Botulinum Toxin A in Postherpetic Neuralgia

A Parallel, Randomized, Double-Blind, Single-Dose, Placebo-controlled Trial

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Objectives: Cumulative evidence support a beneficial effect of botulinum toxin A (BTX-A) in postherpetic neuralgia (PHN). We aimed to assess efficacy, safety, and tolerability of BTX-A in the management of PHN, performing a randomized, double-blind, single-dose, placebo-controlled trial.

Methods: Thirty adults with PHN were randomized either to BTX-A or placebo. Severity of pain was evaluated by patients using a visual analogue scale (VAS) and quality of sleep was assessed using a 5-item questionnaire. Primary outcome was reduction in VAS score, with a greater than 50% reduction being considered clinically significant. Secondary outcomes were reduction in sleep score and maintenance of VAS score after treatment, with over 50% maintenance considered clinically meaningful.

Results: Thirteen patients from the experimental arm achieved an at least 50% reduction in VAS score, compared with none of the placebo patients (NNT = 1.2, 95% CI, 2-1; ARR = 0.87, 95% CI, 055-096; $P < 0.001$). BTX-A patients showed significant reduction in VAS pain scores between baseline and week 2, which persisted for a median period of 16 weeks. BTX-A patients showed significant reduction in sleep scores between baseline and week 2, which remained unchanged until 16th week ($P < 0.001$). Treatment was well tolerated.

Discussion: Data confirm that BTX-A is effective and well tolerated in the treatment of PHN.

Key Words: botulinum toxin, herpes zoster, neuropathic pain, postherpetic neuralgia, randomized clinical trial

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The most common complication of herpes zoster is postherpetic neuralgia (PHN), which can cause chronic and debilitating pain.[1] Furthermore, more than half of the patients with PHN experience sleep disturbances, decrease in daily activity, and significant impairment of their social life.

PHN represents a classic paradigm of neuropathic pain. After reactivation of varicella zoster virus, leading to inflammation of dorsal root ganglia, significant alteration occurs in the molecular, cellular, and connectional state of central nervous system nociceptive pathways, after anatomic deafferentation.[1,2] The viral injury of the peripheral neurons leads to spontaneous discharge and lower activation thresholds, provoking exaggerated responses to stimuli.[1] Pain and temperature detection systems are hypersensitive to light mechanical stimulation, thus leading to severe pain (allodynia). Allodynia may be related to the formation of new connections involving central pain transmission neurons.[2] Previous studies have shown that hyperactivity of A-β afferents after nerve injury can result in touch-evoked pain and spontaneous pain by presynaptic activation of C afferent terminals.[2] However, the precise mechanism of allodynia remains obscure.[1–3]

Prevention of PHN implicates early diagnosis of herpes zoster and early use of antiviral drugs. Treatment modalities for already developed PHN, such as topical analgesics (lidocaine patch 5% and capsaicin), tricyclic antidepressants, gabapentin, pregabalin, and opioid analgesics are often necessary, as in many patients pain continues despite the early use of antivirals. Furthermore, the use of these drugs may be limited by their side effects or patients' tolerability. The combination of different therapeutic regimens is a common practice, as no single therapy is completely effective.[4,5] Treatment-refractory PHN is a common phenomenon in daily clinical practice.

Botulinum toxin A (BTX-A) blocks acetylcholine by cleaving synaptosome-associated protein-25, which participates in the formation of the exocytic soluble N-ethyl-maleimide–sensitive factor attachment protein receptor complex (SNARE), which is essential for the fusion of acetylocholine-containing vesicles with the presynaptic membrane.[6,7] The local peripheral BTX-A injection may result in reduction of various substances that sensitize nociceptors. This antinociceptive effect is associated with the inhibition of formalin-induced glutamate release and a possible reduction of the peripheral nociceptive input by inhibiting the release of substance P and calcitonin gene–related peptide, which play a significant role in neurogenic inflammation.[8]

On the basis of the encouraging preliminary clinical results with the use of BTX-A in PHN, reported in a previous pilot study,[9] and the cumulative evidence supporting the beneficial effect of BTX-A in neuropathic pain,[10–15] we conducted this clinical trial to assess efficacy, safety, and tolerability of the BTX-A in the treatment of PHN.

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MATERIALS AND METHODS

Study Design

We conducted a randomised, double-blind, 2-arm, single-dose, placebo-controlled clinical trial of parallel design to assess efficacy, safety, and tolerability of the BTX-A as compared with placebo in the treatment of PHN. The trial lasted for 4 weeks followed by an open-label 20-week follow-up phase for complete responders to assess maintenance and pain recurrence. It was approved by the local ethics committee and was conducted in accordance with the latest revision of the Declaration of Helsinki at the First Department of Dermatology, at the Aristotle University of Thessaloniki in Greece. The registration number, given by the Cochrane database for ongoing clinical trials, was CSG Trial No. 54 (date accepted: April 20, 2009). Patients were given both verbal and written information on the purpose and design of the study and provided written consent before any study-related procedure. Patients that entered the study were pooled from the outpatient dermatology clinic of the First Department of Dermatology, at the Aristotle University of Thessaloniki, where they were previously monitored for herpes zoster. Patient enrollment started on 27/04/2009 and ended on 09/11/2009. The last patient completed the study on 12/05/2010.

Men or women over the 18 years of age with pain present for more than 3 months after healing of the herpes zoster rash were eligible for this study. Participants were required to have a visual analogue scale (VAS) score ≥ 7 at baseline. Exclusion criteria included cranial nerves involvement, severe non-PHN pain that might compromise evaluation of pain caused by PHN, and skin disorders that might impair the therapeutic technique. In addition, female patients were required not to be pregnant or lactating. Other therapies had to be discontinued at least 30 days before receiving study medication. During the trial protocol no concomitant treatment was allowed except of paracetamol up to 1000 mg/d.

After inclusion, patients were randomly assigned to either BTX-A (BOTOX, onabotulinumtoxinA; Allergan) or placebo, using a computer-generated random numbers table at a 1:1 allocation ratio. Throughout the study, patient allocation was not available to assessors. Medication kits were sequentially numbered. Dilution of BTX-A and preparation of syringes was performed by an independent nonblinded physician. Placebo syringes were identical in appearance to syringes containing active drug. Medication was blindly administered with subcutaneous injection. A total of 100 IU of BTX-A (5 U/route), diluted with 4 mL of sodium chloride (0.9%), was injected subcutaneously in a chessboard manner, all over the affected area (as designated by the patients), at the first group, using 30 G size needles. Each patient received 40 injections in total, with a minimal distance of 1 cm between injections' sites. Placebo group received normal saline, dispensed exactly the same way. Participants and physicians who administered treatment and assessors were blinded. Drug administration was performed by a single physician, who was not an assessor.

Severity of pain was evaluated by patients using a VAS (anchored at the ends with 0: no pain; 10: unbearable pain), at baseline and daily for the first 2 weeks, always in the same manner. On morning awakening, patients evaluated their pain for the previous 24 hours by circling numbers that best described their pain (worst pain experienced within the previous 24 h) on the VAS numerical scale. After the initial 2-week period, VAS score was recorded every 2 weeks for the next 12 weeks, and every 4 weeks, until week 24. Quality of sleep was assessed using a 5-item questionnaire, answered by the patients at baseline and during the same follow-up appointments. After the initial 4-week period, only patients in the BTX-A group were allowed entering the open-label 20-week period, whereas patients under placebo were scheduled to receive other available treatments. Table 1 illustrates the 5-item questionnaire and the scoring system used for the evaluation of the answers.

Primary outcome was a reduction in VAS score within a 4-week period, with a greater than 50% reduction—compared with the baseline—considered clinically significant. Secondary outcomes were reduction in sleep score and maintenance of VAS score after treatment, with an greater than 50% maintenance considered clinically meaningful.

The safety of BTX-A, for topical and/or systemic adverse effects, was assessed throughout the study. To access tolerability, pain related to injections was rated as mild, moderate, or severe by the participants.

Sample Size Calculation and Statistical Analysis

The primary efficacy analysis was a comparison of the proportion of patients with complete response within the 4-week period after injection between the active treatment group and placebo. Sample size calculations assumed that complete clearance rates were 70% for the active treatment

TABLE 1. Five-Item Questionnaire and Scoring System Used for the Evaluation of Quality of Sleep

	A	B	C	D
How would you describe the overall quality of your sleep during the last week?	Very bad	Rather bad	Rather good	Very good
How many nights during the last week did you feel you could not sleep because of pain?	7 nights	4-6 nights	1-3 nights	None
How many times was your night sleep interrupted because of pain during the last week?	≥ 5 times	3-4 times	1-2 times	None
How many hours of continuous night sleep did you experience during the last week?	1-2 h	3-4 h	5-6 h	7-8 h
How much time did you spend lying on bed before sleeping during the last week?	≥ 60 min	40-60 min	20-40 min	< 20 min

Scoring system. A, 3 points; B, 2 points; C, 1 point; D, 0 points.
Scoring deviation: 0 (min) to 15 (max), 0-5 points: good quality, 5-10: moderate quality, 10-15: bad quality.

group and 20% for the placebo group. Using the 2-sided Fisher exact test with a power level of 80% and a target significance level of 5%, 24 patients (12 patients for active treatment group and 12 patients for the vehicle group) were required to detect a significant difference.

To compensate for nonevaluable patients, we initially planned to enroll 30 patients in total. To compare the magnitude of the treatment effect of both active treatments and the placebo, we calculated standardized effect sizes at posttest by means of Cohen's *d* (subtraction of the mean posttest scores of the groups and dividing the difference by the pooled SD). Cohen's *d* is used to indicate the standardized difference between 2 means. Its interpretation is considered essential when reporting ANOVA results.[16]

Statistical analysis was performed using SPSS 17.0 (Statistical Package for Social Sciences, SPSS Inc., IL) for Windows. All analyses were performed on the intent-to-treat population. Normality and homoscedasticity tests were performed for all dependent variables. For comparison of continuous variables between (1) baseline characteristics and (2) treatments' effect in the same time point, the independent samples *t* test was used for variables after normal distribution, whereas for others Mann-Whitney *U* test or the Pearson χ^2 test where appropriate was performed. For comparison of dichotomous variables the 2-sided Fisher exact test was used. All *P*-values were 2-tailed, and differences were considered significant when the *P*-value was ≤ 0.05. Summary data are expressed as mean ± SD, unless otherwise stated. For variables after normal distribution, Cohen's *d* effect size was also calculated for confidence interval of 95%.

For comparison of the effect of each treatment in the different time points, 1-way repeated measures ANOVA was performed for variables after normal distribution, else the Friedman test was performed. The Mauchly test of sphericity was performed. If sphericity assumption was violated, the Huynh Feldt correction was used, which corrects for violations of assumptions by adjusting the degrees of freedom downward by an appropriate amount. Post hoc analysis was performed using Bonferroni test for the 1-way repeated measures ANOVA to evaluate differences across the different time points. Summary data are expressed as mean ± SD. We analyzed the primary and secondary outcome measures by means of General Linear Model 3×2 repeated measures ANOVAs, with the time points (baseline, 2 wk, and 4 wk) as within-subject factors and the treatment group (placebo or experimental) as between-subject factors.

This study is doubly multivariate because there are multiple observations of multiple measures. We broke down within-subject factors (different weeks) further into repeated contrasts. Contrasts compared mean changes from baseline to 2- to 4-week treatment scores of within-subject factors for between-subject factor treatment groups. Another contrast compared mean changes from baseline to 2- to 4-week treatment scores for the experimental group with between-subjects factor for those patients with at least 50% reduction in VAS score. Life table analysis was performed in the experimental group to analyse the effect of time on response (in d), defining as the main event 50% reduction in VAS score, and on maintenance (in wk), defining as the main event maintenance of 50% reduction of VAS score.

RESULTS

We included 30 patients with PHN, in 2 equal arms (15 to experimental BTX-A and 15 to placebo, Table 2; Fig. 1).

TABLE 2. Baseline Characteristics of the 2 Treatment Groups

	Botox	Placebo	*P*
Age (y)	73.2 ± 10.5	77.5 ± 8.2	0.22
Sex	Male: 8	Male: 10	0.71
	Female: 7	Female: 5	—
Duration of pain (d)	102.2 ± 10.4	105.6 ± 11.7	0.55
VAS score	8.8 ± 1	8.7 ± 0.8	0.71
Sleep score	8.7 ± 2.2	9 ± 2.2	0.68
Previous antiviral treatment			
Yes	Yes: 11	Yes: 11	—
No	No: 4	No: 4	—
Affected neurotome	Thoracic: 12	Thoracic: 13	—
	Sciatic: 1	Sciatic: 0	—
	Brachiplex: 2	Brachiplex: 2	

VAS indicates visual analogue scale.

Four patients were excluded because of low pain score at baseline. There were 8 males in the experimental arm versus 10 in the placebo arm (Pearson χ^2, $P = 0.456$). Patients' age covered normality assumption. Mean age of patients was 73.2 ± 10.5 years in the experimental versus 77.5 ± 8.2 years in the placebo arm (Student *t* test, $P = 0.244$). Affected neurotomes were thoracic: 12; sciatic: 1; brachial plexus: 2 in the experimental arm versus thoracic: 13; sciatic: none; brachial plexus: 2 in the placebo arm (Pearson χ^2, $P = 0.595$).

VAS Score

Overall, 13 patients (87% of all patients in active treatment) from the experimental arm achieved an at least 50% reduction in VAS pain score, compared with none of the placebo patients (Pearson χ^2, $P < 0.001$). This statistic yielded a single patient number need to treat (NNT = 1.2; 95% CI, 2-1) and an absolute risk reduction (ARR) of 0.87 (95% CI, 055-096). Figure 2 shows mean VAS scores at the follow-up visits, range, and SD.

BTX-A-treated patients showed significant reduction in VAS score between baseline and week 2 (tests of within-patient contrasts, $P < 0.001$), which then remained stable between weeks 2 and 4 (tests of within-patient contrasts, $P = 0.334$) (Fig. 3). Placebo-treated patients also showed a marginally significant reduction in VAS score between baseline and week 2 (tests of within-patient contrasts, $P = 0.045$), which then remained stable between weeks 2 and 4 (tests of within-patient contrasts, $P = 0.751$). We further examined the curve of VAS score in the experimental arm. It seems that after the initial decline, VAS scores remain unchanged until week 10 ($P < 0.001$), when a raise begins which continues also on 16th week ($P < 0.001$, Fig. 3). Between treatment groups analysis reported estimated marginal means of VAS pain scores for baseline, second week, and fourth week: for BTX-A-treated patients 8.8 (8.3 to 9.3), 4.1 (3.5 to 4.7), and 3.9 (3.3 to 4.5) as opposed to 8.7 (8.2 to 9.2), 8.1 (7.5 to 8.7), and 8.2 (7.6 to 8.8) for controls, respectively (tests of between-subjects effects, $P < 0.001$). A plot of the estimated marginal means is presented in Figure 4. Cohen's *d* effect size was 3.6 (3.2 to 5.0) for second week and 4.2 (3.6 to 5.2) for fourth week.

Through life table analysis, we recorded a median time to achievement of >50% reduction in VAS score of 7.44 days (Fig. 5). Maintenance of VAS pain score was noted for a median period of 16 weeks (Fig. 6).

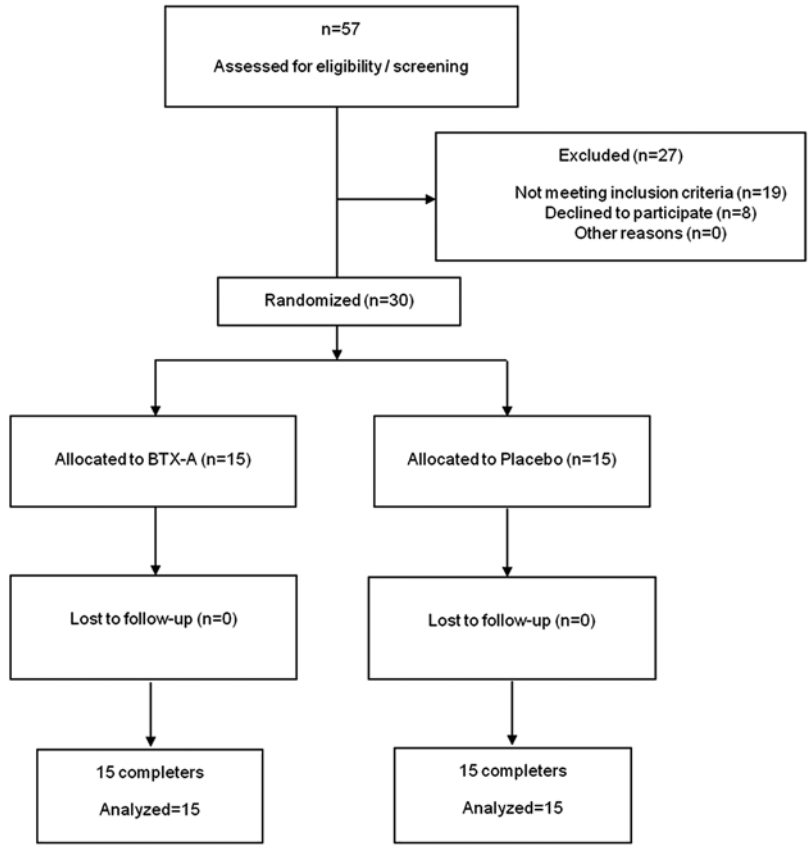

FIGURE 1. Flow diagram depicting enrollment, allocation, treatment completers, and number of patients per treatment group. Values are expressed as numbers of patients.

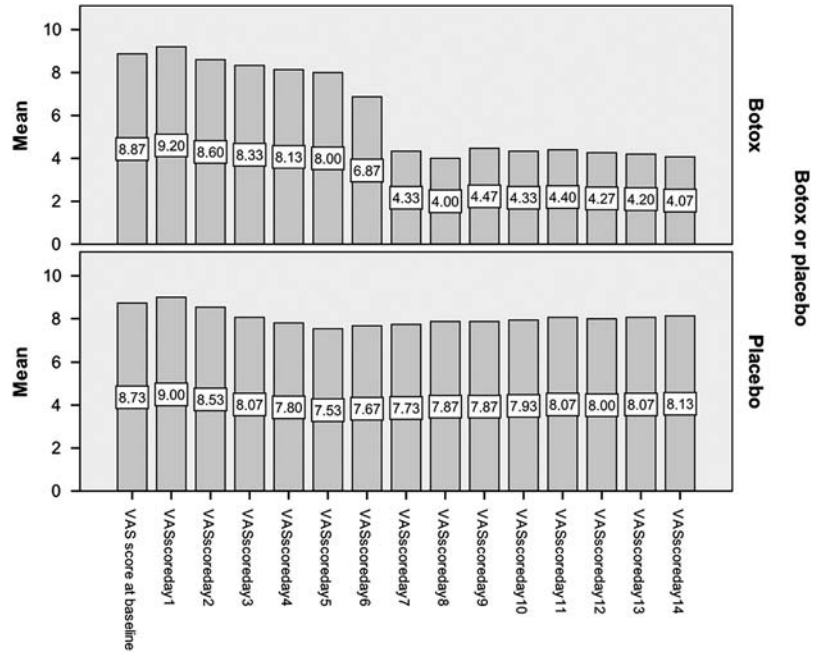

FIGURE 2. Mean visual analogue scale (VAS) scores at the follow-up visits, range, and SD.

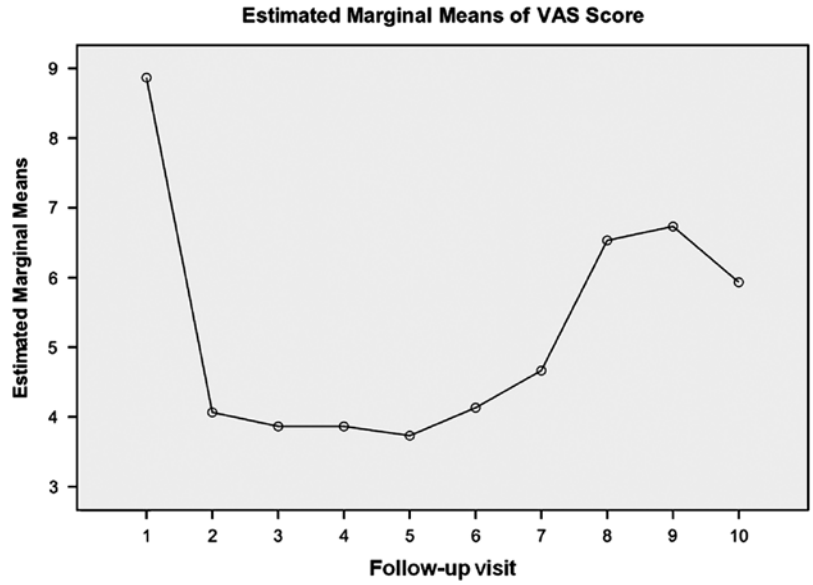

FIGURE 3. Botox-treated patients with postherpetic neuralgia. The profile plot for the visual analogue scale (VAS) scores in subsequent time visually confirms the steady decrease in VAS scores over and after the course of the Botox treatment, as noted in the estimated marginal means. Follow-up visit (FUV) 1: baseline, FUV 2: second week, FUV 3: fourth week, FUV 4: sixth week, FUV 5: eighth week, FUV 6: 10th week, FUV 7: 12th week, FUV 8: 16th week, FUV 9: 20th week, FUV 10: 24th week from baseline.

Sleep Score

BTX-A-treated patients showed significant improvements in sleep scores between baseline and week 2 (tests of within-patient contrasts, $P < 0.001$), which then remained stable between weeks 2 and 4 (tests of within-patient contrasts, $P = 0.546$) (Fig. 7). Placebo-treated patients also showed a trend toward a nonsignificant improvement in sleep score between baseline and week 2 (tests of within-patient contrasts, $P = 0.068$), which then remained unchanged between weeks 2 and 4 (tests of within-patient contrasts, $P = 1.000$). We further examined the curve of sleep score in the experimental arm. It seems that after the initial decline ($P < 0.001$), sleep scores remain unchanged until week 12 ($P < 0.001$, Fig. 6). Between treatment groups analysis reported estimated marginal means of sleep scores for baseline, second week, and fourth week: for BTX-A-treated patients 8.7 (7.5 to 9.9), 4.2 (3.2 to 5.3), and 4.1 (3.2 to 5.1) as opposed to 9.0 (7.5 to 9.9), 8.5 (7.5 to 9.6), and 8.5 (7.6 to 9.5) for controls, respectively (tests of between-patients effects, $P < 0.001$). A plot of the estimated marginal means

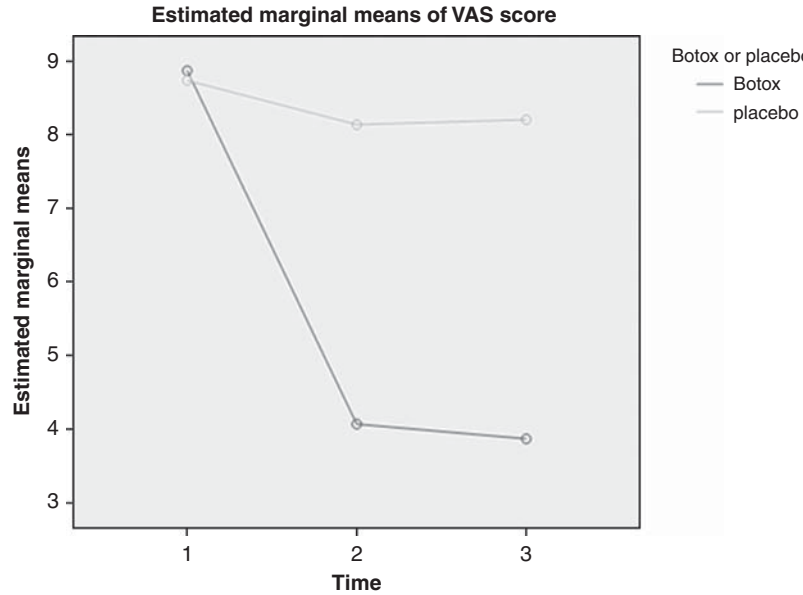

FIGURE 4. Patients with postherpetic neuralgia treated either by BTX-A or placebo. The profile plot for the visual analogue scale (VAS) pain scores in subsequent time visually confirms the steady decrease in VAS pain scores over the course of the treatment, as noted in the estimated marginal means. The marked difference in effect size between treatments is evident (tests of between-patiens effects, $P < 0.001$).

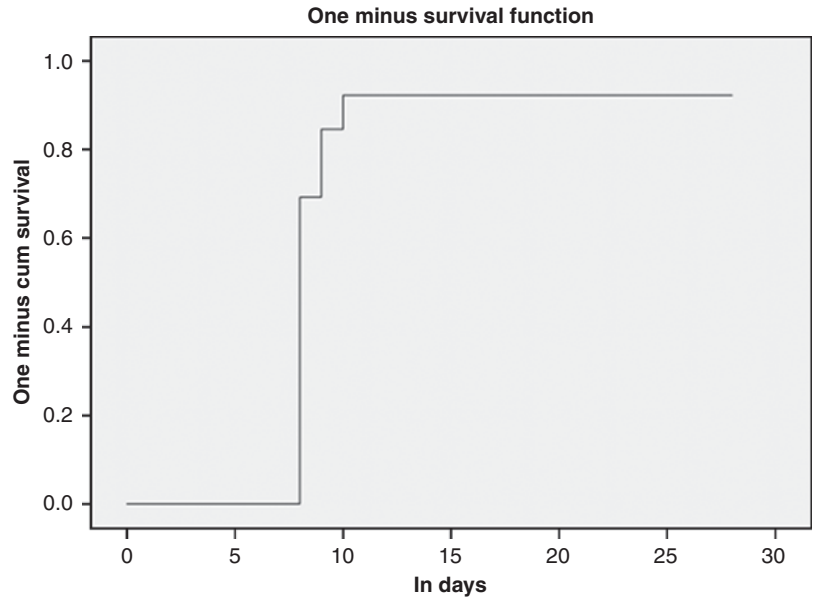

FIGURE 5. Life table analysis. One minus survival function of time to achievement of >50% reduction in visual analogue scale (VAS) pain score (median time = 7.44 d) in BTX-A-treated patients with postherpetic neuralgia.

is presented in Figure 8. Cohen's *d* effect size was 2.2 (2.8 to 5.8) for second week and 2.4 (3.0 to 5.8) for fourth week.

Tolerability and Safety

All individuals reported pain during injections, with no difference between BTX-A (mild pain: 2; moderate pain: 7; severe pain: 6) and placebo (mild pain: 3; moderate pain: 6; severe pain: 6; Pearson χ^2, $P = 0.778$). No patient had to discontinue the treatment because of discomfort and no patient was able to recognize the active treatment based on side effects. The pain was transient and gradually declined within the next 24 hours from treatment administration. No other local or systemic side effects were recorded during the procedure or at any other time during the study.

DISCUSSION

Our data clearly demonstrate that BTX-A is beneficial in the management of PHN, in terms of pain reduction and improvement of patients' quality of sleep. Pain relief occurred as early as 1 week after BTX-A administration, and it was preserved until the week 16 of the study. The therapeutic effect on pain was consistent with improvement on the secondary outcome of sleep disturbance by week 1, which was sustained throughout a 12-week period.

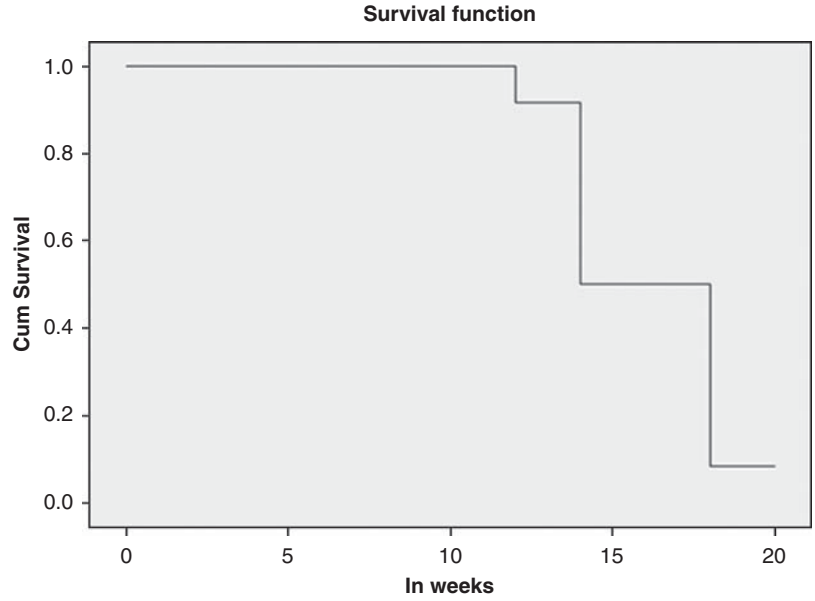

FIGURE 6. Life table analysis. Survival function of maintenance >50% of reduction in visual analogue scale (VAS) pain score (median time = 16.0 wk) in BTX-A-treated patients with postherpetic neuralgia.

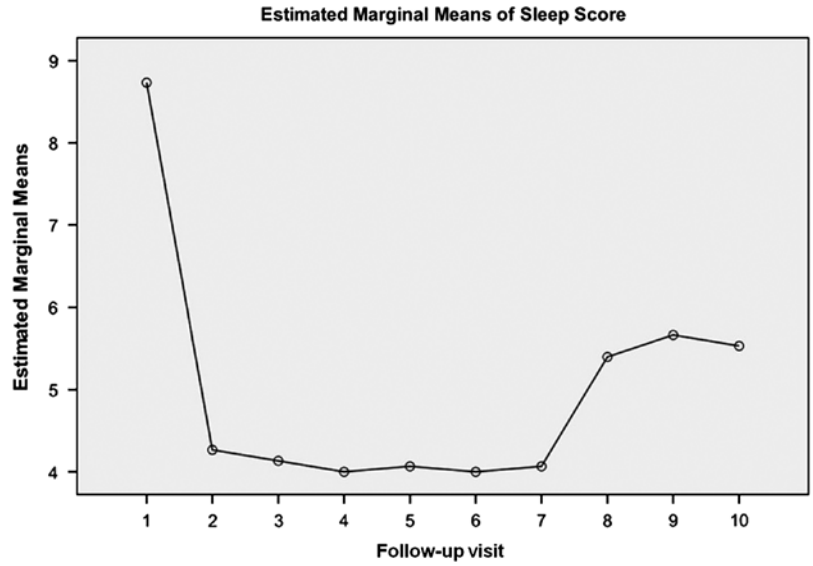

FIGURE 7. Botox-treated patients with postherpetic neuralgia. The profile plot for the sleep scores in subsequent time visually confirms the steady decrease in sleep scores over and after the course of the Botox treatment, as noted in the estimated marginal means. Follow-up visit (FUV) 1: baseline, FUV 2: second week, FUV 3: fourth week, FUV 4: sixth week, FUV 5: eighth week, FUV 6: 10th week, FUV 7: 12th week, FUV 8: 16th week, FUV 9: 20th week, FUV 10: 24th week from baseline.

The therapeutic effect, based on VAS score, gradually declined to $< 50\%$ of that initially achieved by the 16th week. This finding might justify a second course of BTX-A injections to prolong the achieved effect in pain management. Future studies focusing in the optimal dosage schema for maintenance of treatment outcome are warranted.

On the basis of our results, the risk/benefit ratio of BTX-A usage was shown to be very low, as no significant safety issues were raised. Similarly to previous studies,[9,11,12,14,15] the most frequently reported side effect was pain during injections, with no difference between BTX-A and placebo group, which resolved spontaneously few hours later. No other local or systemic adverse events were reported during the therapeutic procedure, or at any other time during the study.

Currently available evidence concerning efficacy of BTX-A in the management of neuropathic pain is controversial. Analytically, Ranoux et al[11] investigated analgesic effect of BTX-A in 29 patients with focal painful neuropathies and mechanical allodynia using a randomized, double-blind, placebo-controlled design. According to their results, BTX-A treatment, when compared with placebo, was associated with persistent improvement on pain intensity, which sustained in the proportion of responders

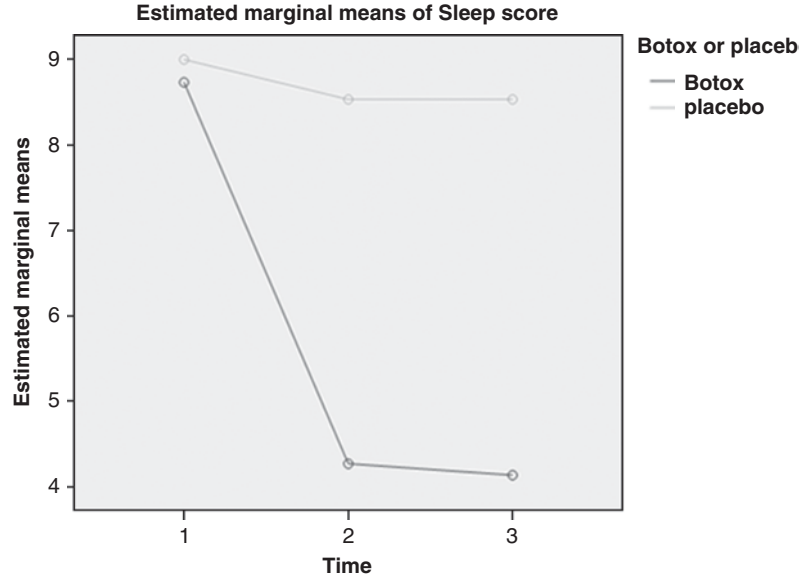

FIGURE 8. Patients with postherpetic neuralgia treated either by BTX-A or placebo. The profile plot for the sleep scores in subsequent time visually confirms the steady decrease in sleep scores over the course of the treatment, as noted in the estimated marginal means. Time 1: baseline, 2: week 2, 3 week 4.

(number needed to treat for 50% pain relief: 3.03 at 12 wk), neuropathic symptoms, and general activity. Investigators concluded that BTX-A may induce direct analgesic effects in patients with chronic neuropathic pain independent of its effects on muscle tone, suggesting for the first time BTX-A as a novel indications for analgesia.[11] Similarly, BTX-A was shown to be efficacious in improvement of allodynia and hyperesthesia in 20 patients with diabetic neuropathy.[14] In addition, preliminary results of a pilot study evaluating BTX-A in 14 individuals suffering from complex regional pain syndrome showed lack of efficacy.[13] As the authors of the latter study postulate, this discrepancy may be partially explained by differences in the pathophysiology of allodynia in complex regional pain syndrome when compared with allodynia after simple trauma, PHN, or diabetic neuropathy.[13] With regard to PHN, available data from case series[5,9,10] and randomized controlled trials[11,14,15] uniformly converge to the conclusion that BTX-A has a definite therapeutic benefit in this subgroup of neuropathy patients. In the largest—in terms of sample size—trial, a total of 60 patients were randomized to receive either BTX-A or placebo or lidocaine. The investigators found that the results were significantly better with BTX-A, at both 7 days and 3 months, on the primary outcome (pain intensity) as compared with the other 2 groups.[15] The latter conclusion is further established by our trial, which is the first study assessing efficacy of BTX-A in PHN, using the 50% reduction of VAS as a primary dichotomous outcome. This outcome has been reported to be one of the most clinically relevant efficacy outcomes in neuropathic pain trials.[17] In this context, results from the current study support the benefit of BTX-A in PHN.

Increased efficacy of BTX in our patients might be related to various study parameters. A large proportion of naive individuals in this trial might account for a better response to the treatment. Other reasons might include the short to moderate pain duration, as well as the very high pain score at baseline that it perhaps might be quite relevant to optimize the response to the active drug and reduce the placebo response. Possible limitations include the lack of qualitative evaluation of pain, as well as the fact that the sleep scale was not previously validated.

A main consideration to BTX-A use is that, unlike other therapeutic modalities for PHN, it induces antitoxin antibodies, an event that could probably lead to limitation of clinical effectiveness after repetitive long-term use.[18] Possible clinically significant loss of efficacy, as a result of antibodies-associated deactivation of the toxin, in conjunction with a potential need for dosage adjustment after repetitive treatments, might represent another interesting field for study.

In summary, our results demonstrate that in terms of efficacy, safety, and tolerability, BTX-A is a very promising therapeutic modality for PHN, and could be a welcome addition to the armamentarium of agents used to treat herpes-associated pain. Further studies are warranted to optimize and establish treatment protocols for long-term pain management (Supplementary Digital Content 1, http://links.lww.com/CJP/A55).

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REFERENCES

1. Bennett GJ. Hypotheses on the pathogenesis of herpes zoster-associated pain. *Ann Neurol*. 1994;35:S38–S41.
2. Cervero F, Laird JMA. Mechanisms of touch-evoked pain (allodynia): a new model. *Pain*. 1996;68:13–23.
3. Woolf CJ, Shortland P, Coggeshall RE. Peripheral nerve injury triggers central sprouting of myelinated afferents. *Nature*. 1992;355:75–78.
4. Sugeng MW, Yosipovitch G, Leok GC. Post herpetic neuralgia and the dermatologist. *Int J Dermatol*. 2001;40:6–11.
5. Tyring SK. Management of herpes zoster and postherpetic neuralgia. *J Am Acad Dermatol*. 2007;57:S136–S142.
6. Blasi J, Chapman ER, Link E, et al. Botulinum neurotoxin A selectively cleaves the synaptic protein SNAP-25. *Nature*. 1993;365:160–163.
7. Hay JC. SNARE complex structure and function. *Exp Cell Res*. 2001;271:10–21.
8. Cui M, Khanijou S, Rubino J, et al. Subcutaneous administration of botulinum toxin A reduces formalin-induced pain. *Pain*. 2004;107:125–133.
9. Sotiriou E, Apalla Z, Panagiotidou D, et al. Severe postherpetic neuralgia successfully treated with botulinum toxin a: three case reports. *Acta Derm Venereol*. 2009;89:214–215.
10. Liu HT, Tsai SK, Kao MC, et al. Botulinum toxin a relieved neuropathic pain in a case of post-herpetic neuralgia. *Pain Med*. 2006;7:89–91.
11. Ranoux D, Attal N, Morain F, et al. Botulinum toxin type a induces direct analgesic effects in chronic neuropathic pain. *Ann Neurol*. 2008;64:274–283.
12. Ruiz Huete C, Bermejo PE. Botulinum toxin type a in the treatment of neuropathic pain in a case of postherpetic neuralgia. *Neurologia*. 2008;23:259–262.
13. Safarpour D, Salardini A, Richardson D, et al. Botulinum toxin a for treatment of allodynia of complex regional pain syndrome: a pilot study. *Pain Med*. 2010;11:1411–1414.
14. Yuan RY, Sheu JJ, Yu JM, et al. Botulinum toxin for diabetic neuropathic pain: a randomized double-blind crossover trial. *Neurology*. 2009;72:1473–1488.
15. Xiao L, Mackey S, Hui H, et al. Subcutaneous injection of botulinum toxin a is beneficial in postherpetic neuralgia. *Pain Med*. 2010;11:1827–1833.
16. Wilkinson L. Statistical methods in psychology journals: guidelines and explanations. *Am Psychol*. 1999;54:594–604.
17. Attal N, Cruccu G, Baron R, et al. EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. *Eur J Neurol*. 2010;17:1113–e88.
18. Naumann M, Carruthers A, Carruthers J, et al. Meta-analysis of neutralizing antibody conversion with onabotulinumtoxinA (BOTOX®) across multiple indications. *Mov Disord*. 2010; 25:2211–2218.

