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Ultrasound-Guided Percutaneous Cryoneurolysis to Treat Chronic Post-Amputation Phantom Limb Pain:

A Multicenter, Randomized, Controlled Trial

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Abstract

Background: Post-amputation phantom pain is notoriously persistent with few validated treatments. Cryoneurolysis involves the application of low temperatures to reversibly ablate peripheral nerves. We tested the hypothesis that a single cryoneurolysis treatment would decrease phantom pain 4 months later.

Methods: We enrolled patients with a lower-limb amputation and established phantom pain. Each received a single-injection femoral and sciatic nerve block with lidocaine and was subsequently randomized to receive either ultrasound-guided percutaneous cryoneurolysis or sham treatment at these same locations. The primary outcome was the change in average phantom pain intensity between baseline and 4 months as measured with a Numeric Rating Scale (0–10), after which an optional crossover treatment was offered. Investigators, participants, and clinical staff were masked to treatment group assignment with the exception of the treating physician performing the cryoneurolysis who had no subsequent participant interaction.

Results: Pretreatment phantom pain scores were similar in both groups, with a median [quartiles] of **5.0** [4.0, 6.0] for active treatment and **5.0** [4.0, 7.0] for sham. After 4 months, pain intensity decreased by **0.5** [−0.5, 3.0] in patients given cryoneurolysis (n=71) versus **0** [0, 3] in patients given sham (n=73): estimated difference (95% CI) −0.1 (−1.0, 0.7), **P=0.759**. Following our statistical gatekeeping protocol, we did not make inferences or draw conclusions on secondary endpoints. One serious adverse event occurred following a protocol deviation in which a femoral nerve cryolesion was induced just below the inguinal ligament—instead of the sensory-only

saphenous nerve—which resulted in quadriceps weakness, and possibly a fall and clavicle fracture.

Discussion: Percutaneous cryoneurolysis did not decrease chronic lower extremity phantom limb pain 4 months following treatment. However, these results were based upon our specific study protocol; and since the optimal cryoneurolysis treatment parameters such as freeze duration and anatomic treatment location remain unknown, further research is warranted.

Introduction

Tens-of-millions of people are living with a lower limb amputation,[1] with up to 50–85% developing chronic, intractable pain perceived as originating from the missing limb, often described as “phantom limb pain”.[2] Phantom pain is notoriously persistent,[3] with few adequately powered randomized controlled trials to guide treatment.[4] The precise etiology of phantom pain is unclear. However, neural restructuring frequently occurs following limb amputation, and the degree of cortical reorganization is associated with phantom pain intensity.[5]

One study suggested that a single-injection local anesthetic peripheral nerve block can resolve both phantom pain and cortical abnormalities, although the improvements disappeared following block resolution.[6] Nevertheless, this demonstrated that at least in some cases, persistent cortical abnormalities and phantom pain may be maintained from abnormal input from the peripheral nervous system.[7] A recent randomized, controlled trial reported that prolonging a peripheral nerve block using a 6-day continuous perineural local anesthetic infusion extended limb analgesia for at least 1 month.[8] These findings suggest that a peripheral nerve block of extended duration—lasting weeks or months rather than days—may allow prolonged cortical reorganization and provide lasting relief from phantom pain.

A prolonged neural block is provided with cryoneurolysis which entails the application of very low temperatures (approximately −70°C using nitrous oxide) to *reversibly* ablate peripheral nerves.[9] Guided using real-time imaging, a percutaneously-inserted probe has gas circulated throughout its length, inducing cold at the distal end and freezing the target nerve.[10] There is no implanted device, and there is no external equipment to prepare, manage, or malfunction—a single administration results in effects measured in weeks to months without any subsequent patient or clinician interventions. While multiple uncontrolled case series suggest a possible analgesic benefit in treating phantom and residual limb pain with percutaneous cryoneurolysis,[11–13] the technique has not been validated for post-amputation pain in a randomized, controlled study.

We therefore designed this multicenter, randomized, observer- and participant-masked, sham-controlled, parallel-arm, partial crossover clinical trial to determine if a single treatment of ultrasound-guided percutaneous cryoneurolysis would provide effective and lasting analgesia for established lower extremity phantom limb pain. Specifically, we tested the primary hypothesis that the change in average phantom limb pain intensity between baseline and 4 months would be greater following cryoneurolysis versus sham treatment (as measured with the Numeric Rating Scale of the Brief Pain Inventory).

Methods

This study was conducted within the ethical guidelines outlined in the Declaration of Helsinki and followed Good Clinical Practice. The trial was prospectively registered at clinicaltrials.gov (NCT03449667; Principal Investigator: Brian M. Ilfeld, MD, MS; initial posting: February 28, 2018). The protocol was approved by the Institutional Review Board at each of the 6 enrolling centers as well as the United States Army Medical Research and Development Command Human Research Protection Office. Responsible for the oversight and conduct of the investigation was an independent Data Safety Monitoring Board (Appendix A). Written, informed consent was obtained from all participants.

Participants.

Six medical centers enrolled patients, including public and private civilian, Veterans Affairs, and military treatment facilities. Potential participants were presented with the study in chronic pain clinics and advertisements were posted in amputee-centered national print and web-based publications. Enrollment was offered to adult patients of at least 18 years of age with a lower limb traumatic or surgical amputation at least 12 weeks prior to enrollment. The amputation had to be distal to the hip (femoral head remaining) and patients had to experience at least moderate phantom limb pain defined as a 3 or higher on the Numeric Rating Scale (NRS; 0–10, 0= no pain; 10=worst imaginable pain) at least daily for the previous 2 months. Patients had to agree to avoid both changes to their analgesic regimen as well as elective surgical procedures from 1 month prior to, and at least 4 months following, the initial study intervention. Patients were excluded for an amide local anesthetic allergy, pregnancy, incarceration, inability to communicate with the investigators, morbid obesity (body mass index greater than 40 kg/m^2); and possessing any contraindication specific to cryoneurolysis such as a localized infection at the treatment site, cryoglobulinemia, cold urticaria and Raynaud's Syndrome.

Intervention.

Subjects were asked to not eat or drink after midnight prior to the procedure. For women of childbearing age with the possibility of pregnancy, a sample of urine was collected before any study interventions to rule-out pregnancy. All subjects had a peripheral intravenous catheter inserted, standard noninvasive monitors applied (blood pressure cuff, pulse oximeter, 5-lead ECG), and oxygen administered *via* a facemask or nasal cannula. Oral and/or intravenous sedatives and analgesics such as midazolam, diazepam, and fentanyl were titrated for patient comfort, if necessary, while ensuring that patients remained responsive to verbal cues.

The specific nerves targeted were the sciatic and femoral (or their distal branches), with the most distal location clearly visualized with ultrasound treated (but prior to the sciatic bifurcation and at the level of the medial epicondyle for the saphenous nerve). The potential cryoneurolysis entry sites were prepared with chlorhexidine gluconate and isopropyl alcohol and a sterile, fenestrated drape. Using the appropriate ultrasound transducer for the specific anatomic location and subject anatomy (linear vs curvilinear array), the target nerves were identified in a transverse cross-sectional (short axis) view. A local anesthetic skin wheal was

raised adjacent to the ultrasound transducer and a Tuohy-tip needle was inserted through the skin wheal in-plane beneath the ultrasound transducer and directed until the needle tip was immediately adjacent to the target nerve. Local anesthetic (1–3 mL, lidocaine 2%) was injected in divided doses with frequent aspiration. This was repeated for the additional target nerve(s). Within 20 minutes of the last injection, the subject's limb pain level was evaluated on the 0–10 NRS and if higher than at baseline prior to injection, the subject did not continue with treatment and their participation in the study ended.

Treatment group assignment (randomization).

Remaining subjects were allocated to one of two possible treatments: *active* cryoneurolysis or *sham (placebo)*. Randomization was stratified by institution in randomly chosen block sizes using computer-generated lists by the informatics group of the Department of Outcomes Research at the Cleveland Clinic. Treatment group assignment was conveyed to the enrolling sites *via* the same secure web-based system used to collect and collate all post-intervention outcomes (Research Electronic Data Capture, Cleveland Clinic, Cleveland, Ohio).[14]

A cryoneurolysis console device was used for all participants (PainBlocker, Epimed, Farmers Branch, Texas). Cryoneurolysis probes are available that either (1) pass nitrous oxide to the tip inducing freezing temperatures (approximately −70°C); or (2) vent the nitrous oxide at the base of the probe so that no gas reaches the probe tip, resulting in no temperature change. Importantly, these 16 gauge, trocar-tipped probes are indistinguishable in appearance and audible cues, and therefore investigators, participants, and all clinical staff were masked to treatment group assignment (with the exception of the treating physician performing the cryoneurolysis). Following repeated sterile preparation and draping, an angiocatheter-like introducer was inserted beneath the ultrasound transducer and directed until immediately adjacent to the target nerve. The appropriate probe (active vs sham) was inserted through the introducer and the cryoneurolysis device was triggered using 3 cycles of 2-minute gas activation separated by 1-minute defrost periods.[15] The process was repeated for each treated nerve using the same probe for all applications (e.g., all nerves received either active cryoneurolysis or sham/placebo, and not a mix of the two possible treatments).

Of note, the treating physician was not masked to treatment group assignment during the cryoneurolysis procedure. This was because the ice ball forming at the distal end of the probe—with active treatment—is clearly visible by ultrasound; and the lack of an ice ball for placebo subjects is equally clear.[16] We believe it is essential to continuously visualize the probe and target nerve throughout the freeze/thaw cycles to ensure (1) the entire nerve diameter is fully encompassed by the sphere of ice and (2) the ice ball remains relatively motionless to prevent it tearing surrounding tissue. This cannot be achieved if the ultrasound is turned off during nitrous oxide administration to mask the provider; and we prioritized patient safety over provider masking. Treating physicians did not have subsequent contact with study participants, or data collection, management, and analysis.

Prior to discharge, participants and their caretakers were provided with verbal and written instructions as well as the contact information for an investigator. Patients were informed that any sensory deficits from the short-acting lidocaine bolus that they may be experiencing

would regress, and that they should not be alarmed by any subsequent increase in pain. Participants were provided with crutches if they so desired, although prior experience suggested that nearly all patients treated with cryoneurolysis continue to ambulate using their prosthesis without difficulty.

Optional crossover treatment.

Up to 2 months following the primary outcome measurement at Month 4, participants could return for an optional repeated intervention procedure ("crossover") with the alternative treatment (either active cryoneurolysis or sham/placebo), using the same protocol as described for the initial intervention. The crossover treatment was not required for study participation, as the primary analysis included a parallel study design for the initial intervention evaluated prior to any crossover treatment. However, the optional crossover treatment was offered for two reasons: (1) to ensure that all subjects had access to the proposed treatment, regardless of the treatment to which they were initially assigned; and (2) to permit intra-subject differences between treatments to be analyzed (e.g., assessing treatment-effect heterogeneity, or the variability of the causal effect across individuals, which will would not be available from the parallel-group portion of the study alone). These intra-subject differences were secondary analyses, as there would be patient-selection bias regarding which subjects decided to have the crossover treatment.

The main results of the study were provided to all participants following final analysis.

Outcome measurements.

We selected outcome measures that have established reliability and validity, with minimal inter-rater discordance, and are recommended for chronic pain clinical trials by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) consensus statement.[17] Outcomes were evaluated at baseline (prior to intervention), on days 1 and 7, as well as months 1, 2, 3, and 4 relative to the initial and optional crossover treatment(s). In addition, outcomes were evaluated 12 months following the initial intervention. Outcome measures were collected in person for the baseline measurements immediately prior to the initial intervention as well as the crossover treatments. All other outcomes were collected by investigators at the University of California, San Diego by telephone regardless of enrolling center.

The questionnaires differentiated multiple dimensions of limb sensations/pain:

Residual limb ("stump") pain: Painful sensations localized to the portion of limb still physically present [18]

Phantom limb pain: Painful sensations referred to the lost body part [18]

Phantom limb sensations: *Non*-painful sensations referred to the lost body part [18]

Each type of pain/sensation was defined for patients immediately prior to questionnaire application at each time point, and patients were instructed to address phantom limb pain when responding to questions unless otherwise specified. Each time the questionnaire was applied, participants were instructed to respond for the previous 3 days.[19] Exceptions

included Day 1 for both the initial and crossover treatments because at these time points the interest was in participants' experiences subsequent to the intervention. At these time points, participants were instructed to respond for the period since the intervention the previous day.

The primary instrument was the Brief Pain Inventory (short form) which assesses pain and its interference with physical and emotional functioning.[20] The form includes three domains: (1) *pain,* with four questions using an NRS to evaluate 4 pain levels: "current", "least", "worst", and "average"; (2) percentage of *relief* provided by pain treatments with one question; and, (3) *interference* with physical and emotional functioning using a 0–10 scale (0 = no interference; 10 = complete interference). The seven interference questions involve general activity, mood, walking ability, normal work activities (both inside and outside of the home), relationships, sleep, and enjoyment of life.[20] The seven functioning questions can be combined to produce an interference subscale (0–70). The use of both single items (e.g., mood) and the composite scores is supported by the IMMPACT recommendations for assessing pain in clinical trials.[17,21] Because phantom limb and residual limb ("stump") pain have been correlated, the latter was assessed with the same four pain intensity questions.

To provide a global measure of worsening or improvement, the Patient Global Impression of Change was administered allowing patient evaluation of integrated treatment effects.[17] This measure is a 7-point ordinal scale requiring the patient to rate the current intensity of phantom limb pain compared to their pre-treatment baseline: 1 for "very much worse" to 7 for "very much improved" (4 is "no change"). Additional psychosocial factors were evaluated using the Beck Depression Inventory, a 21-item instrument measuring characteristic symptoms and signs of depression.[22] Each of the 21 factors is rated on a 0–3 scale, and then summed to produce the total score of 0–63. Mild, moderate, and severe depression are defined with scores of 10–18, 19–29, and 30–63, respectively.[23] Lastly, the frequency and average duration of non-painful phantom sensations as well as phantom and residual limb pain were assessed.

Statistical analysis.

Treatment group assignment was unmasked only following completion of the statistical analysis. We used descriptive statistics to compare the treatment groups for baseline variables. Groups were considered well-balanced on a particular baseline variable if the absolute standardized difference (difference in means, mean ranks or proportions divided by the pooled standard deviation) was less than $1.96\sqrt{(n_1+n_2)/(n_1n_2)} = 0.46$, where n_1 and n_2 are the per-group sample sizes.[24] All analyses were modified intention-to-treat, in which all randomized subjects who received any of the study treatment were included and retained in their respective treatment groups.[25] Confidence intervals were adjusted for the group sequential design with overall alpha of 0.05, such that 95.6% confidence intervals are reported throughout (referred to as "95% confidence intervals"). The study was designed with 90% power to detect a mean change of 1.7 or more on the NRS for "average phantom pain" while adjusting for 3 interim analyses. Missing data were imputed using last observation carried forward for the primary outcome and using multiple imputation for secondary outcomes and sensitivity analysis on the primary outcome.

Aim 1: Primary outcome.

We assessed the average causal effect of cryoneurolysis (active) versus sham/placebo on phantom limb pain intensity (average pain over previous 72 hours) at 4 months after the initial treatment using analysis of covariance to adjust for clinical site, baseline average pain intensity, clinical site, and any imbalanced baseline variable. We also assessed the treatment effect on the change from baseline average pain intensity (instead of adjusting for baseline pain score) in an analogous linear regression model. As a sensitivity analysis we assessed the median difference (95% CI) of active vs placebo using the Hodges-Lehmann estimator of location shift and compared groups with a Wilcoxon rank sum test stratified by study site. We also assessed the treatment-by-clinical site interaction in the linear regression models.

Assessing treatment effect heterogeneity.—We assessed whether the treatment effect on the primary outcome (phantom limb pain intensity over past 72 hours) varied across levels of specific baseline variables [besides clinical site] using linear regression as in the primary analysis and testing the treatment-by-covariate interaction. We assessed treatment effect heterogeneity across level of sex, body mass index, amputation level, phantom pain 20 minutes after 2nd lidocaine injection, and baseline average phantom and residual limb pain, with a pre-determined significance criterion of $P<0.10$ for the interaction, without correction made for these multiple covariate analyses. For the last 3 variables, which are continuous/ordinal pain scores, the interaction was assessed on a continuous scale, although the results are shown in the forest plot dichotomizing pain into mild (NRS ≤ 3) versus moderate to severe (NRS > 3).

Secondary outcomes (at 4 months).

Randomized groups were compared at 4 months on the global measure of improvement (Patient Global Impression of Change scale; Aim 2a) using the Wilcoxon rank sum test and Hodges Lehman estimation of location shift, stratified by study site. We used a mixed effects regression model with a fixed effect for treatment and an unstructured correlation matrix adjusted for study site and baseline pain interference components to assess the treatment effect across the 7 components of the Brief Pain Inventory pain interference (Aim 2b). Randomized groups were compared on the Beck Depression Inventory using the Wilcoxon rank sum test and estimating the treatment effect using the Hodges-Lehmann estimator of location shift, stratified by study site (Aim 2c).

The crossover treatment 4–6 months following the initial intervention allowed all subjects the opportunity to receive the study treatment, but because it was optional also introduced selection bias from this time point forward. For crossover patients, we assessed the treatment effect using a linear mixed effects regression model with a fixed effect for treatment and random effect for patient, adjusted for treatment sequence and period. We tested for evidence of differential carry-over effect with the treatment-by-period interaction. We also descriptively assessed (no treatment effects were estimated) the change from the initial baseline to 12 months for the initial active and sham participants for those who both did and did not receive the crossover treatment.

Interim analyses.

We conducted interim analyses to assess efficacy (rejecting null) and futility (rejecting alternative) at each 25% of the maximum enrollment using a group sequential procedure. Specifically, a gamma spending function was used with parameters −4 and −2 for efficacy and futility, respectively.[26] Thus, boundaries at the 1st through 4th analyses for efficacy (futility in parentheses) were P≤ 0.0016 (P>0.9572), P≤ 0.0048 (P>0.7186), P≤ 0.0147 (P>0.2389) and P≤ 0.0440 (P>0.0440) (Supplemental Table A and Figure A).

Type I error and Gatekeeping.—We designed the study to use a parallel gatekeeping procedure to control the study-wide type I error at 0.05.[27] For this procedure, we therefore *a priori* prioritized the study outcomes into ordered sets, as Aim 1, Aim 2a, Aim 2b and then Aim 2c. Analysis would proceeded in that order, and testing would proceed through each "gate" to the next set if and only if at least one outcome in the current set reaches significance. The significance level for each set would be 0.05 times a cumulative penalty for non-significant results in previous sets (i.e., a "rejection gain factor" equal to the cumulative product of the proportion of significant tests across the preceding sets). Within a set, a multiple comparison procedure (Bonferroni correction) was planned to control the type I error at the appropriate level.

Sample size considerations.—Our sample size estimate was based on the primary specific aim of whether the addition of cryoneurolysis decreases phantom limb pain intensity resulting from an amputation compared with current standard-of-care treatment at 4 months following cryoneurolysis. Receiver operating characteristic curve analyses demonstrated that changes from baseline of at least 1.7 along a 10-point NRS accurately identified patients who rated improvements as "much improved" or more, compared with those who perceived no change or worsening following analgesic interventions.[28] Multiple additional studies confirm this degree of reduction as clinically meaningful to individual patients with chronic pain.[29–31] Although meaningful group differences in the mean change would be somewhat smaller than important changes for individuals, we took a very conservative approach and powered our study to be able to detect group differences in mean change from baseline of 1.7 points or more on the NRS.

Based on a conservative standard deviation estimate for each group of 3.0 at 4 months, a correlation of 0.50 between baseline and follow-up NRS, a two-sided test at the 0.05 significance level, power of 0.90, and 4 equally spaced analyses (3 interim and 1 final, as needed), a maximum of 72 subjects in each group (N=144 total) is required. The expected sample size for this group sequential design (i.e., average sample size over thousands of such trials, stopping when a boundary is crossed) was a total of 100 under the alternative and 102 under the null hypotheses. East 5.3 software (Cytel Inc., Cambridge, MA, USA) was used for sample size calculations and all analysis.

Results

Between March 2018 and March 2021, a total of 144 patients were enrolled at 6 medical centers (Figure 1). Phantom limb pain fell from a median [quartiles] of 4.0 [2.0, 6.0] immediately prior to the initial single-injection lidocaine bolus to 0 [0, 3.0] for the active

group, 0 [0, 2.0] for the placebo group 20 minutes following the bolus. No participant experienced an increase in limb pain in the 20 minutes following the lidocaine injections. Therefore, all participants were randomized to either active treatment with cryoneurolysis (n=71) or sham/placebo (n=73). Regarding baseline characteristics, all the variables were balanced between the two randomized groups with ASD ≤ 0.33 (Table 1).

Primary outcome.

Pretreatment phantom pain scores were a median [quartiles] of **5.0** [4.0, 6.0] for active treatment (cryoneurolysis) and **5.0** [4.0, 7.0] for sham/placebo. At 4 months average phantom limb pain scores were 4.3 [1.5, 6] for active and 4.5 [2, 6] for placebo, with estimated difference in means (95% CI) of −0.12 (−0.95, 0.7), P=0.759, adjusting for baseline pain score and clinical site while using last-observation-carried-forward (for n=1 cryoneurolysis and n=7 placebo patients); the futility boundary was crossed (Supplemental Figure A). We also assessed change from baseline: pain intensity decreased by **0.5** [−0.5, 3.0] in patients given cryoneurolysis (n=71) versus **0** [0, 3] in patients given sham (n=73): estimated difference (95% CI) −0.1 (−1.0, 0.7), **P=0.759**. Finally, the nonparametric Hodges-Lehman estimator comparing active and placebo on 4 month average phantom limb pain scores gave a similar result, with median difference (95% CI) of −0.25 (−1, 0.5), P=0.565.

Treatment effect heterogeneity.—There was little notable evidence of treatment effect heterogeneity across levels of most of the selected baseline (pre-randomization) variables, except for amputation level (interaction P=0.003, Figure 2). Active cryoneurolysis was better for a trans-tibial amputation level, but worse for trans-femoral and ankle/foot amputations (Table 2).

Gatekeeping rules.—Since the primary outcome was not significant, based on our a priori statistical plan to use a parallel gatekeeping approach to control study-wide type I error at 5% we cannot make inference on any of the secondary endpoints. Therefore, secondary outcome results are given in the form of estimated difference and confidence interval (not P-value), but we do not make any formal inference or conclusions on them—only on the primary outcome.

Secondary end points.

Using the 1–7 Global Impression of Change Scale at Month 4, participants who received active treatment rated their phantom pain as a median of **4** (“no change”) [4, 7] versus **4** (“no change”) [4, 6] for placebo subjects with an estimated median difference (95% CI) of 0 (0, 0) at 4 months (Aim 2A). Using the Brief Pain Inventory interference subscale to measure pain’s interference with physical and emotional functioning at Month 4, patients who received active cryoneurolysis scored **23** [0, 39] versus **22** [3, 34] for sham: median difference (95% CI) of 0 (−5, 6) (Aim 2B, Table 3, Figure 3). The mixed effects model suggested no treatment-by-component interaction, and the estimated difference in means (95% CI) [scale 0–10] was 0.2 (−0.5, 0.9). Using the Beck Depression Inventory (Aim 2C), subjects receiving active treatment reporting a median change from baseline of **−2** [−7, 0] vs. **−2** [−5, 0] for sham: difference (95% CI) of 1 (−1, 3). Descriptively, cryoneurolysis did not

demonstrably improve phantom and residual limb pain outcomes at any time point compared with the sham treatment (Table 3, Figures 4 and 5).

Crossover treatments.

The crossover treatment administered 0–2 months following the measurement of the primary outcome was optional, resulting in selection bias on patients who did not cross over and, on those who did cross over, potential interference with the longer-term effects of the initial treatment. Therefore, outcomes following the 4-month time point are reported descriptively only. Ninety-one patients participated in the crossover phase, receiving either an active (n=49) or sham (n=42) treatment (Supplemental Table B). Active treatment appeared to be similar to sham on 4 months average phantom limb pain intensity, pain's interference on physical and psychological functioning, and Patient Global Impression of Change (Supplemental Table C). The period by treatment interaction P-value of 0.04 suggested that there was some evidence of differential carryover effect between the first and second periods. These results would be generalizable to patients like those who chose to receive the crossover, which may differ from the main trial population. As well, active treatment had a larger reduction from baseline in average phantom limb pain intensity compared to placebo, with a mean difference (95% CI) of −1 (−2, −0.5). The variability in the individual causal effects of active versus placebo as measured by the standard deviation of the individual treatment effects was 1.3. Outcomes at 12 months following randomization did not appear to differ between treatment groups (Table 4).

Serious adverse events and major protocol deviations.

There were two deaths within the year following treatment, neither determined to be related to study participation: one myocardial infarction and one related to COVID-19 infection with severe acute respiratory syndrome. One participant developed dementia of unknown etiology within the 6 months following his initial treatment per an adult child's report. The only adverse event deemed related to study participation was a woman with a trans-tibial amputation who first received a sham treatment and subsequently crossed over with an active treatment that was performed just distal to the inguinal ligament for the femoral nerve. This protocol deviation resulted in profound quadriceps femoris weakness and some insensate areas of skin on the medial thigh. These deficits resolved slowly until complete resolution following 12–15 months. However, three months following the active crossover treatment she fell while climbing stairs and fractured a clavicle which required three subsequent surgical fixation procedures.

Discussion

This multicenter, randomized, sham-controlled trial failed to identify a benefit in treating established post-amputation phantom limb pain with ultrasound-guided percutaneous cryoneurolysis. This is a somewhat surprising and disappointing finding considering that cryoneurolysis has been used to treat post-amputation pain for decades with favorable outcomes reported in *un*controlled case series.[11–13] We can only speculate on the reasons for these contrasting findings.

The most obvious potential explanation is that cryoneurolysis does not, in fact, result in lasting, measurable analgesic benefits, and previous reports of post-treatment improvement in uncontrolled series are due to a placebo effect, selective reporting, and/or natural resolution of pain over time.[11–13] As possible evidence of a placebo effect, 29% of the sham group experienced a decrease in pain score of at least 1.7, the threshold we prospectively defined as the smallest clinically-relevant improvement for individuals based on previously-published data (similar to the 36% who had received active cryoneurolysis).[28]

It is also possible that the improvement in about one-third of all patients was not a placebo response to the study intervention, but rather a consequence of the single-injection of local anesthetic administered to all participants immediately prior to the study intervention.[6] This would help to explain why participants who chose to cross over did not experience the analgesic benefits of those who responded to the initial treatment and who therefore, presumably, chose not to undergo the crossover treatment. While a possible explanation for our results over the first few months, it is doubtful that a single injection of lidocaine is responsible for the finding that most of these “responders” reported continued improvement after 12 months.[6,8]

Alternatively, our negative results may be explained by the locations we applied cryoneurolysis. In the subset of patients with a trans-tibial amputation—a majority of participants (n=92)—cryoneurolysis *was* associated with an improved outcome compared with sham at 4 months (P=0.003 overall; pairwise comparisons: trans-tibial vs ankle, P=0.017; trans-tibial vs trans-femoral, P=0.006). Conversely, patients with a trans-femoral or foot/ankle amputation who received active treatment fared worse than their sham counterparts. These could be spurious findings (Type I error), but it is worth exploring given that medical progress is usually iterative, and 3 major differences between trans-tibial and trans-femoral amputations may help inform future research: (1) duration of effect; (2) impact on abnormal input from the peripheral to central nervous system; and (3) target nerve cross-sectional area.

Regarding the first—a reduced duration of a treatment effect for trans-femoral amputations—it is important to note that the optimal point for cryoneurolysis along a target nerve remains unknown. We chose to treat both the sciatic and femoral nerves at the most distal locations clearly visualized with ultrasound as low as the bifurcation of the sciatic nerve and medial femoral epicondyle for the saphenous nerve. Our reasoning was that a more proximal lesion could increase sensory, motor, and proprioception deficits in the residual limb, increasing the risk of falls when using a prosthesis for the entire treatment effect duration, usually measured in months. Of 144 participants, the only serious adverse event deemed related to study participation may be seen as supporting this decision: an investigator chose to treat the femoral nerve at the inguinal ligament for a patient with a trans-tibial amputation instead of more distally at the medial femoral epicondyle, resulting in profound quadriceps femoris muscle weakness lasting over one year and possibly contributing to a fall three months following treatment.

However, using a distal cryoneurolysis location for the remaining participants likely decreased the duration of effect for trans-femoral amputations. The primary determinant

of cryoneurolysis duration is a function of the distance between the cryolesion and nerve endings, with nerves regrowing at approximately 1–2 mm/day.[32] Therefore, if our theory that block duration and analgesic benefits are correlated, the short cryolesion-nerve ending distance for trans-*femoral* amputations would greatly decrease both the duration of cryoneurolysis effects and the impact on phantom limb pain. In contrast, because we did not apply cryoneurolysis distal to the medial femoral epicondyle, for trans-*tibial* amputations there was a greater length of remaining nerve distal to the cryolesion, resulting in an increased treatment effect duration and therefore possibly analgesic effects.[12]

The second difference between the two amputation locations—reduced impact on abnormal input from the peripheral to central nervous systems—is also based on our chosen protocol. We did not treat the obturator or posterior femoral cutaneous nerves which contribute to the innervation at the level of trans-femoral amputations, so the coverage provided by the cryoneurolysis intervention was inherently incomplete. In contrast, afferent sensory input for trans-tibial amputations is carried by the two nerves we did treat—the sciatic nerve and saphenous branch of the femoral nerve. Effective treatment for trans-femoral amputations may require the administration of cryoneurolysis to all nerves innervating the lower extremity.

The third difference between the two amputation locations is the target nerve cross-sectional area which is larger the more proximal within the lower extremity. The premise of our study hypothesis is that phantom limb pain is at least partially sustained by abnormal input from the peripheral to the central nervous systems. Therefore, interrupting the abnormal input with cryoneurolysis requires a thorough neural lesion with a prolonged duration. Reducing the temperature of a nerve below −20°C (but not colder than −100°C) results in a Sunderland second-degree nerve injury characterized by a reversible degeneration of axons known as Wallerian degeneration.[33] In contrast, temperatures warmer than −20°C result in a first-degree nerve injury,[34] which induces a shorter, unpredictable neuropraxia that can itself result in dysesthesias.[35] In other words, an inadequate freeze can actually induce pain. In our study, while treating physicians visualized the ice ball with ultrasound to ensure it encompassed the entire nerve, there is no guarantee that the entire sphere of ice cooled below −20°C, possibly inducing a variable duration neuropraxia that could result in analgesia initially but increase pain subsequently.[36] In addition, without Wallerian degeneration, the entire nerve could have remained functional since myelinated fibers can conduct “over” small lesions: while a lesion length of 3–6 mm is adequate to severe conduction in laboratory animals, the length in humans remains unknown.[37] Since the sciatic nerve is the largest in the human body, cryoneurolysis of more proximal application for the trans-femoral amputations may have resulted in incomplete cryolesions. Supporting this theory is our seemingly counterintuitive finding that patients with a trans-femoral amputation who received active treatment fared *worse* than their sham counterparts.

Evidence contradicting these last 2 interpretations is that amputations at or below the ankle had the same cryoneurolysis administration levels as trans-tibial yet were *not* associated with improved outcomes. Possibly explaining this apparent contradiction is that there were only 9 ankle cases and therefore this finding may itself be spurious, with confidence in the result far lower than for the trans-femoral (n=43) and trans-tibial (n=92) subgroups.

Limitations.

The major limitation of our trial is the reality that the optimal cryoneurolysis treatment parameters such as duration of freeze, duration of thaw, number of freeze/thaw cycles, freeze temperature, probe design, and anatomic treatment location all remain unknown.[10] The specific technique used in our trial was based on published (successful) pilot studies and decades of prior clinical experience,[9,11,12] but whether other techniques might have different effects is unknown.

A second limitation is the local anesthetic administered prior to the study intervention for both active cryoneurolysis as well as sham treatment groups. As such, even participants who underwent the sham procedure had a single-injection peripheral nerve block which may decrease post-amputation pain for up to a few weeks.[38] We provided local anesthetic to participants who would receive the active treatment because (1) it negates the discomfort experienced by many patients undergoing cryoneurolysis; and (2) we wanted to confirm that a peripheral nerve block would not induce paradoxical pain: a rare response, but one which could result in months of increased pain following a cryoneurolysis procedure (which we did not observe).[39] We provided the local anesthetic block to patients who would receive sham to retain masking of treatment group assignment: we presumed that patients who experienced absolutely no sensory changes during the (sham) study intervention without a peripheral nerve block would assume they had received the sham. While this protocol does not decrease confidence in our primary outcome—we can conclude that the addition of cryoneurolysis failed to improve pain outcomes 4 months after treatment—it does make interpretation of our negative results and designing subsequent research more challenging.

In summary, ultrasound-guided percutaneous cryoneurolysis did not reduce phantom limb pain 4 months after treatment. Although we do not make inferences or draw conclusions on the secondary endpoints due to our gatekeeping procedure and negative primary outcome, assessment of treatment effect heterogeneity remains important. Exploratory *post hoc* analysis revealed that treatment effect after 4 months was associated with the level of amputation, with trans-tibial amputation responsive to cryoneurolysis as opposed to ankle/foot and trans-femoral which fared worse than sham. The reasons for this difference remain unclear and future research is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R: Estimating the prevalence of limb loss in the United States: 2005 to 2050. Arch Phys Med Rehabil 2008; 89: 422–429 [PubMed: 18295618]
2. Hsu E, Cohen SP: Postamputation pain: epidemiology, mechanisms, and treatment. J Pain Res 2013; 6: 121–36 [PubMed: 23426608]
3. Wartan SW, Hamann W, Wedley JR, McColl I: Phantom pain and sensation among British veteran amputees. Br J Anaesth 1997; 78: 652–659 [PubMed: 9215014]
4. Richardson C, Kulkarni J: A review of the management of phantom limb pain: challenges and solutions. J Pain Res 2017; 10: 1861–1870 [PubMed: 28860841]
5. Flor H, Elbert T, Knecht S, Wienbruch C, Pantev C, Birbaumer N, Larbig W, Taub E: Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. Nature 1995; 375: 482–484 [PubMed: 7777055]
6. Birbaumer N, Lutzenberger W, Montoya P, Larbig W, Unertl K, Topfner S, Grodd W, Taub E, Flor H: Effects of regional anesthesia on phantom limb pain are mirrored in changes in cortical reorganization. J Neurosci 1997; 17: 5503–5508 [PubMed: 9204932]
7. Silva S, Loubinoux I, Olivier M, Bataille B, Fourcade O, Samii K, Jeannerod M, Demonet JF: Impaired visual hand recognition in preoperative patients during brachial plexus anesthesia: importance of peripheral neural input for mental representation of the hand. Anesthesiology 2011; 114: 126–34 [PubMed: 21169795]
8. Ilfeld BM, Khatibi B, Maheshwari K, Madison SJ, Esa WAS, Mariano ER, Kent ML, Hanling S, Sessler DI, Eisenach JC, Cohen SP, Mascha EJ, Ma C, Padwal JA, Turan A, Investigators PA: Ambulatory continuous peripheral nerve blocks to treat postamputation phantom limb pain: a multicenter, randomized, quadruple-masked, placebo-controlled clinical trial. Pain 2021; 162: 938–955 [PubMed: 33021563]
9. Trescot AM: Cryoanalgesia in interventional pain management. Pain Physician 2003; 6: 345–60 [PubMed: 16880882]
10. Ilfeld BM, Finneran JJ: Cryoneurolysis and Percutaneous Peripheral Nerve Stimulation to Treat Acute Pain. Anesthesiology 2020; 133: 1127–1149 [PubMed: 32898231]
11. Moesker AA, Karl HW, Trescot AM: Treatment of phantom limb pain by cryoneurolysis of the amputated nerve. Pain Pract 2014; 14: 52–6 [PubMed: 23279331]
12. Prologo JD, Gilliland CA, Miller M, Harkey P, Knight J, Kies D, Hawkins CM, Corn D, Monson DK, Edalat F, Dariushnia S, Brewster L: Percutaneous Image-Guided Cryoablation for the Treatment of Phantom Limb Pain in Amputees: A Pilot Study. J Vasc Interv Radiol 2017; 28: 24–34 e4 [PubMed: 27887967]
13. Neumann V, O'Connor RJ, Bush D: Cryoprobe treatment: an alternative to phenol injections for painful neuromas after amputation. AJR Am J Roentgenol 2008; 191: W313; author reply W314 [PubMed: 19020222]
14. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of biomedical informatics 2009; 42: 377–81 [PubMed: 18929686]
15. Gill W, Fraser J, Carter DC: Repeated freeze-thaw cycles in cryosurgery. Nature 1968; 219: 410–3 [PubMed: 5662164]

16. Kastler A, Gruber H, Gizewski E, Loizides A: Ultrasound Assessment of Ice-Ball Formation by Cryoneurolysis Device in an Ex Vivo Model. Reg Anesth Pain Med 2018; 43: 631–633 [PubMed: 29781931]

17. Dworkin RH, Turk DC, Peirce-Sandner S, Baron R, Bellamy N, Burke LB, Chappell A, Chartier K, Cleeland CS, Costello A, Cowan P, Dimitrova R, Ellenberg S, Farrar JT, French JA, Gilron I, Hertz S, Jadad AR, Jay GW, Kalliomaki J, Katz NP, Kerns RD, Manning DC, McDermott MP, McGrath PJ, Narayana A, Porter L, Quessy S, Rappaport BA, Rauschkolb C, Reeve BB, Rhodes T, Sampaio C, Simpson DM, Stauffer JW, Stucki G, Tobias J, White RE, Witter J: Research design considerations for confirmatory chronic pain clinical trials: IMMPACT recommendations. Pain 2010; 149: 177–93 [PubMed: 20207481]

18. Manchikanti L, Singh V: Managing phantom pain. Pain Physician 2004; 7: 365–75 [PubMed: 16858476]

19. Broderick JE, Schwartz JE, Vikingstad G, Pribbernow M, Grossman S, Stone AA: The accuracy of pain and fatigue items across different reporting periods. Pain 2008; 139: 146–57 [PubMed: 18455312]

20. Cleeland CS, Ryan KM: Pain assessment: Global use of the Brief Pain Inventory. Ann Acad Med Singapore 1994; 23: 129–38 [PubMed: 8080219]

21. Turk DC, Dworkin RH, Burke LB, Gershon R, Rothman M, Scott J, Allen RR, Atkinson JH, Chandler J, Cleeland C, Cowan P, Dimitrova R, Dionne R, Farrar JT, Haythornthwaite JA, Hertz S, Jadad AR, Jensen MP, Kellstein D, Kerns RD, Manning DC, Martin S, Max MB, McDermott MP, McGrath P, Moulin DE, Nurmikko T, Quessy S, Raja S, Rappaport BA, Rauschkolb C, Robinson JP, Royal MA, Simon L, Stauffer JW, Stucki G, Tollett J, von Stein T, Wallace MS, Wernicke J, White RE, Williams AC, Witter J, Wyrwich KW: Developing patient-reported outcome measures for pain clinical trials: IMMPACT recommendations. Pain 2006; 125: 208–15 [PubMed: 17069973]

22. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J: An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561–571 [PubMed: 13688369]

23. Dworkin RH, Turk DC, Wyrwich KW, Beaton D, Cleeland CS, Farrar JT, Haythornthwaite JA, Jensen MP, Kerns RD, Ader DN, Brandenburg N, Burke LB, Cella D, Chandler J, Cowan P, Dimitrova R, Dionne R, Hertz S, Jadad AR, Katz NP, Kehlet H, Kramer LD, Manning DC, McCormick C, McDermott MP, McQuay HJ, Patel S, Porter L, Quessy S, Rappaport BA, Rauschkolb C, Revicki DA, Rothman M, Schmader KE, Stacey BR, Stauffer JW, von Stein T, White RE, Witter J, Zavisic S: Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. J Pain 2008; 9: 105–21 [PubMed: 18055266]

24. Austin PC: Balance diagnostics for comparing the distribution of baseline covariates between treatment groups in propensity-score matched samples. Stat Med 2009; 28: 3083–107 [PubMed: 19757444]

25. Moher D, Schulz KF, Altman D: The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA 2001; 285: 1987–1991 [PubMed: 11308435]

26. Hwang IK, Shih WJ, De Cani JS: Group sequential designs using a family of type I error probability spending functions. Stat Med 1990; 9: 1439–45 [PubMed: 2281231]

27. Dmitrienko A, Tamhane AC: Gatekeeping procedures with clinical trial applications. Pharm Stat 2007; 6: 171–80 [PubMed: 17583553]

28. Farrar JT, Young JP Jr., LaMoreaux L, Werth JL, Poole RM: Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 2001; 94: 149–158 [PubMed: 11690728]

29. Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL: Defining the clinically important difference in pain outcome measures. Pain 2000; 88: 287–294 [PubMed: 11068116]

30. Dworkin RH, Turk DC, McDermott MP, Peirce-Sandner S, Burke LB, Cowan P, Farrar JT, Hertz S, Raja SN, Rappaport BA, Rauschkolb C, Sampaio C: Interpreting the clinical importance of group differences in chronic pain clinical trials: IMMPACT recommendations. Pain 2009; 146: 238–44 [PubMed: 19836888]

31. Gilron I, Jensen MP: Clinical trial methodology of pain treatment studies: selection and measurement of self-report primary outcomes for efficacy. Reg Anesth Pain Med 2011; 36: 374–81 [PubMed: 21610560]
32. Trojaborg W: Rate of recovery in motor and sensory fibres of the radial nerve: clinical and electrophysiological aspects. J Neurol Neurosurg Psychiatry 1970; 33: 625–38 [PubMed: 5478945]
33. Sunderland S: A classification of peripheral nerve injuries producing loss of function. Brain 1951; 74: 491–516 [PubMed: 14895767]
34. Evans PJ, Lloyd JW, Green CJ: Cryoanalgesia: the response to alterations in freeze cycle and temperature. Br J Anaesth 1981; 53: 1121–7 [PubMed: 7326160]
35. Goulding K, Beaule PE, Kim PR, Fazekas A: Incidence of lateral femoral cutaneous nerve neuropraxia after anterior approach hip arthroplasty. Clin Orthop Relat Res 2010; 468: 2397–404 [PubMed: 20532717]
36. Young S, Sharma S, Rubin N, Hall D, Anderson JK: Ablation zone considerations in cryoablation of RCC: accuracy of manufacturer provided model and change from first to second freeze cycle. Clin Imaging 2022; 89: 10–15 [PubMed: 35660642]
37. Douglas WW, Malcolm JL: The effect of localized cooling on conduction in cat nerves. J Physiol 1955; 130: 53–71 [PubMed: 13278885]
38. Ilfeld BM, Khatibi B, Maheshwari K, Madison SJ, Ali Sakr Esa W, Mariano ER, Kent ML, Hanling S, Sessler DI, Eisenach JC, Cohen SP, Mascha EJ, Yang D, Padwal JA, Turan A, Investigators PA: Immediate Effects of a Continuous Peripheral Nerve Block on Postamputation Phantom and Residual Limb Pain: Secondary Outcomes From a Multicenter Randomized Controlled Clinical Trial. Anesth Analg 2021; 133: 1019–1027 [PubMed: 34314392]
39. Martin G, Grant SA, MacLeod DB, Breslin DS, Brewer RP: Severe phantom leg pain in an amputee after lumbar plexus block. Reg Anesth Pain Med 2003; 28: 475–478 [PubMed: 14556141]

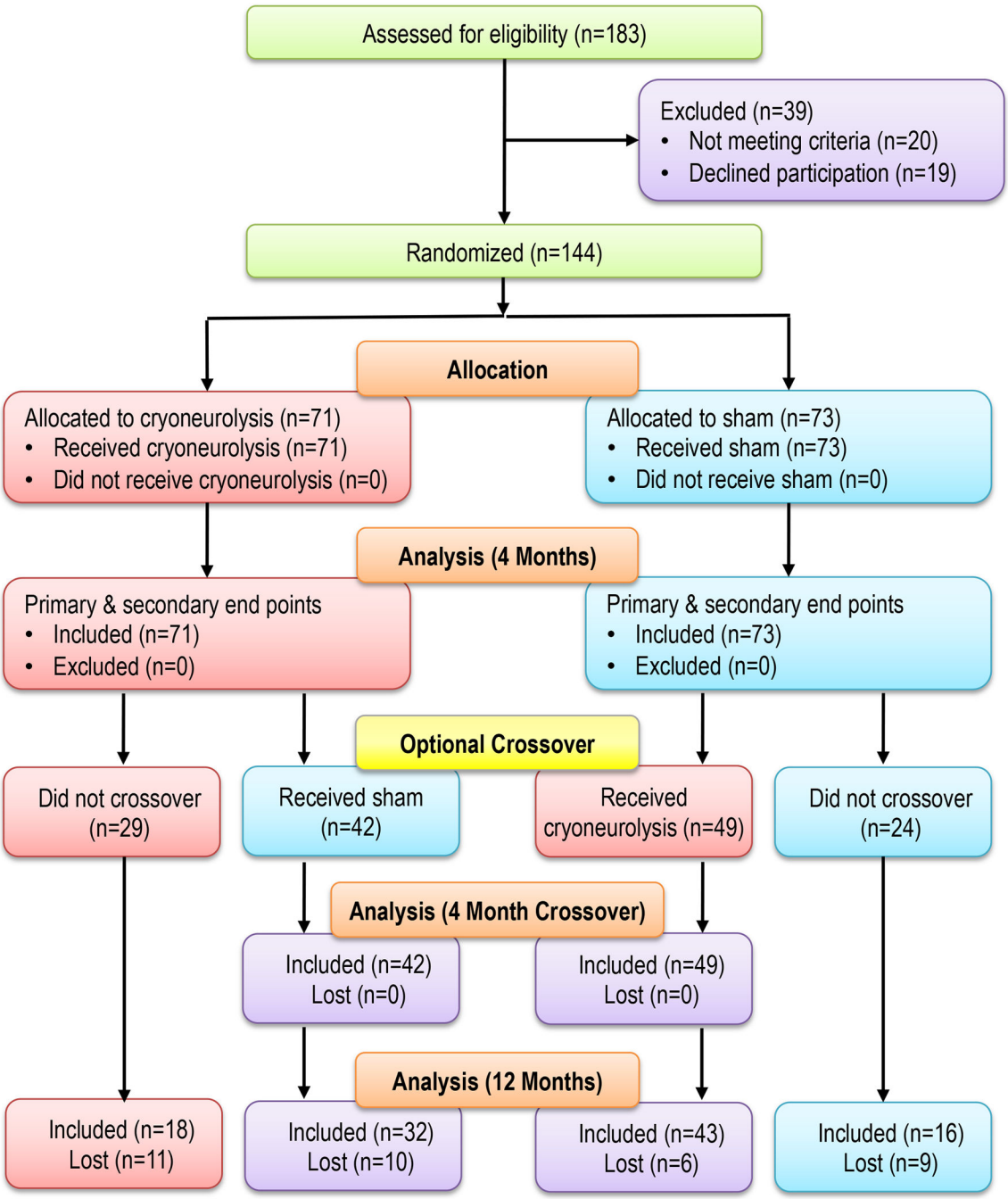

Figure 1.
CONSORT diagram.

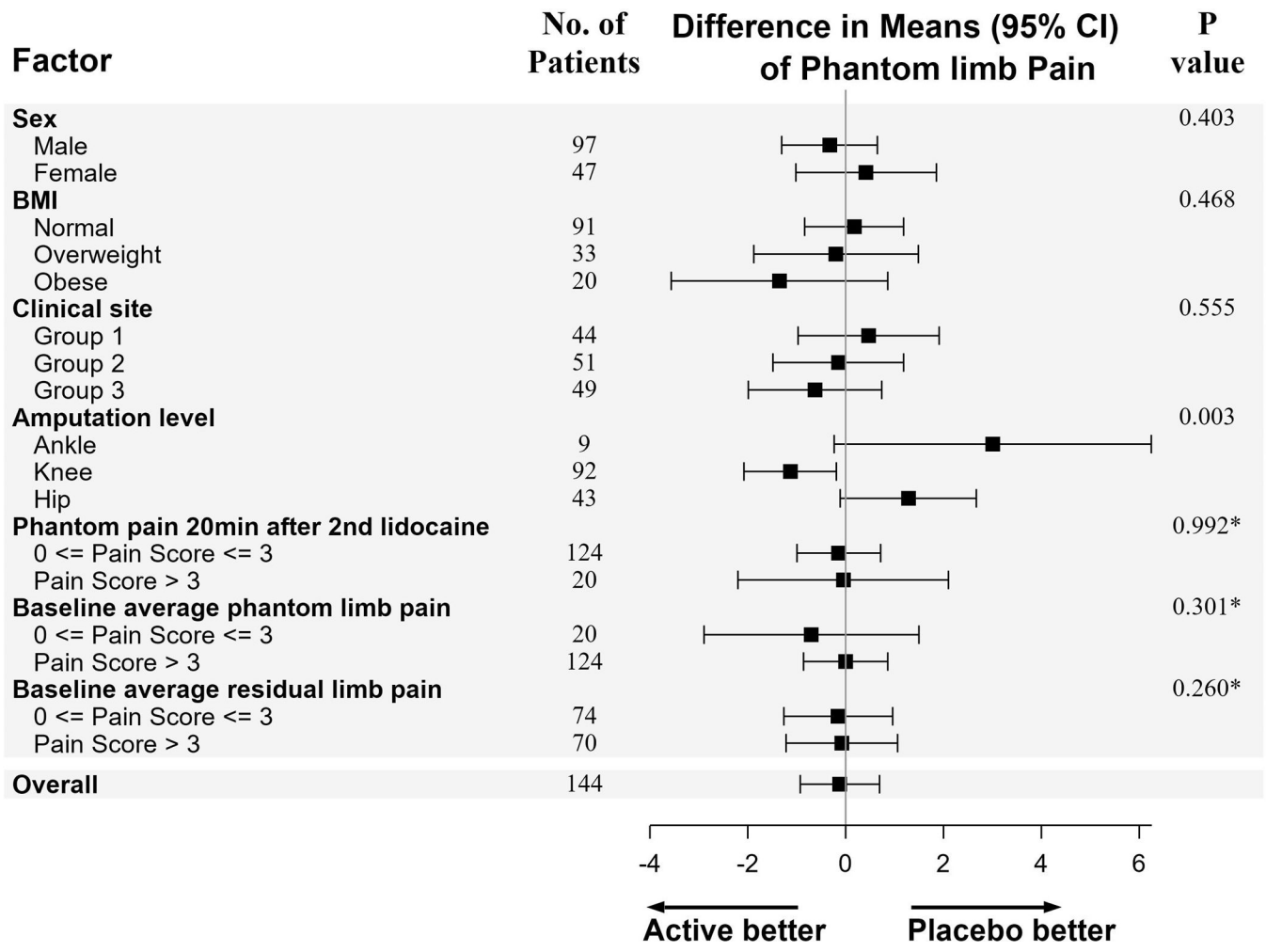

Figure 2.

Forest plot assessing interactions between prespecified baseline factors and the effect of ultrasound-guided percutaneous cryoneurolysis on phantom limb pain at 4 months. * P value was estimated from continuous pain score by multivariable linear regression adjusted for study site and day 0 average phantom limb pain.

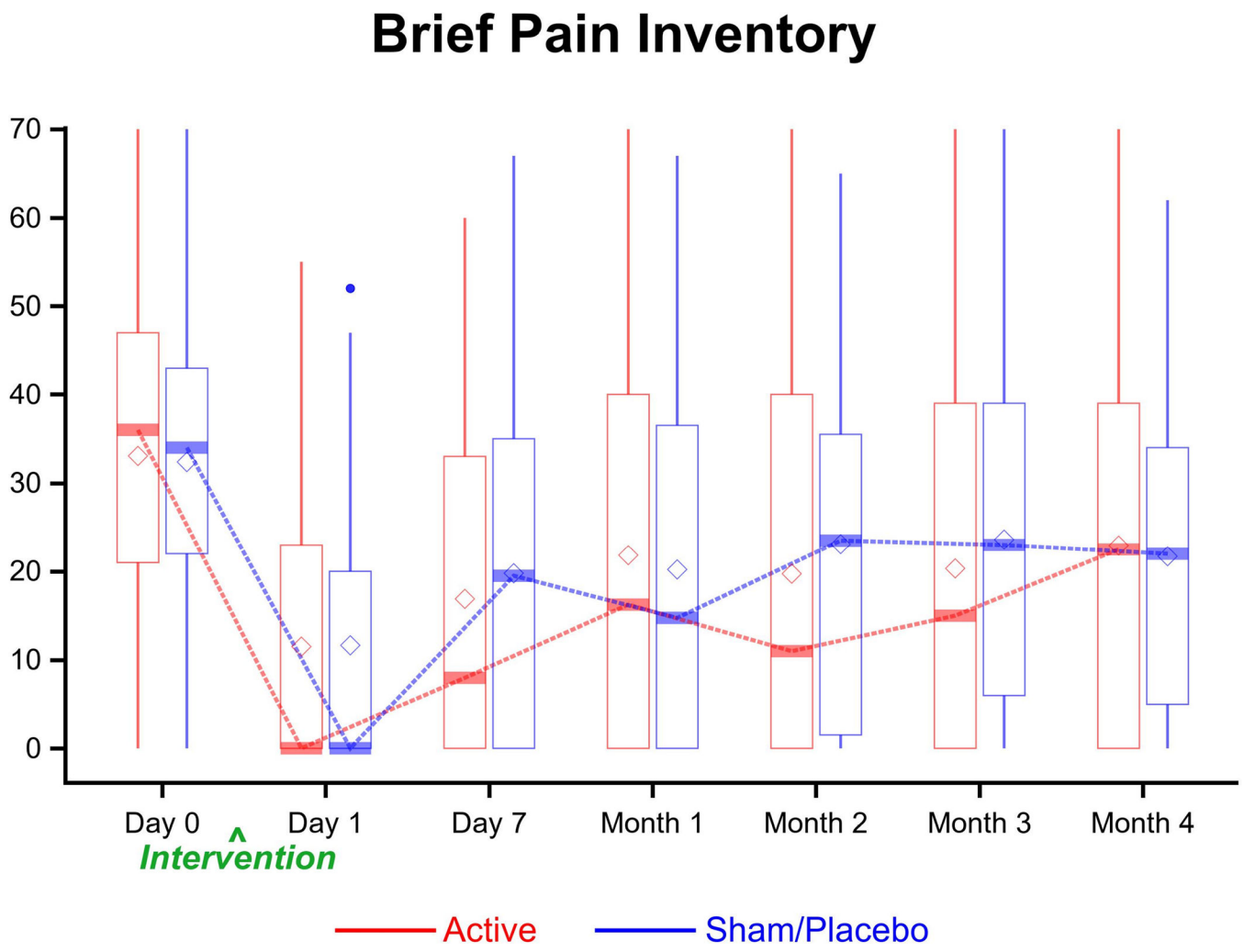

Figure 3.

Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on the Brief Pain Inventory (BPI) interference domain over time. Data expressed as pain's interference on each of 7 components (higher scores = more interference) demarked as median (dark horizontal bars) with 25th-75th (box), 10th-90th (whiskers), mean (diamonds), and outliers (circles). Following our statistical gatekeeping protocol, we do not make inference or draw conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

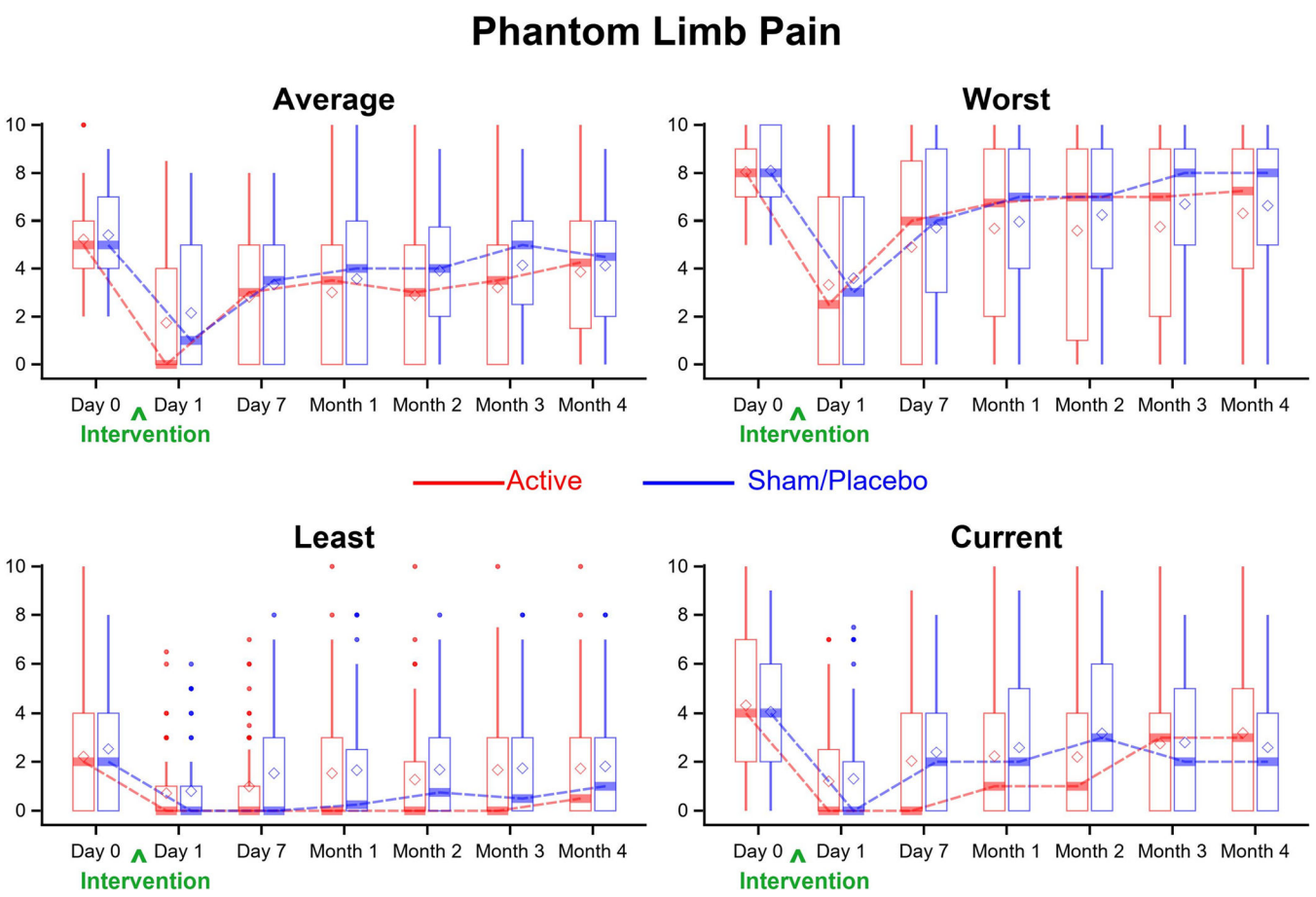

Figure 4.

Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on worst, average, least and current *phantom* limb pain over time (primary outcome: *average* phantom limb pain at 4 months). Pain intensity indicated using a numeric rating scale of 0–10, with 0 equal to no pain and 10 being the worst imaginable pain. Data expressed as median (dark horizontal bars) with 25^{th}-75^{th} (box), 10^{th}-90^{th} (whiskers), mean (diamonds), and outliers (circles). Following our statistical gatekeeping protocol, we do not make inference or conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

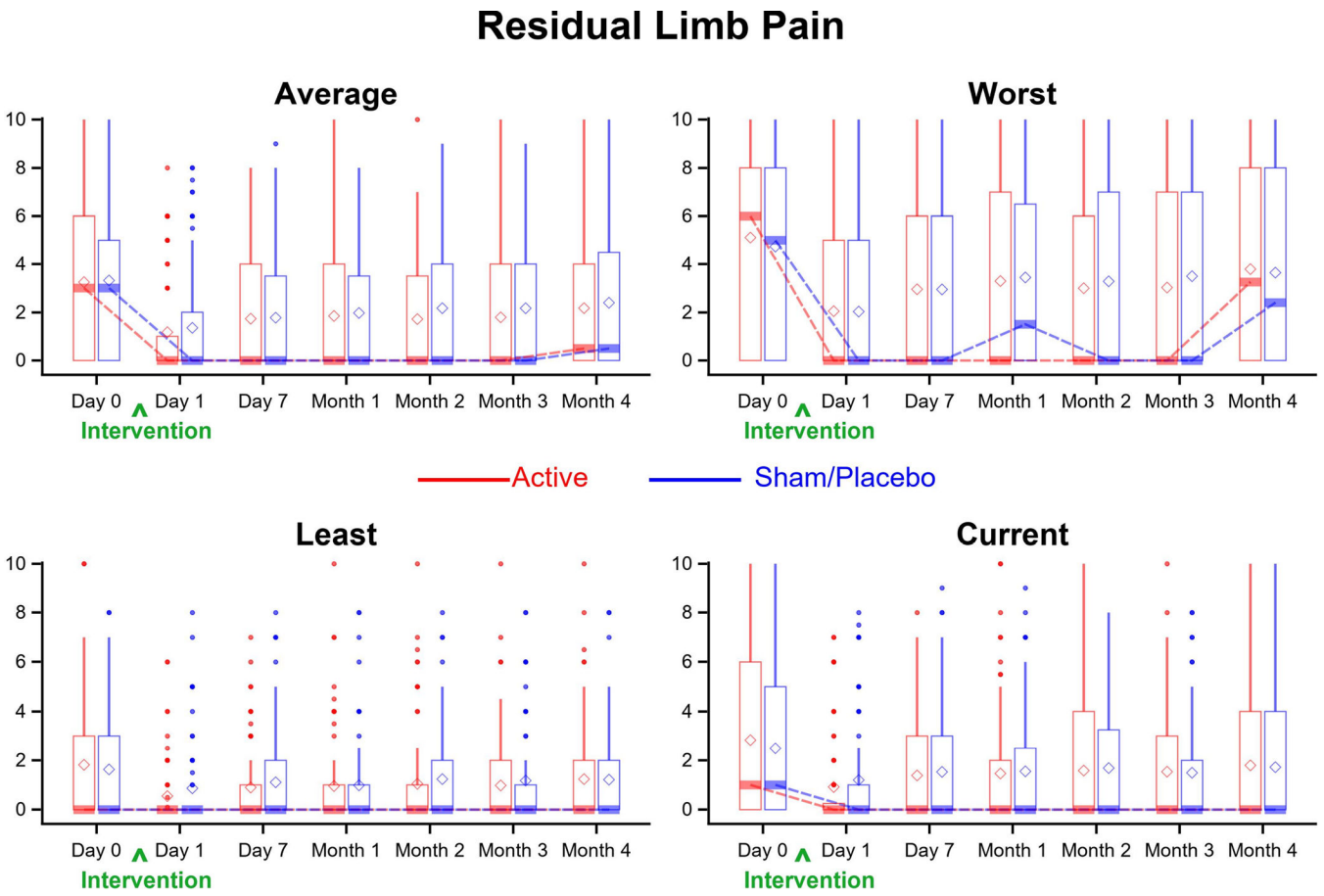

Figure 5.

Effects of ultrasound-guided percutaneous cryoneurolysis (denoted in green) on worst, average, least and current *residual* limb pain over time. Pain intensity indicated using a numeric rating scale of 0–10, with 0 equal to no pain and 10 being the worst imaginable pain. Data expressed as median (dark horizontal bars) with 25^{th}-75^{th} (box), 10^{th}-90^{th} (whiskers), mean (diamonds), and outliers (circles). Following our statistical gatekeeping protocol, we do not make inference or conclusions on the secondary endpoints, since no difference was found on the primary endpoint.

Table 1.

Patient characteristics by treatment group (n=144). Any variable with an absolute standardized difference (ASD) > 0.33 was considered unbalanced.

	Active (n = 71)	Placebo (n = 73)	ASD
Anthropometrics and Demographics			
Age (years)	58 ± 13	58 ± 13	0.020
Female (%)	18 (25)	29 (40)	0.310
Body mass index (kg/m^2)	29 ± 5.8	28 ± 5.3	0.049
Marital status (%) *			0.028
Single (never married)	16 (23)	16 (22)	
Single (divorced)	18 (25)	12 (16)	
Currently married	33 (47)	36 (49)	
Others (separated and widowed)	4 (5)	9 (13)	
Military status (%)			0.182
Civilian (never in military)	59 (83)	55 (75)	
Veteran	11 (16)	18 (25)	
Active Duty	1 (1)	0 (0)	
Years of education	14 [12, 16]	14 [12, 16]	0.075
Study Limb Information			
Right (v. left) side (%)	32 (45)	32 (44)	0.025
Level of amputation (%)			0.100
Trans-femoral	22 (31)	21 (29)	
Trans-tibial	46 (65)	46 (63)	
Foot/ankle	3 (4)	6 (8)	
History of residual limb pain (%)	55 (78)	55 (75)	0.050
Current residual limb pain (%)	44 (62)	45 (62)	0.007
Current prosthesis use (%)	66 (93)	67 (92)	0.044
Intervention Information			
Pain score in limb			
After premed but before procedure	4 [2, 6]	4 [2, 6]	0.034
Twenty min after lidocaine injections	0 [0, 3]	0 [0, 2]	0.005
Phantom limb pain prior to discharge	0 [0, 1]	0 [0, 0]	0.064
Residual limb pain prior to discharge	0 [0, 0]	0 [0, 0]	0.041
Distance of treatment from end of residual limb			
Sciatic nerve (cm)	15 [11, 23]	17 [12, 23]	0.132
Femoral nerve (cm) $^{\Phi}$	22 [15, 31]	22 [16, 33]	0.021
Enrollment Center			
Cleveland Clinic	20 (50%)	20 (50%)	
Naval Medical Center San Diego	0 (0%)	1 (100%)	
Palo Alto Veterans Affairs	1 (50%)	1 (50%)	
University of California San Diego	25 (50%)	25 (50%)	

	Active (n = 71)	Placebo (n = 73)	ASD
University of Florida	24 (49%)	25 (51%)	
Walter Reed National Military MC	1 (50%)	1 (50%)	

Statistics presented as Mean ± SD, Median [P25, P75] or N (column %)

Some groups do not total 100% due to rounding error

$^{\Phi}$One missing value from the sham treatment group

MC: Medical Center

Table 2

Assessing interactions between treatment and specified baseline factors on primary outcome of month 4 average phantom limb pain.

Factor	Active[1]	Placebo[1]	Difference in Means[2] A-P (95% CI)	P value[2]	P value [3]
Sex					0.403
- Male	3.5 ± 2.7	3.9 ± 2.7	−0.33 (−1.32, 0.65)	0.506	
- Female	4.6 ± 2.6	4.3 ± 3.2	0.42 (−1.04, 1.87)	0.573	
BMI					0.468
- Normal	3.7 ± 2.8	4.0 ± 2.9	0.17 (−0.85, 1.19)	0.742	
- Overweight	4.2 ± 2.4	3.7 ± 2.4	−0.20 (−1.89, 1.49)	0.815	
- Obese	3.7 ± 2.7	5.1 ± 3.7	−1.35 (−3.58, 0.87)	0.230	
Clinical site					0.555
- Group 1	3.9 ± 2.2	3.7 ± 3.1	0.47 (−0.98, 1.92)	0.525	
- Group 2	3.8 ± 2.7	3.9 ± 2.7	−0.15 (−1.50, 1.20)	0.827	
- Group 3	3.7 ± 3.1	4.6 ± 2.9	−0.63 (−2.01, 0.75)	0.367	
Amputation level					0.003 [4]
- Ankle	4.3 ± 3.8	2.4 ± 3.8	3.00 (−0.25, 6.26)	0.070	
- Knee	3.1 ± 2.5	4.4 ± 2.7	−1.14 (−2.09, −0.18)	0.020	
- Hip	5.3 ± 2.3	3.9 ± 2.9	1.28 (−0.13, 2.68)	0.074	
Phantom pain 20 mins after 2nd lidocaine					0.992
- Pain score in [0,3]	3.5 ± 2.6	3.9 ± 2.8	−0.14 (−1.01, 0.72)	0.744	
- Pain score > 3	5.5 ± 2.8	5.1 ± 3.3	−0.05 (−2.21, 2.11)	0.961	
Baseline average phantom limb pain					0.301
- Pain score in [0,3]	1.5 ± 1.8	2.5 ± 1.4	−0.7 (−2.91, 1.5)	0.529	
- Pain score > 3	4.1 ± 2.6	4.4 ± 3.0	0 (−0.88, 0.87)	0.992	
Baseline average residual limb pain					0.260
- Pain score in [0,3]	3.5 ± 2.6	3.9 ± 2.6	−0.16 (−1.28, 0.97)	0.784	
- Pain score > 3	4.1 ± 2.7	4.3 ± 3.2	−0.08 (−1.24, 1.07)	0.887	
Overall	3.8 ± 2.7	4.1 ± 2.9	−0.12 (−0.95, 0.70)		0.759

[1] Mean ± SD for month 4 average phantom limb pain.

[2] Difference in means of active vs. placebo and P-value (significant if P< 0.05) estimated using a linear mixed effects regression model adjusted for study site, factor and baseline pain interference components.

[3] Interaction P-value from same linear model assessing treatment-by-covariate interaction.

[4] Since the overall interaction was significant we report pairwise interactions here as well: ankle vs hip (P=0.34), knee vs ankle (P = 0.017), knee vs hip (P= 0.006). In summary, the treatment effect for knee was statistically different from ankle and hip.

Table 3.

Effect of treatment group on secondary outcomes (N=144)

	Active (n = 71)	Placebo (n = 73)	Difference in Means[1] or Medians [2] A-P (95% CI) [3]
Patient Global Impression of Change Scale (Month 4) [Φ]			
Score (1–7)	4 [4, 7]	4 [4, 6]	0 (0, 0) [2]
Score ≤ 4 (worse or no change)	47 (66%)	42 (58%)	
Score > 4 (improved)	23 (32%)	24 (33%)	
Brief Pain Inventory (Interference Subscale) [Φ]			
Total score	23 [0, 39]	22 [3, 34]	0 (−5, 6)[2]
Overall treatment effect	3.4 (0.4) [4]	3.0 (0.4) [4]	0.2 (−0.5, 0.9)[1]
General Activity	2.5 [0, 6]	3.5 [0, 6]	
Mood	3.0 [0, 6]	1.5 [0, 5]	
Walking ability	2.5 [0, 6]	2.5 [0, 5]	
Normal work	2.0 [0, 6]	2.0 [0, 5]	
Relations with other people	1.5 [0, 4]	0 [0, 4]	
Sleep	3.0 [0, 7]	5.0 [0, 8]	
Enjoyment of life	3.0 [0, 7]	3.5 [0, 6]	
Beck Depression Inventory			
Total score	4 [0, 14]	2 [0, 9]	
Change from baseline	−2 [−7, 0]	−2 [−5, 0]	1 (−1, 3)[2]

[Φ] One and 7 missing values from the active and sham treatment groups, respectively

Summary statistics presented as median [Q1, Q3] or Mean ± SD with complete dataset. Last observation carried forward method was applied for all analysis, if month 3 measurements are available.

[1] Overall treatment effect: Difference in means between two groups across the 7 components was estimated from a linear mixed effects regression model. The model adjusted for study site, baseline pain interference components. Treatment by component interaction was non-significant (P=0.202). Per-group mean (SE) across components is also reported.

[2] Median difference (confidence interval) of placebo vs. active was estimated with the Hodges-Lehmann estimator of location shift between groups stratified by study site; P-value from Wilcoxon-Mann-Whitney test.

[3] Confidence intervals adjusted for group sequential design to maintain overall study alpha of 0.05. P-value of 0.044 or less was considered significant for treatment effect on all outcomes.

[4] Estimates (Standard error) were reported for each group

Table 4.

Long-term follow-up at **12 months** post-randomization. Values represent the change from initial baseline with the exception of the Patient Global Impression of Change which are presented as raw values (n=144).

Initial Treatment:	**Active**	**Sham**	**Active**	**Sham**
	No Crossover		***Had Crossover***	
	(n = 29)	**(n = 24)**	**(n = 42)**	**(n = 49)**
Phantom pain				
Worst pain	−6 [−7, 0] **	−4 [−6, 1] *	−1 [−5, 0] †	−1 [−4.3, 0] §
Average pain	−4 [−5, 0] **	−4 [−5, −1.5] *	−3.5 [−5, −1] †	−1.3 [−5, 0] §
Residual limb pain				
Worst pain	0 [−4, 0] **	−2 [−5, 0] *	−0.3 [−5.5, 0] †	0 [−2, 0] §
Average pain	0 [−3, 0] **	−2 [−5, 0] *	−1.5 [−3.5, 0] †	0 [−2, 0] §
Brief Pain Inventory Components				
General Activity	−4 [−7, −1] **	−3 [−4, 0] *	−2 [−5, 0] †	−1 [−5, 0] ‡
Mood	−2 [−5, 0] **	−3 [−6, 0] *	−2 [−5, 0] †	0 [−3, 0] ‡
Walking ability	−2 [−5, −1] **	−3 [−10, −2] *	−1.5 [−5, −0.5] †	−1 [−5, 0] ‡
Normal work	−2 [−7, −1] **	−3 [−7, −1] *	−2 [−5, 0] †	0 [−3, 0] ‡
Relations with others	−1 [−4, 0] §	−1 [−5, 0] *	−1 [−1.5,0] †	0 [−2, 0] ‡
Sleep	−3.5 [−8, −1] **	−5 [−9, 0] *	−2 [−4, −0.5] †	−2.5 [−7, 0] ‡
Enjoyment of life	−2 [−6, 0] §	−1 [−7, 1] *	−2.5 [−5, 0] †	−1 [−4, 0] ‡
Global Impression of Change	7 [4, 7] **	7 [4, 7] *	5 [4, 7] †	4 [4, 7] ‡
Beck Depression Inventory	−7 [−11, −5] **	−4 [−10, −1] *	−7 [−10, −1] †	−6 [−9.5, −3] §

Data presented as median [interquartile range]

* 9 missing values

** 11 missing values

§ 12 missing values

† 10 missing values

§ 5 missing values

‡ 6 missing values