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GENERAL GYNECOLOGY

Subcutaneous botulinum toxin type A injections for provoked vestibulodynia: a randomized placebo-controlled trial and exploratory subanalysis

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Abstract

Background Previous studies using botulinum toxin type A (BT) to treat provoked vestibulodynia (PVD) reported conflicting findings, possibly attributable to singular injections or low doses. We assessed PVD treatment effectiveness with high-dose single injections of BT (50 or 100 units) versus placebo, and then repeat BT 100 U injections over 6 months.

Methods This was a randomized, double-blind, three-arm, placebo-controlled study with 33 PVD patients. BT 50 U (arm A), 100 U (arm B) or saline (arm C) were injected subcutaneously into the dorsal vulvar vestibulum and pain was assessed after 3 months. The investigation proceeded as an unblinded exploratory analysis, in which symptomatic patients received a BT 100 U injection. Symptomatic patients in arm C received a second BT 100 U injection at the 6-month visit. Symptoms were measured at 3-month cycles using: (1) cotton swab-provoked visual analogue scale (VAS), (2) von Frey filaments, and (3) Marinoff dyspareunia scale.

Results The three groups were comparable in terms of demographics and baseline clinical characteristics. Three months after the initial injection, no significant differences in pain were observed among the study arms, yet significant improvements occurred within all groups using the von Frey filaments test. Results from the exploratory analyses showed repeat injections of 100 U BT over 6 months led to significant pain reduction (VAS and von Frey filaments). Fifty-eight percent (7/12) of patients assessable after repeat injections were symptom-free or had ≥ 2 VAS reduction. Adverse events were minor and no serious adverse events occurred during the RCT or exploratory analysis.

Conclusions PVD symptoms after one subcutaneous injection of BT (50 or 100 units) did not significantly differ compared to placebo, yet all three study arms experienced a reduction in pain 3 months after a single injection. Exploratory analyses indicated that repeat high-dose BT injections may significantly reduce pain over 6 months.

Trial registration This trial was registered with the Swiss Medical Agency (reference number: 2007DR2102) in 2007.

Keywords Provoked vestibulodynia · Vulvodynia · Botulinum toxin type A · von Frey filaments · Dyspareunia · Sexual intercourse

Abbreviations

BT	Botulinum toxin type A
IQR	Interquartile range
PVD	Provoked vestibulodynia
RCT	Randomized controlled trial
SD	Standard deviation
VAS	Visual analogue scale

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Background

The International Society for the Study of Vulvar Disease defines vulvodynia as chronic vulvar discomfort characterized by burning, stinging, irritation, or rawness [1]. According to consensus terminology published in 2015, vulvar pain is likely the result of a multifactorial process [2]. Estimated prevalence rates of vulvodynia vary from 8.3 to 16% [3–5]. Provoked vestibulodynia (PVD), previously known as vulvar vestibulitis syndrome (VVS), is the most frequently reported sub-category of vulvodynia, and is the most common reason for sexual pain in young women [5–7]. PVD is different from generalized vulvodynia in that symptoms of pain

are localized within the vestibule and provoked by touch or pressure [2].

Although a wide range of therapeutic options is currently available, diagnosis and treatment of PVD remain a challenge. Therapies are often selected on an individual basis and lack sufficient evidence of the underlying pathology [8, 9]. One off-label treatment for PVD, botulinum toxin type A (BT), can be injected in the dorsal vestibulum subcutaneously or in the bulbocavernosus body. The use of BT has been widely tested on other pain and spastic conditions, and has been proven to be an effective treatment option [10–13]. The hypothesized mechanism of action is a decrease in neuropeptides involved in pain, which results in a lower threshold of activation of C-afferent nociceptors whose endings are increased in PVD patients [14, 15].

Most published studies examining the efficacy of botulinum toxin type A in the treatment of vulvodynia used single injections of 10–80 units [16–21]. Findings have been conflicting, and many suggested a placebo effect. Petersen et al. identified the use of low dosages of BT and the lack of repeat doses as limitations of their randomized controlled trial (RCT) [20].

The primary aim of this randomized, double-blind, three-arm, placebo-controlled study was to assess the effectiveness of high-dose injections of BT (50 or 100 units) or placebo to treat PVD. The secondary aim was to explore the effects of repeat BT injections (100 units) over a 6 month period to generate hypotheses for future investigations. Using a two-step study design we were able to benefit from the scientific strength of a RCT, although limited to assessments of only a single injection, and then continue with a less robust exploratory design, which primarily served to address patient's expectations of receiving treatment rather than placebo.

Methods

The investigation was conducted from June 2008 to September 2014 at the vulva clinic, a tertiary referral center of the Department of Gynecology of the University Hospital Zurich, Switzerland. The study was approved by the Ethics Commission of Zurich (KEK-ZH StV 16/2005) and the Swiss Medical Agency (# 2007DR2102) in 2007. All patients provided written informed consent to participate.

This two-step study design combined a placebo-controlled RCT with an exploratory analysis to gather information about the effectiveness of treatment with botulinum toxin. The first design phase had the advantage of scientific rigor yet it was limited by the use of a single injection. The second phase enabled continuation of the research by assessing the use of multiple injections, and it offered all patients an opportunity to receive treatment rather than placebo. Although an exploratory design is less robust than a RCT, its merit lies in the generation of hypotheses for future investigations.

The inclusion criteria for the study were as follows: age ≥ 18 years, fulfilled criteria for diagnosis of provoked vestibulodynia according to Friedrich [22], normal vulvoscopy, lacking signs and negative swabs for common infections (i.e., chlamydia, ureaplasma, yeast, streptococcus type A and gonorrhea), no concurrent therapy for vulvodynia, cessation of corticoid creams ≥ 2 weeks before enrollment, and use of contraceptives. Exclusion criteria were the presence of vulvar dermatoses, myasthenia gravis or Lambert Eaton Syndrome; use of antidepressants, neuroleptic medication or other drugs (i.e., D-penicillamine, chloroquine, aminoglycoside) with the potential to cause interactions with botulinum toxin type A. Pregnant or lactating women were also excluded.

Procedures

Eligibility screening was conducted at the clinic and included a physical exam and completion of a questionnaire. The questionnaire was used to gather information on the patients' general medical history and demographics such as employment, education level, parity, co-morbidities, smoking habits, history of sexual abuse, contraception, concomitant medication, history of pain (other than PVD), and urinary tract and yeast infections. In addition, the following information regarding PVD was collected: duration of symptoms (in months), previous treatment (including surgery), dyspareunia, perception of PVD etiology and whether patients knew other women with similar complaints. If a patient fulfilled the eligibility criteria, the study nurse assigned the woman to one of three study arms using computerized randomization software. Given that a double-blind design was used for the initial phase (3 months) of the study, treatment allocation was kept confidential by the nurse in the study records.

Patients returned for the baseline visit 1 week following the screening visit. The initial injection was administered immediately after the three pain measurements were completed. First, sensitivity levels of pain were determined using a cotton swab-provoked visual analogue scale (VAS) (0–10). Second, the tactile and mechanical pain stimulation was measured using von Frey filaments (Touch Test™ Sensory Evaluators, Semmes–Weinstein Von Frey Aesthesiometers, Stoelting Co., Wood Dale, Illinois, USA), a set of 20 monofilaments with increasing log force value ranging from 1.65 to 6.65. Filaments of increasing thickness were progressively applied to the mucosal surface until a mechanical pain stimulus was felt. Testing locations on the vulvar vestibulum were at 1, 3, 5, 6, 7, 9 and 11 o'clock (Fig. 1) [22]. Third, patients graded dyspareunia using the Marinoff dyspareunia scale (0 = no problems, 1 = discomfort that does not affect

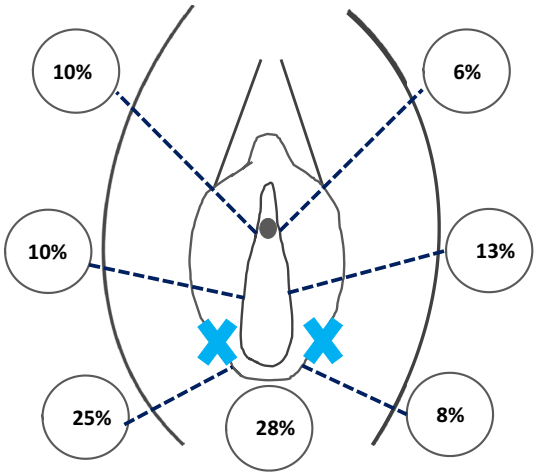

Fig. 1 Distribution of reported maximum pain location and the sites of the subcutaneous injections

completion, 2 = pain interrupts or prevents completion, 3 = pain prevents any attempts at intercourse). Additional parameters such as pain quality, trigger events for pain, and the frequency of intercourse were also assessed during the study visit. Once the baseline assessments were completed, the study nurse prepared the syringes in a separate room to ensure that both the physician and patient were unaware of the treatment arm. After vulvar disinfection with chlorhexidine, 50 (arm A) or 100 (arm B) units botulinum toxin type A (Allergan AG, Zurich, Switzerland) diluted in 1 ml saline was injected using a 25 gauge syringe in the subcutaneous layers of the dorsal vestibulum (each side 0.5 ml) (Fig. 1). One milliliter of saline was used as placebo (arm C) and administered as described above.

At the 3-month visit, the pain assessments were repeated. Following these exams, the RCT design ended and the study was unblinded. At this point, the study nurse informed the patient and the physician to which treatment arm the patient had been assigned. As part of an exploratory analysis into the effects of repeat high-dose injections, a second injection with 100 U BT was administered to symptomatic patients of the entire cohort.

Study assessments were repeated at the 6-month visit for all treatment arms. At this point, only symptomatic patients in the baseline placebo group (arm C) received an additional injection of 100 U botulinum toxin A. Three months following this third injection (9 months from study initiation), patients in arm C underwent final assessments. Patients in the other study arms ended their participation in the exploratory analysis after 6 months and proceeded with further treatment according to the physician's discretion.

Participants

Response rates from previous case and pilot studies using BT to treat vulvodynia/genital pain [16, 18, 19, 27] with one BT injection between 20 and 80 units showed a mean significant difference of 64% in pain reduction. Using the statistical power analysis program G*Power version 3.1.9.2, the sample size was calculated using an ANOVA repeated within–between interactions statistical test for three groups over three comparative time points (alpha 0.05, power 0.80, effect size 0.27). The total sample size estimation was 30 patients. Taking into account the potential for a 5% drop-out rate, the target sample size per group was 11 patients ($n = 33$).

Thirty-three women meeting the inclusion criteria were enrolled and randomized (Table 1). However, due to the randomization process, the distribution of patients per study arm was imbalanced, which resulted in the following group allocations: $A = 12$, $B = 9$, $C = 12$. Furthermore, one patient in arm B withdrew consent following baseline assessments but immediately before drug injection, and one patient in arm C was lost to follow-up at the 3-month visit.

Table 1 Schedule of treatment and assessments according to study arm for randomized controlled trial and exploratory analysis

Time point	Design	Study arm		
		A	*B*	*C*
Randomization	Double-blind RCT	$n = 12$	$n = 9$	$n = 12$
Baseline	Double-blind RCT	A0, BT-50 ($n = 12$)	A0, BT-100 ($n = 8$)	A0, PI ($n = 12$)
3 months	Double-blind RCT	A3 ($n = 12$)	A3 ($n = 8$)	A3 ($n = 11$)
	Unblinded exploratory analysis	BT-100 ($n = 12$)	BT-100 ($n = 7$)	BT-100 ($n = 11$)
6 months	Unblinded exploratory analysis	A6 ($n = 10$)	A6 ($n = 5$)	A6 ($n = 11$) BT-100 ($n = 10$)
9 months	Unblinded exploratory analysis	–	–	A9 ($n = 7$)

IQR interquartile range, *SD* standard deviation, *PVD* provoked vestibulodynia, *VAS* cotton swab-provoked visual analogue scale; *A6* 6 month assessment; *A9* 9 month assessment; *BT* botulinum toxin A injection—units; *PI* placebo injection (saline)

*Fisher's exact test, §Kruskal–Wallis test, **one-way ANOVA

Outcome measures

The study endpoints were to assess differences in pain levels according to the following three measurement tools: (1) cotton swab-provoked VAS (0–10), (2) von Frey filaments, and (3) Marinoff dyspareunia scale (0–3). The primary endpoint was to determine treatment effectiveness after 3 months following a single injection. For the exploratory analyses, intragroup comparisons were performed for patients having received two consecutive injections of 100 units of botulinum toxin A. Information on pain location and quality, and frequency of sexual intercourse were also collected. For purposes of this study, 'treatment success' was defined as patients with ≥ 2 point improvement on the cotton swab-provoked VAS assessment or patients who reported being symptom-free. Rowbotham et al. reported a two-point decrease on the VAS was meaningful for patients [24]. Lastly, adverse events related to the injection (such as pain at the injection site, duration of pain, use of pain medication due to discomfort from injection, the occurrence of urinary or stool incontinence, or other complaints) were also documented during study visits.

Statistical analyses

Descriptive statistics were calculated according to treatment arms. Inferential statistics of intergroup comparisons were performed with one-way ANOVA, Kruskal–Wallis or Fisher's exact test (p values adjusted with Bonferroni correction). Intragroup changes over time were assessed using the paired sample t test or Wilcoxon signed-ranks test. Two-sided p values less than 0.05 were considered statistically significant. Multivariate logistic regression analysis was performed using a combined categorical outcome of 'successful treatment' defined as ≥ 2 point improvement on the cotton swab-provoked VAS assessment or patients who reported being symptom-free. Variables included in this model were age, previous surgery for PVD, duration of symptoms, and study arm. Statistical analyses were performed with IBM SPSS Statistics 22 (IBM Corporation, Armonk, New York).

Results

Patient characteristics

Clinical and demographic characteristics of patients according to the three treatment arms, including medical history and prior therapy, are presented in Table 2. No statistically significant differences were detected at baseline measurements. Patients attributed the primary cause for their condition to the following: 34% to a yeast infection, 34% to an unknown cause, 22% to a psychological reason, and 7% to an anatomical problem. All patients had some form of prior

Table 2 Baseline demographics, medical history and previous PVD therapy by study treatment arm

Characteristic	Cohort ($n=32$)	Arm A ($n=12$)	Arm B ($n=8$)	Arm C ($n=12$)	p value
Age in years—median (IQR)	27 (24–30)	27 (25–28)	28 (23–35)	27 (23–30)	0.97§
BMI—mean, SD (range)	20.81 ± 2.37 (17–26)	20.9 ± 3.75 (17–26)	20.3 ± 0.42 (20–21)	21.03 ± 1.56 (20–23)	0.951**
Married, *n* (%)	10 (31%)	5 (42%)	3 (38%)	2 (17%)	0.403*
Nulliparity, *n* (%)	28 (88%)	9 (75%)	7 (88%)	12 (100%)	0.16*
Smoker, *n* (%)	7 (22%)	3 (25%)	2 (25%)	2 (17%)	0.99*
Currently employed, *n* (%)	25 (78%)	11 (92%)	7 (88%)	7 (58%)	0.16*
Use of oral contraceptive, *n* (%)	23 (72%)	8 (67%)	4 (50%)	11 (92%)	0.14*
History of physical abuse, *n* (%)	2 (6%)	1 (8%)	0 (0%)	1 (8%)	1.00*
Has friend(s) with similar symptoms	2 (6%)	1 (8%)	0 (0%)	1 (8%)	1.00*
Primary PVD, *n* (%)	7 (22%)	3 (25%)	2 (25%)	2 (17%)	0.99*
Intercourse within last month, *n* (%)	28 (88%)	10 (83%)	4 (50%)	11 (92%)	0.12*
VAS during intercourse (0–10)—median (IQR)	8 (5–9)	8 (7–9)	8.5 (7–9)	8 (8–9)	0.858§
Duration (months) of PVD—median (IQR)	54 (32–96)	42 (17–92)	90 (36–174)	54 (29–96)	0.368§
Duration (months) of previous PVD treatment—median (IQR)	48 (24–60)	42 (26–69)	48 (27–111)	48 (24–54)	0.691§
Bladder complaints, *n* (%)	10 (31%)	6 (50%)	1 (13%)	3 (25%)	0.189*
Other pain syndromes, *n* (%)	15 (47%)	4 (33%)	5 (63%)	6 (50%)	0.45*
Prior vulvar surgery, *n* (%)	7 (22%)	4 (33%)	2 (6%)	1 (8%)	0.42*

A0 baseline assessment, *A3* 3 month assessment

*Mean, standard deviation; **Median, interquartile range; §Paired sample t test; ¥Wilcoxon signed-ranks test; ***ANOVA; ****Kruskal–Wallis

local therapy. Additional previous treatment types included: ten patients had physiotherapy, seven had prior vulvar surgery, two received psychological counseling and one patient underwent acupuncture.

RCT pain outcomes: inter- and intragroup comparisons

Table 3 shows a summary of all pain outcomes according to treatment arm during the randomized controlled trial. Although pain reduction was observed in all treatment arms, the difference between the groups was not statistically significant at baseline or 3 months.

With regard to intragroup changes at 3 months, no statistically significant improvements in cotton swab-provoked VAS scores were found. When using the von Frey filaments, all treatment groups' pain levels were reduced significantly, including women treated with a single injection of placebo. Findings from the Marinoff dyspareunia scale indicated statistically significant improvements in arm A from baseline to 3 months. No statistically significant changes in the frequency of sexual intercourse were reported between or within the treatment groups at any point during the study.

Findings from the multivariate logistic regression analyses using the outcome 'successful treatment' (≥ 2 point improvement on the cotton swab-provoked VAS assessment or symptom-free patients) did not indicate any statistically significant associations. Variables in the model were age, previous surgery, duration of symptoms, and study arm. Success rates at end of the RCT were as follows $A = 8.3\%$ (1/12), $B = 50\%$ (4/8) and $C = 36.3\%$ (4/11) ($p = 0.116$).

Exploratory analysis of patients with repeat 100 units BT

A total of 17 patients from arms B and C received two consecutive doses of 100 units of BT within 3 months. Complete follow-up data (VAS and/or symptom status) were available for analysis for 12 of the 17 women. Statistically significant improvements were observed regarding both the cotton swab-provoked VAS and von Frey filaments (Table 4). The success rate (symptom-free or ≥ 2 VAS point improvement) in this group was 58% (7/12). If the five women lost to follow-up were considered non-responders, the rate of improvement would still be 41% (7/17).

Pain location and pain quality

The majority of patients described pain as mixed forms of nociceptive pain (45%), 36% of women tended to have burning pain and 19% reported stabbing pain. Figure 1 shows the distribution of reported maximum pain location and the sites of the injections.

Safety and adverse effects

No serious adverse events were reported during or after injection up to the final assessments performed 3 months

Table 3 Pain measurements according to treatment arm during randomized controlled trial ($n = 31$)

Test Type	Arm	Results		*p* value *(intra-group)*
		A0*	A3*	A0/A3
Cotton swab provoked VAS	A	6.6 (±2.01)	6.2 (±2.60)	0.41
	B	7.4 (±1.85)	6 (±1.77)	0.239
	C	7 (±2.22)	6.5 (±1.31)	0.623
p value (*intergroup*)		0.735	0.857	
		A0**	A3**	A0/A3
Von Frey Filaments	A	4.31 (4.25–4.61)	4.74 (4.63–4.93)	*0.028*
	B	4.17 (3.9–4.31)	4.695 (4.14–5.18)	*0.017*
	C	4.17 (3.84–4.74)	4.56 (4.17–4.93)	*0.016*
p value (*intergroup*)		0.257	0.616	
		A0**	A3**	A0/A3
Marinoff dyspareunia scale	A	2 (2–3)	1.5 (0–2)	*0.031*
	B	2.5 (1–3)	1.5 (0–3)	0.276
	C	2 (2–3)	2 (1–2)	0.102
p value (*intergroup*)		0.838	0.927	

Italic values indicate significance of *p* value ($p < 0.05$)

A0 baseline assessment; *A3* 3 month assessment

*Mean, standard deviation; **Median, interquartile range

Table 4 Pain levels assessed before and after two consecutive injections of 100 units botulinum toxin type A during the exploratory analysis ($n = 10$)

Test type	Baseline	Three months after second injection	p value
Cotton swab-provoked VAS*	7.35 (±2.06)	5 (±2.21)	0.029
von Frey filament test**	4.17 (3.84–4.56)	4.93 (4.74–5.46)	0.003
Marinoff Dyspareunia Scale**	2 (2–3)	1 (1–2)	0.059

*Mean, standard deviation, paired sample t test; **median, interquartile range, Wilcoxon signed-ranks test

after last study drug administration. No episodes of urinary or stool incontinence were reported. The majority of the patients (88%) reported some pain immediately after injection, which subsided in most cases in less than 24 h.

Discussion

The randomized controlled trial showed no significant differences in pain levels when comparing treatment arms at 3 months. Hence, the efficacy of a single 50 or 100 units BT injection subcutaneously in the dorsal vestibulum compared to placebo could not be demonstrated. However, significant improvements in the von Frey filament measurements were detected within all groups at 3 months after all single injections. Likewise, findings from our supplemental exploratory analysis with repeat injections of 100 U BT over 6 months led to significant pain reduction. Between 41% and 58% of patients reported ≥ 2 VAS score reduction or no dyspareunia (symptom-free) after repeat injections of 100 U BT.

Finding a lack of efficacy of a single BT injection, when compared to placebo, is consistent with other studies. In a randomized controlled trial with 64 patients, Petersen et al. [20] could not demonstrate improved outcomes of 20 units BT injected into the bulbocavernosus body when compared with placebo. All patients experienced pain reduction yet differences between the groups were not significant. Likewise, no dose-dependent increase in efficacy could be demonstrated in our study. Although disappointing in terms of further elucidating effective treatment alternatives for PVD, this result is not surprising given our study population had a long history of dyspareunia, most patients had sought medical advice for over 3 years prior to study participation, and 22% had surgery of the vulva to treat PVD.

We believe there are four potential explanations for the lack of effect of BT injections compared to saline injection. First, the hypothesis that BT leads to a decrease in neuropeptides involved in pain, which results in a lower threshold of activation of C-afferent nociceptors, is not clinically relevant. Second, the injection of BT in the dorsal vestibulum may be too localized to have an effect on the ventral skin of the vestibulum. As shown in Fig. 1, a high number of patients identified the ventral part of the vestibulum as the area of maximum provoked pain. The location at 5 and 7 o'clock at the vestibulum, where the pudendal nerve enters the vulva posteriorly, was considered to be the most beneficial point for treatment. However, it is debatable if site-specific injections at the point of maximum sensory threshold levels would yield increased pain relief. Furthermore, BT injection using a volume higher than 1 ml of dissolving saline might have enhanced the distribution of the drug to more ventral parts of the vestibulum.

A third possible explanation for the lack of effect of BT injections is that a heightened awareness of the disease may have altered patients' perception of pain, which may have led to reports of significant improvement of symptoms at 3 months following saline injection. Lastly, the 3-month time span for assessment of efficacy after initial drug injection may have been too long. We chose a 3-month reinjection interval because previous studies using BT to treat PVD reported a treatment response of this duration [16, 18, 19]. Since we were able to demonstrate a beneficial effect of repeat injections, shorter reinjection intervals may be more suitable, which has also been suggested by other researchers [23].

In addition to assessing single injections, our study included an exploratory analysis to evaluate the effects of repeat injections over time. This part of the study, which was unblinded and not randomized, was used for hypothesis generation only and conclusions based on these findings are limited. Nevertheless, repeat subcutaneous injections of 100 units BT did indeed show some promise, with a significant reduction in cotton swab-provoked VAS and an increase in pain thresholds using the von Frey filament test. However, this improvement over time may also be explained by spontaneous regression of PVD that has been described for primary and secondary vestibulodynia in up to 30% of the cases [25, 26]. One drawback of our trial's design was that it did not include repeat BT injections versus repeat placebo injections because a significant effect after a single injection was anticipated. Another limitation of our study was the low sample size in arm B due to unequal allocation by the randomization process. This resulted in decreased statistical power that may have reduced our ability to detect a true effect. We also faced challenges with patient recruitment and drop-out, which have also been observed by other researchers working with this patient population [28]. Issues were

patients' concerns about allocation to the placebo arm and periodic fluctuations in pain levels.

The treatment of vestibulodynia remains intriguing because the cause of this pain syndrome is still not known and may due to multiple etiologies [2, 9]. Established treatment options, such as desensitization exercises and cognitive behavioral therapy, should be routinely offered [29]. Whether repeat subcutaneous BT injections can contribute to a successful treatment regimen stills needs to be elucidated.

Conclusions

In conclusion, we could not demonstrate a beneficial effect on pain reduction following a single subcutaneous injection of botulinum toxin type A at doses of 50 and 100 U when compared with placebo. In the exploratory analysis with repeat injections of 100 U BT, a significant reduction of the cotton swab-provoked VAS could be shown, as well as an increase in the pain threshold level. However, this beneficial effect could not be compared to an exclusively placebo study arm since this investigation was not designed to test repeat placebo injections. Given the safety of 100 U botulinum toxin injections and the positive effect of repeat injections, future PVD studies ought to compare repeat BT injections with placebo within shorter time intervals.

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Author contributions ID: data collection, analysis and manuscript writing. NG: protocol finalization, study initiation, and manuscript editing. MK: concept idea, study protocol, and manuscript editing. GPG: patient recruitment, and manuscript editing. JW: data analysis, and manuscript editing. DF: concept idea, and manuscript editing. MKF: concept idea, study protocol, data analysis, manuscript writing and editing. CB: study organization, reports to authorities (Swissmedic, KEK), data collection and analysis, and manuscript writing. MKF and CB contributed equally to the manuscript.

Compliance with ethical standards

Ethics approval and consent to participate Study design was approved by Ethics Commission of Zurich (KEK-ZH StV 16/2005). All patients provided written consent to participate.

Availability of data and material The datasets used and/or analyzed during this current study are available from the corresponding author on reasonable request.

Conflict of interest The authors declare that they have no competing interests.

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