previously reported the short-term results of the “first-in-man” prospective randomized double-blind study to compare the efficacy and safety of botulinum toxin injection
into epicardial fat pads for the prevention of postoperative atrial tachyarrhythmia in patients with paroxysmal AF (PAF) undergoing coronary artery bypass graft (CABG) surgery. This study demonstrated that botulinum toxin injection substantial suppressed postoperative atrial tachyarrhythmias during the first postoperative month after CABG without any serious adverse events. We hypothesized that by suppression of the ganglionated plexi (GP) activity in the epicardial fat pads, longer term antiarrhythmic effects can be achieved.

Methods

The methodologic details of this study were published previously. Briefly, this was a randomized, double-blind, parallel-group, two-center study that compared treatment with botulinum toxin to normal saline (placebo). The study protocol was approved by the local Ethics Committee and conducted in compliance with the protocol, and in accordance with standard institutional operating procedures and the Declaration of Helsinki. All patients enrolled in the study provided written informed consent. The study was registered with ClinicalTrials.org (NCT01842529).

Patient Population

Patients were eligible based on the following criteria:

Inclusion criteria:

(i) Preoperative history of PAF

(ii) Indication for CABG according to the American College of Cardiology/American Heart Association (ACC/AHA) guidelines for CABG surgery

Exclusion criteria:

Previous heart surgery or AF ablation procedure

Emergency CABG
Unstable angina or heart failure

Persistent AF or AF at the time of screening

Planned maze procedure or pulmonary vein isolation

Use of class I or III antiarrhythmic drugs within 5 elimination half-lives of the drug (or within 2 months for amiodarone)

Need for concomitant valve surgery

Unwillingness to participate

Patients were randomized to botulinum toxin (n=30) or placebo (n=30) injection into epicardial fat pads using a coded envelope system opened on the day of the surgery. Patients were followed for one year to assess maintenance of sinus rhythm (Fig. 1).

The primary endpoint was recurrence of any atrial tachyarrhythmia, including AF and atrial tachycardia, during the follow-up after CABG procedure on no antiarrhythmic drug. The secondary endpoints included time intervals from end of surgery to extubation and discharge from ICU, CK-MB levels, post-CABG length of stay, congestive heart failure, sustained ventricular arrhythmias, myocardial infarction, infection, renal failure, respiratory failure, stroke or transient ischemic attack, rehospitalization, readmission to ICU, death, heart rate variability (HRV) and AF burden during the 12-month follow-up based on implantable loop recorder (ILR) findings. All end points were independently adjudicated by 2 blinded cardiologists.

**Botulinum Toxin/Placebo Injection**

All patients underwent conventional CABG. After the main stage of the surgery, botulinum toxin (Xeomin, incobotulinumtoxin A, Merz Pharma GmbH & Co KGaA, Germany; 50 U/1 mL at each fat pad; botulinum toxin group) or 0.9% normal saline (1 mL at each fat pad; placebo group) was injected into the entire visible area of the four major epicardial fat pads (Fig. 2).
Injections were performed using a conventional insulin syringe (1 ml) in two stages. First stage was a needle puncture at 45 degrees relative to the surface of the fat pad at a depth of 2 mm; the second included an additional 2 mm advancement of the needle parallel to the surface of the pad. After needle placement, adipose tissue infiltration was performed by injection of solution at volume 0.2-0.3 ml per puncture. The infiltration zone had a distinctive whitish color and clear boundary, and was 5-6 mm in diameter. To cover the entire surface of the fat pad, it was necessary to create 3 to 5 such infiltration zones. The first epicardial left atrial fat pad was located anterior to the right superior pulmonary vein and corresponded to the anterior right GP (Fig. 2A); the second epicardial fat pad was located infero-posterior to the right inferior pulmonary vein and corresponded to the inferior right GP (Fig. 2B); the third fat pad was located anterior to the left superior PV and left inferior PV (between the PVs and LAA) and corresponded to the Marshall tract GP and superior left GP (Fig. 2C); and the fourth fat pad was located inferiorly to the left inferior PV and extended posteriorly and corresponded to the inferior left GP (Fig. 2D).

**Implantable Loop Recorder**

The ILR (Reveal XT, Medtronic, Inc., Minneapolis, MN) was implanted on the day of the CABG procedure in all patients. The ILR protocol has been defined in detail previously. The device stores the amount of AF per day (daily AF burden, hours of AF in 1 day) and the AF burden of the overall follow-up period, defined as the percentage of time spent in AF (AF%). In addition, the ECG is stored for the visual confirmation of AF episodes. AF was visually verified by investigators through the analysis of the stored ECGs. By compiling data from multiple follow-up sessions, it was possible to discern the trend in the AF burden over prolonged periods.

**Patient Follow-up**

All antiarrhythmic drugs were discontinued before the CABG procedure, regardless of the
cardiac rhythm. Re-hospitalization and treatment for AF were monitored. The data stored by the ILR were collected at 7, 14, 21 and 30 days and then at 3, 6, 9, and 12 months. At each follow-up visit, all AF recordings were manually reviewed to confirm automatic detections. Any erroneous designations were removed. Patients were provided with the Patient Assistant, a tool that allows each patient to store the ECG through the implanted device during symptoms; data were collected in order to analyze heart rhythm during symptomatic events.

**Definition of Responders**

Patients with an AF% $\leq 0.5\%$ were considered AF-free (Responders), while those with an AF% $> 0.5\%$ were classified as patients with AF recurrences (non-Responders). This cut-off of 0.5% corresponds to a maximum cumulative time in AF of 3.6h in 1 month and to more than 99.5% of the time spent in sinus rhythm during the overall follow-up period. AF was visually verified by investigators through the analysis of the stored ECGs and all follow-up visits. ILR interpretations were made by physicians not aware of the randomized ablation treatment.

**Heart Rate Variability**

HRV as an indicator of autonomic nervous system activity was evaluated by a standard technique before and after CABG procedure when on no antiarrhythmic drugs. Analysis of 24-h monitoring data was performed by considering SDNN, the standard deviation of RR intervals over the entire analyzed period, and rMSSD, the root-mean-square of differences between successive RR intervals. The absolute values (ms) of the power of the low frequencies (LFs) and high frequencies (HFs) were investigated. The autonomic balance was assessed by the spectral LF to HF ratio (LF/HF). Mean sinus rate and time domain and frequency domain analyses of HRV were obtained from 24-h Holter records before and after the surgical procedure.
Statistical Analysis

Results are expressed as mean values ± SD for continuous, and frequencies and percentages for categorical variables. Continuous variables were compared by Student’s t test or Wilcoxon test as appropriate. Categorical secondary end points and categorical demographic, clinical, adverse events and CABG variables were compared by either χ² test or Fisher’s exact test. Differences in arrhythmia-free survival were assessed by using the log-rank test. Linear mixed effects models with fixed (treatment and time) and random (subject) effects were used for analysis of treatment by time interactions. Based on Mauchly’s test, the assumption of sphericity was considered as valid for all mixed-effects models. After establishing overall significance of a model, Tukey’s all-pair post-hoc comparisons with adjustment for multiplicity were carried out.

All reported p values were based on two-sided tests and a p-value of <0.05 was considered significant. All statistical calculations were performed by using the SPSS version 13.0 software (SPSS Inc, Chicago, Il, USA).

Results

Study Patients

There were no baseline differences between groups (Table 1). There were no differences in the number of grafts placed, duration of aorta cross-clamping, or number of off-pump CABGs performed between groups (all P > 0.05) (Table 2).

AF Freedom during Follow-up

Based on ILR data, the incidence of early postoperative AF (up to 30 days follow-up) was 2 of 30 patients (7%) in the botulinum toxin group and 9 of 30 patients (30%) in the placebo group (P=0.024, log-rank). After 30 days and up to the 12-month follow-up examination, 7 of the 30 patients in the placebo group (27%) and none of the 30 patients in the botulinum toxin group
(0%) had recurrent AF (P=0.002, log-rank). AF% was significantly higher in the placebo group than in the botulinum toxin group through the entire follow-up period: the mean AF burden was 1.7±3.1% and 0.3±0.5%, respectively (p<0.001; Fig. 3).

All patients were completely asymptomatic in the botulinum toxin group and 2 patients were symptomatic in the placebo group. In the placebo group at the end of follow-up, 6 patients with recurrences had paroxysmal AF and required treatment with antiarrhythmic drug; 2 patients had progression to persistent AF and underwent catheter ablation. None of the patients in the botulinum toxin group received any form of antiarrhythmic therapy or intervention, and none had developed persistent AF.

**Safety Data**

There were no significant differences in the median time of extubation, or intervals from the end of surgery to discharge from the ICU (all P > 0.05) (Table 3). There were no significant differences in CK-MB levels in the postoperative period. There was no significant difference in the postoperative hospital length of stay between groups (P=0.12). Other postoperative complications (see Methods), including death, were similar between groups (all P > 0.05; Table 4).

**Heart Rate Variability**

The results of time- and frequency-domain HRV are shown in Table 5. After surgery, all the time- and frequency-domain HRV parameters decreased precipitously in both groups. At 1-month follow-up, SDNN decreased from baseline by 45% (P<0.05) after botulinum toxin injection and by 49% (P<0.05) in the placebo group; rMSSD dropped by 69% (P<0.05) and 55% (P<0.05) respectively; LF decreased by 77% (P<0.05) and 71% (P<0.05) respectively; HF fell to the lowest levels in both groups by 76% and 66% respectively; LF/HF decreased by 43% and
23% respectively (P<0.05 in both cases). At the same time, mean HR increased more in the botulinum toxin group than in the placebo group, by 22% and 13% respectively (P<0.05 for both within and between-group comparisons).

At 3-months, HRV parameters relatively recovered in the placebo group, whereas they remained largely depressed in the botulinum toxin group. In the placebo group, SDNN increased by 61% (P<0.001), rMSSD increased by 71% (P<0.001); LF by 175% (P<0.001); HF by 131% (P<0.001); and LF/HF by 35% (P<0.001) as compared to 1 month.

At 6-months in the botulinum toxin group, the time- and frequency-domain HRV parameters remained significantly suppressed as compared to baseline, but demonstrated a strong trend toward recovering. The results in the placebo group were similar to values observed at 3 months.

At the end of the follow-up period, time- and frequency-domain HRV characteristics were not significantly different from those observed at 6 months in both groups, and no between-group differences were detected.

**Discussion**

The main findings of this double-blind, randomized, placebo-controlled study were: (1) botulinum toxin intraoperative injection into the epicardial fat pads prevented recurrences of atrial tachyarrhythmia in patients with prior PAF undergoing CABG surgery; (2) the botulinum toxin injection did not delay timing of discharge from the ICU, increase postoperative hospital length of stay or cause any other identifiable postoperative complication; (3) botulinum toxin injection induced pronounced alteration of HRV components for approximately 6 months, suggesting reduction of both parasympathetic activity and sympathetic activity; (4) despite the
finding that HRV changes dissipated in 6 months, botulinum toxin injection was associated with a lower AF burden during one year of follow-up.

AF is a common complication of cardiac surgery with an incidence of 10% to 50%.\textsuperscript{1-5} In a recent study, Aranki et al. demonstrated that 70% of postoperative AF occurrence was clustered around the first four postoperative days, 94% being diagnosed in the first 6 days and only 6% more than a week after surgery.\textsuperscript{10} Postoperative AF was more often seen in females and in older patients, especially those with a history of chronic heart failure. Furthermore, a higher EuroSCORE and lower EF predicted the development of postoperative AF.\textsuperscript{10,40} Patients with postoperative AF were more likely to be diagnosed with minor as well as major complications (i.e. stroke, MI, acute renal failure requiring dialysis, and sternum dehiscence).\textsuperscript{41} Postoperative AF patients also had longer total length of hospital stay, and specifically the stay in ICU can more than doubled.\textsuperscript{42} Postoperative (<30-day) mortality was 5% in patients who were diagnosed with postoperative AF, as compared to only 0.7% in patients with postoperative normal sinus rhythm. The difference in survival is also reflected in long-term survival: an 83% 5-year survival for postoperative AF patients and 93% for postoperative normal sinus rhythm patients.\textsuperscript{42}

Autonomic imbalance and the interaction between inflammatory substances inducing GP hyperactivity play a major role in the mechanism of postoperative AF.\textsuperscript{43,44} The operative procedure results in release of inflammatory substances that could activate nerve endings of the atrial neural network, instigating AF, as shown in experimental models.\textsuperscript{44,45}

A number of previous studies have shown an advantage of prophylactic beta-blocker therapy for postoperative AF, but owing to their possible negative inotropic effects and a lack of consensus on clinical guidelines, their use has not been universal.\textsuperscript{46} Furthermore, preoperative anti-arrhythmic medications are often withheld for several days, which might further compound
the risk of arrhythmias.

Lu et al., using a rapid atrial pacing model, clearly demonstrated the role of GP ablation in the suppression of AF. They showed that shortening of the effective refractory period, increase of effective refractory period dispersion, and increased AF inducibility caused by rapid atrial pacing were all reversed by ablation of the 4 major atrial GP and the ligament of Marshall. In animals receiving GP ablation first, rapid atrial pacing for 6 hours failed to change the effective refractory period, effective refractory period dispersion, and AF inducibility. They demonstrated that several clinical studies also indicated the benefits of autonomic denervation by targeting the major atrial GP.22,30,47-50

Tsuboi et al. tested selective injection of botulinum toxin A into the fat pad for prevention of bradycardia induced by vagal nervous stimulation in the dog heart. They demonstrated that botulinum neural toxin A selectively injected into the sinoatrial fat pad inhibited a decrease in sinus rate in response to vagus nerve stimulation, and suggested that botulinum toxin can inhibit ganglionic neurotransmission in the dog heart in situ. In a study by Yu et al., a neurotoxic agent (N-isopropylacrylamide monomer) in the anterior right and inferior right GPs reduced GP activity with a subsequent increase in the AF threshold.32 In a recent study, Oh et al. investigated the short- to-mid-term effects of GP suppression by botulinum toxin on AF inducibility in the dog. The vagal stimulation effects on dispersion of ERP and AF inducibility were significantly attenuated at 1 week after botulinum toxin injection and recovered at 2 to 3 weeks. These findings suggest that temporary suppression of AF may be achieved with botulinum toxin in canine models, possibly by reduced dispersion of ERP.33

Suppression of HRV after surgical MAZE or CABG is a well-recognized phenomenon.52-53 Surgical MAZE involves extensive injury to the GP and autonomic nerves, leading to
persistent changes of the HRV beyond a year. The changes in HRV after CABG is thought to result from manipulation of the heart and adjacent structures, prolonged anesthesia, and use of cardioplegics and extracorporeal circulation. In the present study, all the HRV parameters were markedly suppressed in both groups after surgery. In the botulinum toxin group, all the HRV parameters remained suppressed while the same parameters had recovered to the pre-CABG levels in the control group, consistent with prior reports that HRV changes returned to normal within 4 months after CABG. The effects of botulinum toxin injection lasted for at least 3 months but dissipated before 6 months, consistent with the expected duration of its neuro-suppressive effects.

An intriguing finding of the present study is that the antiarrhythmic effects appeared to persist through the one-year follow-up. It is known that autonomic innervation of the heart consists of a large neural network and GP serve as the “integration centers” in the neural network.23,27-32 The major atrial GP, targeted by botulinum toxin in the present study, contain hundreds to thousands of autonomic neurons, mainly post-synaptic cholinergic neurons activated by presynaptic acetylcholine. The neural network controls the regional electrophysiological functions such as the refractory period and dispersion of the refractoriness. While HRV has been used for decades to measure the “global” sympathovagal balance, its activity often does not correlate with the neural activity of the cardiac autonomic neural network. For instance, individual values for LF power and LF:HF are not correlated with cardiac noradrenaline spillover and LF power is not associated with myocardial sympathetic innervation by 11C-hydroxyephedrine scanning.54 The lack of correlation between the neural activity of the atrial neural network and HRV may explain the observation in the present study that the antiarrhythmic effects lasted beyond the HRV changes.
Animal studies demonstrated that autonomic hyperactivity and AF form a vicious cycle. The former can initiate AF and the latter can further enhance the autonomic neural activity. Suppression of the GP activity may be able to break this vicious cycle and suppress AF by prolonging the effective refractory period, reducing the dispersion of refractoriness, inhibiting triggered firing of both pulmonary veins (PV) and non-PV tissue as well as suppressing both the sympathetic and parasympathetic neural activity. It is also possible that reduction in early AF events facilitated reverse remodeling, which then helped diminish subsequent AF events. We hypothesize that these salutary effects led to the antiarrhythmic effects of botulinum toxin reported in the present study. In other words, by breaking the vicious cycle formed by AF and autonomic hyperactivity, the antiarrhythmic effects of botulinum toxin, which was expected to last for 2-3 months, extended to a year.

Limitations
This is the “first-in-man” study of botulinum toxin injection into the epicardial fat pads so that a limited number of patients were enrolled. Although the study was randomized, double-blind and prospective, the results will require validation in additional and larger trials. The denervation effect of the sympathetic and parasympathetic systems was not confirmed by precise objective tests such as quantitative measurements of norepinephrine and acetylcholine, or by intracardiac electrophysiological measurements. Also the study has not confirmed the presence of, or the precise mechanisms, by which functional atrial remodeling by botulinum toxin injection may occur.

The AF burden before procedure of each patient was not available and there might be a difference between the botulinum toxin group and control group. However, the potential bias is minimized by randomization of the study patients. The use of ILR is advantageous for
completeness of AF data collection but may detect more episodes than many centers routinely capture using external ECG methods and does not exactly conform to HRS guidelines for tracking AF outcomes.¹³

Conclusions

Botulinum toxin injection into the epicardial fat pads reduced cardiac autonomic nervous system activity and provided substantial postoperative AF suppression after CABG without any serious adverse events. The favorable reduction of AF was present within the 30-day postoperative window, but also during longer follow-up over 1 year.

Conflict of Interest Disclosures: None

References:


54. Goldstein DS, Bentho O, Park MY, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Exp Physiol. 2011;96:1255-1261.

Table 1: Characteristics of Patient Groups

<table>
<thead>
<tr>
<th></th>
<th>Botulinum toxin (n=30)</th>
<th>Placebo (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.8 ± 7</td>
<td>62.7 ± 6</td>
<td>0.56</td>
</tr>
<tr>
<td>Sex, male</td>
<td>23 (77%)</td>
<td>25 (83%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>18 (60%)</td>
<td>16 (53%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>2 (7%)</td>
<td>2 (7%)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>59 ± 7</td>
<td>58 ± 5</td>
<td>0.89</td>
</tr>
<tr>
<td>Left atrial size, cm</td>
<td>47 ± 8</td>
<td>48 ± 6</td>
<td>0.58</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25 (83%)</td>
<td>26 (87%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>23 (77%)</td>
<td>23 (77%)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (37%)</td>
<td>10 (33%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Obstructive lung disease</td>
<td>2 (7%)</td>
<td>3 (10%)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values are n (%) or mean ± SD

Table 2: CABG Surgery Data

<table>
<thead>
<tr>
<th></th>
<th>Botulinum toxin (n=30)</th>
<th>Placebo (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of saphenous vein grafts</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>0</td>
<td>3 (10%)</td>
<td>4 (13%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (10%)</td>
<td>2 (7%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20 (67%)</td>
<td>21 (70%)</td>
<td></td>
</tr>
<tr>
<td>3-5</td>
<td>4 (13%)</td>
<td>3 (10%)</td>
<td></td>
</tr>
<tr>
<td>Number of internal mammary artery grafts</td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>1</td>
<td>29 (97%)</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Off-pump bypass</td>
<td>4 (13%)</td>
<td>5 (17%)</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values are n (%)
### Table 3: CK-MB Activities and Time Intervals From End of Surgery to Important Postoperative Events

<table>
<thead>
<tr>
<th></th>
<th>Botulinum toxin (n=30)</th>
<th>Placebo (n=30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to begin ventilator weaning (min)</td>
<td>94 (71-132)</td>
<td>98 (62-151)</td>
<td>0.37</td>
</tr>
<tr>
<td>Time to extubation (min)</td>
<td>385 (278-1002)</td>
<td>406 (268-981)</td>
<td>0.65</td>
</tr>
<tr>
<td>Time intervals from end of surgery until discharge from ICU (h)</td>
<td>25.1 (20.1-42.1)</td>
<td>23.9 (20.1-43.6)</td>
<td>0.35</td>
</tr>
<tr>
<td>CK-MB 1 h after end of CPB (U/L)</td>
<td>32.3 ± 15.2</td>
<td>28.3 ± 13.1</td>
<td>0.28</td>
</tr>
<tr>
<td>CK-MB 8 h after end of CPB (U/L)</td>
<td>28.3 ± 13.6</td>
<td>23.1 ± 9.3</td>
<td>0.09</td>
</tr>
<tr>
<td>CK-MB 16 h after end of CPB (U/L)</td>
<td>26.6 ± 13.1</td>
<td>26.1 ± 11.4</td>
<td>0.89</td>
</tr>
<tr>
<td>CK-MB 24 h after end of CPB (U/L)</td>
<td>24.3 ± 10.3</td>
<td>21.6 ± 7.5</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (25th-75th percentile)

### Table 4: Incidence of Adverse Events in Patients Undergoing Botulinum Toxin or Placebo Injection

<table>
<thead>
<tr>
<th></th>
<th>Botulinum toxin (n=30)</th>
<th>Placebo (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post CABG length of stay, d</td>
<td>6 (5-8)</td>
<td>6 (4-8)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sustained ventricular arrhythmias</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Bleeding requiring reoperation or transfusion</td>
<td>2 (7%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Stroke or transient ischemic attack</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Rehospitalization for AF</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Readmission to intensive care unit</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Death</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (25th-75th percentile)
Table 5: Heart rate variability before and after CABG procedure

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>1 months</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Btx group</td>
<td>Placebo group</td>
<td>Btx group</td>
<td>Placebo group</td>
<td>Btx group</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>126±27</td>
<td>117±22</td>
<td>69±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rMSSD, ms</td>
<td>35±8</td>
<td>31±9</td>
<td>11±4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean HR, bmp</td>
<td>67±6</td>
<td>64±5</td>
<td>82±6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF, ms</td>
<td>19.9±4.4</td>
<td>18.5±4.6</td>
<td>4.6±2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF, ms</td>
<td>13.1±3.9</td>
<td>11.2±3.5</td>
<td>3.2±1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF/HF</td>
<td>2.1±0.57</td>
<td>1.8±0.52</td>
<td>1.2±0.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD
*p<0.05 for between-group comparison
†p<0.05 as compared to 1 month
‡p<0.05 as compared to 3 months
||p<0.05 as compared to baseline
All p-values are adjusted for multiplicity
Figure Legends:

**Figure 1:** Study design and patient flow.

**Figure 2:** Botulinum toxin or 0.9% normal saline was injected into the four visible areas of the major epicardial fat pads. The First through Fourth fat pads are shown in A-D, respectively (by red arrows). See text for more details.

**Figure 3:** The trend of the AF% in patients within each group (mean ± SE).
78 patients with PAF and indication for CABG were eligible for study.

18 pts declined to participate.

Randomization:

- botulinum toxin injection (n=30)
- placebo injection (n=30)
Long-Term Suppression of Atrial Fibrillation by Botulinum Toxin Injection into Epicardial Fat Pads in Patients Undergoing Cardiac Surgery: One Year Follow Up of a Randomized Pilot Study

Evgeny Pokushalov, Boris Kozlov, Alexander Romanov, Artem Strelnikov, Sevda Bayramova, David Sergeevichev, Alexander Bogachev-Prokopiev, Sergey Zheleznev, Vladimir Shipulin, Vladimir V. Lomivorotov, Alexander Karaskov, Sunny S. Po and Jonathan S. Steinberg

Circ Arrhythm Electrophysiol. published online October 20, 2015; Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/early/2015/09/17/CIRCEP.115.003199

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Arrhythmia and Electrophysiology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Arrhythmia and Electrophysiology is online at: http://circep.ahajournals.org//subscriptions/