The Efficacy of Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ Versus Placebo in the Treatment of Latrodectism: A Randomized, Double-Blind, Placebo-Controlled, Clinical Trial

Richard C. Dart, MD, PhD*; Sean P. Bush, MD; Kennon Heard, MD, PhD; Thomas C. Arnold, MD; Mark Sutter, MD; Danielle Campagne, MD; Christopher P. Holstege, MD; Steven A. Seifert, MD; Jean C. Y. Lo, MD; Dan Quan, DO; Stephen Borron, MD; David A. Meurer, MD; Randy I. Burnham, MS; Jude McNally, PharmD; Walter Garcia-Ubbelohde, MD; Victoria E. Anderson, MPH

*Corresponding Author. E-mail: Richard.dart@rmpdc.org.

Study objective: The antivenom currently available for treatment of systemic black widow envenomation (latrodectism) is composed of equine whole immunoglobin. Although considered effective, it has been associated with anaphylaxis and 2 reported fatalities. We test the efficacy and safety of new equine antivenom composed of purified F(ab′)$_2$ antibody fragments.

Methods: A randomized, double-blind, placebo-controlled trial was conducted at 16 sites across the United States. Subjects aged 10 years or older with moderate to severe pain because of black widow spider envenomation received F(ab′)$_2$ antivenom or placebo. The primary outcome measure was treatment failure, which was defined as failure to achieve and maintain clinically significant reduction in pain for 48 hours posttreatment. Secondary measures of pain intensity differences and summed pain intensity difference were computed. Adverse events were recorded.

Results: Sixty patients were treated (29 antivenom and 31 placebo). The mean age was 39 years and 68% were male. There were 15 treatment failures in the antivenom group and 24 in the placebo group (P=.019). Differences in pain intensity difference between groups were lower at each postbaseline point, and the mean summed pain intensity difference was greater for the antivenom group (difference 2,133; 95% confidence interval 177 to 4,090). No deaths or serious drug-related adverse events were detected.

Conclusion: The F(ab′)$_2$ antivenom met the predefined primary outcome of reduced treatment failures. Secondary outcomes of pain intensity difference and summed pain intensity difference also supported efficacy. The rate of symptom improvement in the placebo group was higher than expected, which may be related to enrollment criteria or placebo effect. [Ann Emerg Med. 2019;■:1-11.]

Please see page XX for the Editor's Capsule Summary of this article.

0196-0644/$-see front matter

https://doi.org/10.1016/j.annemergmed.2019.02.007

INTRODUCTION

Background

There are 5 species of widow spiders (*Latrodectus*) in North America, with 1,330 bites reported to US poison centers in 2016.[1] The active component of *Latrodectus* venom is latrotoxin, a neurotoxin that is highly conserved among widow spiders worldwide. Latrotoxin causes latrodectism, which was aptly described by Maretic[2]: "Cramping pains in the thighs, lumbar region, abdomen or thorax are often present. The patient may also feel pressure and tightness in these areas. From time to time, contraction in the large muscle masses may occur, culminating in paroxysms of pain. This myopathic syndrome of latrodectism is often manifested by hypertonicity of the musculature, with rigidity of the abdomen, trismus, muscle fibrillations, tonic contractions and tremor." The pain can mimic acute abdominal conditions and acute myocardial infarction. Although considered a nonfatal condition, rare but well-documented instances of death have been reported.[3] The syndrome is most severe for the first 12 to 24 hours and then gradually resolves during several days; persistent symptoms may result in repeated visits to the emergency department (ED).

Editor's Capsule Summary

What is already known on this topic

Latrodectus (black widow) spider bite may cause a painful systemic myopathic syndrome (latrodectism). Existing immunoglobulin-based antivenom is limited in supply and associated with anaphylaxis.

What question this study addressed

Is a newer antibody-based antivenom (antivenin *Latrodectus* equine immune F[ab′]$_2$) safe and efficacious in the treatment of black widow spider envenomation?

What this study adds to our knowledge

In this multicenter randomized trial of 60 patients, F(ab′)$_2$ was associated with fewer treatment failures (<13-mm visual analog scale pain scale decrease, need for commercial antivenom, or need for prescription analgesia) than placebo (51.7% versus 77.4%). Adverse event rates were similar.

How this is relevant to clinical practice

F(ab′)$_2$ offers a potential new treatment for *Latrodectus* envenomation. Larger series are needed to verify its safety and effectiveness. Future studies must develop improved methods for defining latrodectism cases and treatment outcomes.

Importance

Few clinical trials have addressed the treatment of *Latrodectus* envenomation. In the United States, treatment of latrodectism typically includes opioid analgesics and benzodiazepines.[4] However, these measures have been associated with prolonged illness and higher rates of hospital admission.[4]

Antivenin (*Latrodectus mactans*) (Merck & Co, Inc, Whitehouse Station, NJ) was introduced in the 1950s and contains equine whole immunoglobin, as well as other constituents of horse serum. It is considered effective, but has been associated with anaphylaxis and 2 reported fatalities from allergic reactions.[4,5] For many years, the supplier has restricted availability of the antivenom because of insufficient supply. This creates an untenable clinical position: patients in severe pain have to wait 24 to 48 hours or more for antivenom. This prolongs pain and increases health care expense. High doses of benzodiazepines and opioids may be used, but have well-known risks and prolong the duration of illness.[4] In contrast, antivenom binds latrotoxin and prevents its interaction with presynaptic membranes. Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ is an experimental equine Fab$_2$ antibody preparation purified to reduce nonimmunizing serum components such as albumin.[6]

Goals of This Investigation

This study was a phase 3 trial to evaluate the efficacy and safety of Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$.

MATERIALS AND METHODS

Study Design

A multicenter, randomized, double-blind, placebo-controlled trial was performed comparing the efficacy of Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ with placebo for reduction of pain intensity caused by latrodectism (Figure 1). The study was approved by the institutional review board at the central coordinating center and at each participating site.

Setting

Subjects were enrolled in the ED at 16 adult, pediatric, and mixed academic and community-based EDs in the United States between October 2009 and October 2014 (Appendix E1, available online at http://www.annemergmed.com). Enrollment, administration of investigational product, and most data collection were completed in the ED. Follow-up interviews in regard to the subjects' clinical condition were completed by telephone.

Selection of Participants

Patients aged 10 years and older presenting within 24 hours of symptom onset related to presumed black widow spider envenomation were eligible for inclusion. Subjects were required to have moderate to severe pain intensity, defined as a score greater than 40 mm on the visual analog scale (VAS) at both the screening and baseline examinations to be eligible. Similar to previous prospective studies, the diagnosis of latrodectism was based on clinical diagnosis by the site investigator because no validated instruments for the diagnosis of latrodectism exist. To increase the probability of accurate diagnosis, concurrence with the diagnosis by a physician not associated with the trial was required for inclusion.

Subjects meeting any of the following criteria were excluded: hypersensitivity to fentanyl or equine serum; distracting injury with acute pain; inability to make a reliable self-report of pain intensity; history of significant

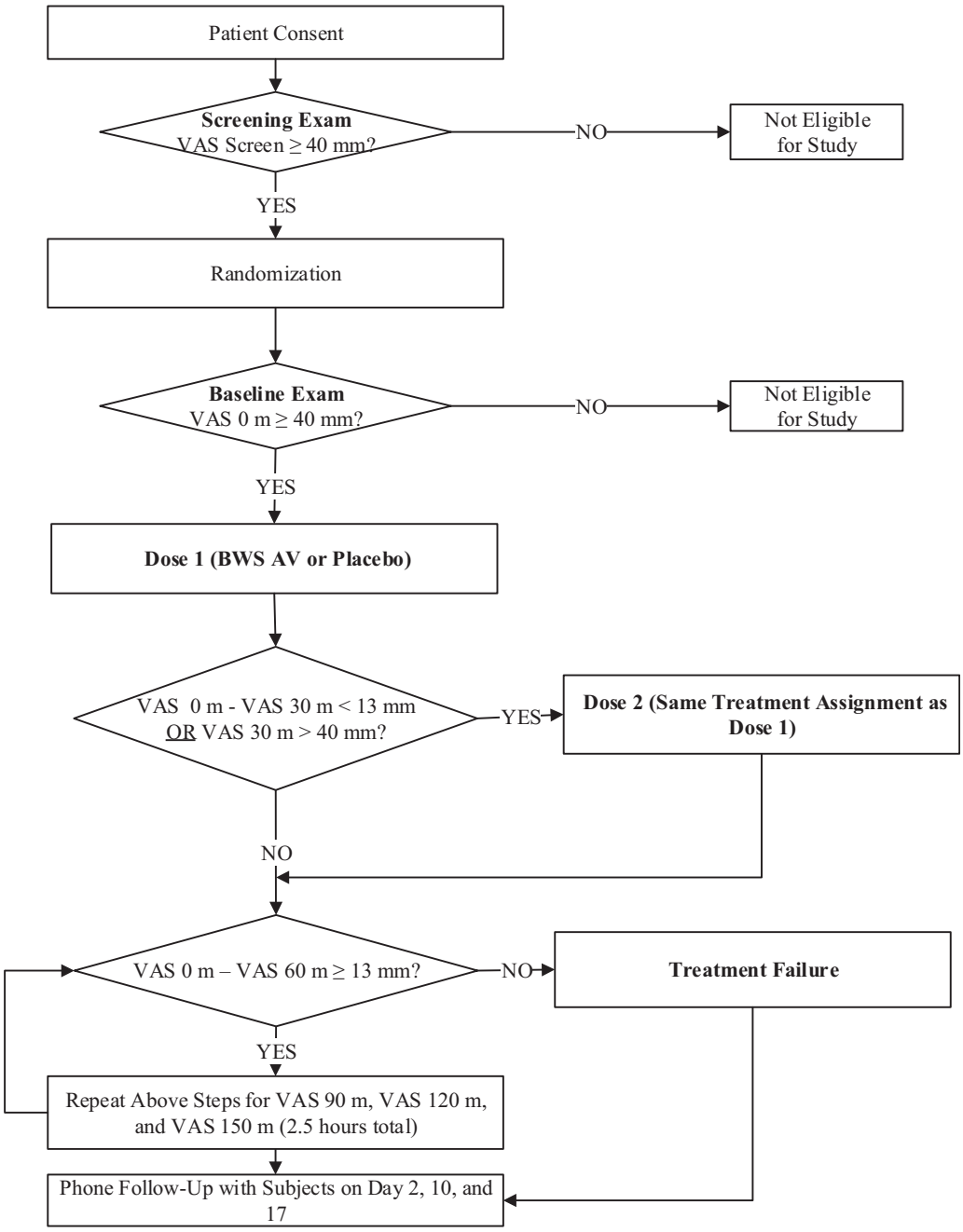

Figure 1. Study schematic. *BWS AV,* Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$.

cardiac, respiratory, hepatic, or renal disease, or psychiatric disorder or chronic pain syndrome, that in the investigator's valuation could limit the efficacy or safety endpoint assessment; history or suspected history of substance abuse; pregnancy or breastfeeding; previous treatment with commercially available antivenom for the current envenomation; or inability to provide a telephone number for follow-up interviews.

Subjects could receive fentanyl for pain relief during the screening phase at a maximum dosage of 1.5 μg/kg. A 30-minute washout period after fentanyl administration was required, and the baseline VAS score had to be 40 mm or greater for administration of the study drug.

Interventions

The study medication was Analatro (Antivenin *Latrodectus* [Black Widow] Equine Immune F[ab′]$_2$), a sterile preparation of black widow spider venom-specific binding fragments. Each 10-mL vial of lyophilized white

powder contains sufficient F(ab′)$_2$ to neutralize not less than 600 lethal dose 50% of *L mactans* venom. The venom-specific binding fragments are enzymatically modified by pepsin cleavage to form F(ab′)$_2$ fragments from hyperimmunized equine serum. The F(ab′)$_2$ content is at least 85% and Fab content is no more than 7%. The product contains 5% or less whole immunoglobulin G and less than 0.5% albumin (Instituto Bioclon, S.A. de C.V., Mexico City, Mexico).

Subjects were randomly assigned to receive either Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ (batches P-9C-01 and P-1L-02), with each dose composed of 3 vials of antivenom reconstituted in a total volume of 50 mL normal saline solution, or the same volume of normal saline solution control (0.9% sodium chloride United States Pharmacopeia), administered intravenously during 10 minutes. The antivenom solution is visually indistinguishable from normal saline solution.[7] This dose is consistent with the recommended dose as confirmed in a previous study and from clinical experience with a similar formulation of this product.[6,7]

Treatment kits containing 6 vials of antivenom or placebo were provided to each center in indistinguishable blinded packaging. The subject, investigators, and site study staff were blinded to treatment assignments. Unblinding of the investigator was allowed if clinically required; however, the procedure was not needed during the study.

A block randomization schedule was determined before the initiation of the trial, using a computerized random-number-generating program. The pharmacy of each investigative site received 1 block containing 4 study kits: 2 kits contained study drug and 2 contained empty vials indicating a placebo assignment (0.9% saline solution was provided by each site). The unblinded pharmacist selected and prepared the next treatment kit in the numeric sequence.

Each subject received an initial dose of blinded study medication. A second dose of blinded study medication was administered 30 minutes after the start of the infusion if subjects met 1 of 2 criteria: they failed to achieve a clinically significant reduction in pain intensity (13-mm decrease) relative to their baseline score, or they still had moderate to severe pain (VAS score 40 mm or greater). Subjects not achieving adequate pain control at any time after the second dose were moved to standard care, which may have included receiving an infusion of the Food and Drug Administration–approved widow spider antivenom (Merck Antivenin *Latrodectus mactans*), at the discretion of the site investigator.

Methods of Measurement and Outcome Measures

The primary outcome of this study was treatment failure, which we defined as a subject who failed to achieve a 13-mm decrease on the VAS scale relative to baseline at 60, 90, 120, or 150 minutes after the start of dose 1; received treatment with commercially available antivenom (Merck Antivenin *Latrodectus mactans*); or received a prescription analgesic medication at any time during the treatment phase through 48 hours after dose 1 (Figure 1).[6] The choice of measures for the evaluation of latrodectism is very difficult because no validated measure exists. It is a subjective experience composed of severe, difficult-to-describe muscle pain; a variety of autonomic effects such as tachycardia, sweating, and hypertension; and psychological effects such as a feeling of impending doom.

Consistent with Food and Drug Administration guidance,[8] we chose pain as the primary evaluation because it is the most commonly reported symptom by a large margin and previous investigators have used it as the primary measure of antivenom efficacy.[4,6] However, the clinical relevance of pain is often questioned because it is subjective, variable, and without established financial cost. Thus, we tested in a preliminary trial and then used in this trial the concept of treatment failure.[6] Treatment failure provided an unambiguous measure: the development or recurrence of a patient's symptoms that resulted in the decision of the treating physician to administer pain medication. This decision has clear medical and financial implications that help illustrate the clinical utility of the antivenom. When an ED provider tries to "cover up" the pain with narcotics or benzodiazepines, patients often soon return to the ED.[4] Another concern is that measure of pain relief at a single point can be misleading. Decreasing pain at an early point is important, but this may not change the patient's overall experience. Following the occurrence of treatment failure for 48 hours establishes a durable response.

We also measured pain response by using the secondary endpoints of pain intensity difference posttreatment relative to baseline, the overall summed pain intensity differences using scores from the VAS, and the proportion of subjects experiencing one or more adverse events determined to be "possibly" or "definitely" related to study drug.

The VAS was used to measure subject self-reported pain intensity. It is a widely accepted, easily administered measure that has been validated in relation to acute pain.[9-11] The VAS score correlates well with numeric ratings of pain in both pediatric and adult ED patients.[12,13] The VAS was administered at baseline and every 30 minutes after the start of the first dose of study drug through the 150-minute treatment phase or treatment failure, whichever occurred first. The VAS was not

continued if a patient failed treatment. The paper-based tool consisted of a 100-mm horizontal line with "no pain" and "worst possible pain" indicated to the left and right ends of the line, respectively. A VAS score greater than 40 mm is generally considered moderate pain. Subjects were instructed to make a single vertical mark on the line to indicate their current pain intensity. The distance between the left end of the line and the subject's mark was independently measured by 2 trained observers and the average score was rounded to the nearest 1.0 mm.

Data were collected by trained research personnel at each site, who used standardized case report forms with source information extracted from the subject's medical record. Each case report form was monitored by trained personnel from the coordinating center and all data were source-document verified. Data were entered into a Research Electronic Data Capture[14] system (version 5.0; Vanderbilt University, Nashville, TN), with double visual verification of entry. Adverse events were coded by body system and preferred term with the *Medical Dictionary for Regulatory Activities* (version 17.0).[15]

Subjects were monitored for adverse events throughout the ED treatment period and were also contacted on days 2, 10, and 17 after discharge. Safety variables included manifestations (or worsening) of adverse events from the start of the infusion to the end of the study period. Adverse events were elicited by periodic questioning of the subject, per study protocol, using a standardized checklist.

Primary Data Analysis

All statistical analyses were planned a priori and applied to the modified intention-to-treat population, which was defined as all patients who received any study drug. The traditional intention-to-treat analysis (including all patients enrolled and randomized) was not used because administration of study drug was based on the VAS score at baseline evaluation. It was possible for a patient to be enrolled and randomized according to the screening VAS score but not receive study drug because his or her baseline VAS score was less than 40 mm (Figure 2). Original sample size estimates were based on data from a phase 2 trial[6] and included Food and Drug Administration feedback requesting that a one-tailed test of the primary hypothesis be conducted at the .025 probability level. A sample size of 28 subjects per group provided 80% power to detect a difference of 40.5% in the proportion of treatment failures between treatment groups. No interim analyses or adjustments to the sample size were conducted. Analyses were performed with SAS (versions 9.3 and 9.4; SAS Institute, Inc., Cary, NC). A data and safety monitoring board met according to predefined enrollment milestones.

The proportion of treatment failure was compared with a one-sided χ^2 test (statistical significance defined as $P<.025$), assuming that randomization was effective and that any measurement bias was similar in both groups. Mean time-response curves for pain intensity difference were compared with a fixed-effects model. Summed pain intensity difference scores were calculated with the area under the curve trapezoidal method and compared with an independent-sample one-sided t test. Missing VAS scores were imputed by using the method of last observation carried forward.

RESULTS

Characteristics of Study Subjects

A total of 66 subjects at 11 sites provided consent. Six patients were subsequently excluded because of a VAS score of less than 40 mm at the baseline measurement. Demographic and clinical characteristics were similar between groups, with most subjects reporting local discomfort at the bite site, radiation of pain from the bite site to larger muscle groups, muscle cramps, and weakness (Tables 1 and 2). Localized erythema with diaphoresis and piloerection was the most commonly observed clinical sign of latrodectism in both groups. The median time between initial symptom onset and first dose of study drug treatment was 8.3 hours for the black widow spider study medication group and 9.6 hours for the placebo group (Table 1).

Treatment failure was more common in the placebo group compared with the black widow spider study medication group. In the placebo group, 24 of 31 subjects (77.4%; 95% confidence interval [CI] 58.9% to 90.4%) failed to achieve and maintain adequate pain control compared with 15 of 29 subjects (51.7%; 95% CI 32.5% to 70.6%) in the antivenom group (P=.019) (Table 3). The number needed to treat was 3.9 patients to prevent 1 additional treatment failure.

The mean VAS score at baseline was 79.7 mm for the antivenom group and 73.8 mm for the placebo group. Individual changes in VAS scores varied by subject and treatment group. The VAS score decreased 40.5 mm in the antivenom group and 19.4 mm in the control group (Figure 3). The VAS score decreased progressively throughout the ED observation period for both groups, but remained higher in the control group after the treatment phase (Figure 4).

Three subjects (2 receiving black widow spider study medication and 1 receiving placebo) had a total of 4 VAS score values imputed with the method of last observation carried forward for determination of the primary endpoint.

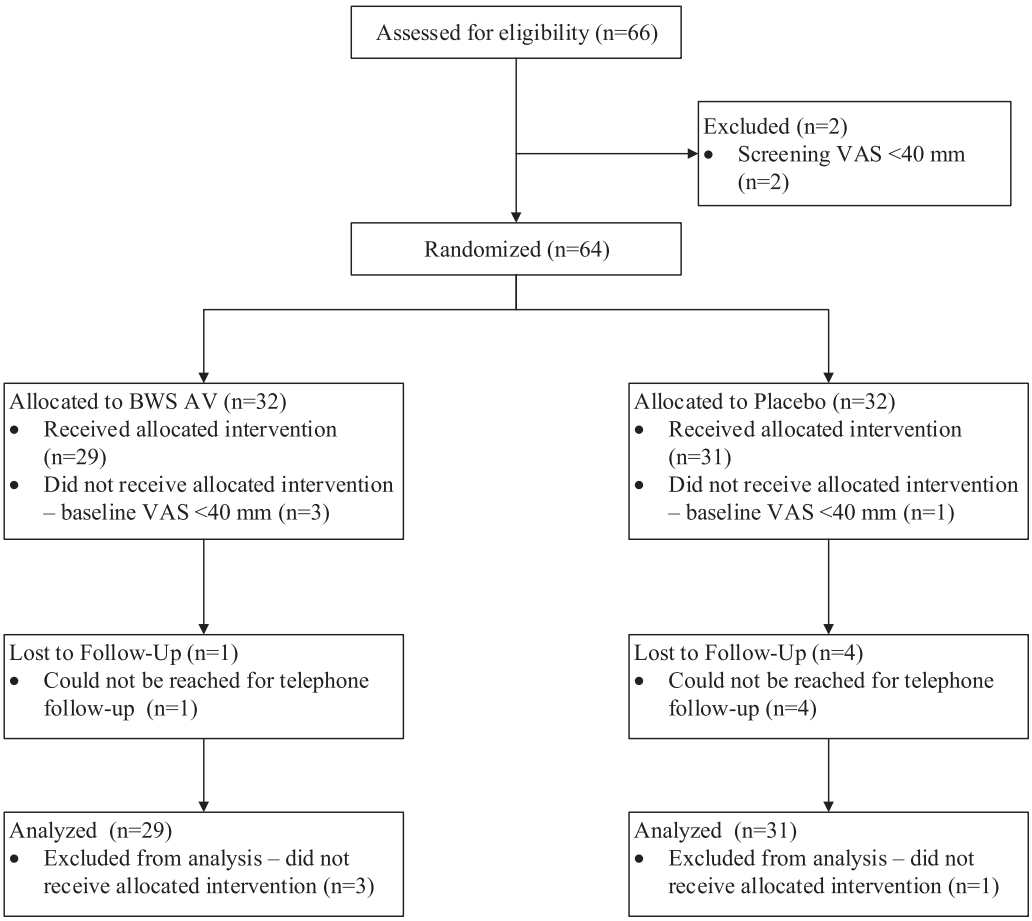

Figure 2. Patient disposition schematic. The unblinded pharmacist at each investigative site accessed the randomized treatment assignment for each patient. The patient, investigator, and site personnel were blinded to the treatment assignment. All subjects lost to follow-up had a known treatment failure disposition at loss to follow-up.

Two additional methods of imputation were used, with no change in the proportion of treatment failures in either group regardless of imputation method.

Overall, 16 subjects with treatment failure (5 receiving black widow spider study medication and 11 receiving placebo) received the commercially available antivenom. Subjects meeting criteria for treatment failure were not evaluated for response to additional therapies administered after failure of the study drug.

The pain intensity difference represents the change in VAS score within each subject. Model-estimated mean pain intensity difference increased over time within both the antivenom and placebo groups. The difference was greater among the antivenom-treated subjects at 60, 90, 120, and 150 minutes (Table 3 and Figure 5).

The summed pain intensity difference is a commonly used measure to better represent the patient's overall pain relief. The mean summed pain intensity difference was greater in the antivenom group (black widow spider study medication 4,521; placebo 2,387; 95% CI 176.9 to 4,089.6) (Table 3), indicating substantially lower pain intensity in the antivenom group.

The antivenom group experienced a median of 3 adverse events (interquartile range 1 to 5) compared with 3 events (interquartile range 1 to 6) in the placebo group. No deaths occurred. Two placebo subjects experienced serious adverse events resulting in hospitalization (cellulitis and inadequate analgesia). Neither event was determined to be related to the study drug. In the antivenom group, 55% (95% CI 35.7% to 73.6%) experienced 1 or more adverse events possibly or definitely related to the study drug compared with 58.1% in the placebo group (95% CI 39.1% to 75.5%). These were most commonly skin and subcutaneous tissue disorders (38.3%), musculoskeletal and connective tissue disorders (31.7%), and general and administration site conditions (15.0%). Pruritus (58.3%), arthralgia (21.7%), and rash (20.0%) were the most frequently reported adverse events overall, although the frequency of events was similar between treatment groups (Appendix E2, available online at http://www.

Table 1. Demographic description of subjects receiving study drugs.

Variable	Response	BWS AV (N=29)	Placebo (N=31)
Age, y	Mean (SD)	37.3 (15.18)	41.0 (17.84)
	Median (range)	34.0 (12.0–69.0)	41.0 (11.0–79.0)
	IQR	25.0–49.0	27.0–52.0
Sex	Male patients, No. (%)	18 (62.1)	23 (74.2)
Ethnicity	Not Hispanic or Latino, No. (%)	16 (55.2)	14 (45.2)
Race*	American Indian or Alaska Native, No. (%)	3 (10.3)	0
	Black, No. (%)	2 (6.9)	0
	White, No. (%)	14 (48.3)	20 (64.5)
	Unknown/missing, No. (%)	10 (34.5)	11 (35.5)
Baseline VAS score, mm	Mean, No. (SD)	79.7 (13.16)	73.8 (16.14)
	Median, No. (range)	79.0 (52.0 to 100.0)	74.0 (40.0 to 100.0)
	IQR	72.0 to 89.0	63.0 to 85.0
Time from first symptoms to initial presentation, h	Mean (SD)	2.6 (3.53)	4.7 (5.83)
	Median (range)	1.3 (0.1 to 15.0)	2.5 (−0.3 to 23.0)
	IQR	0.7 to 2.7	0.8 to 5.8
Time from first symptoms to study drug administration, h	Mean (SD)	9.6 (7.21)	13.0 (12.42)
	Median (range)	8.3 (2.4 to 35.6)	9.6 (2.1 to 63.2)
	IQR	4.48 to 11.20	4.95 to 17.12
Received fentanyl before study drug	No. (%)	10 (34.5)	11 (35.5)
Fentanyl dose, μg	Mean (SD)	48.3 (11.44)	76.8 (22.92)
	Median (range)	50.0 (25.0 to 75.0)	75.0 (50.0 to 100.0)
	IQR	50.0 to 50.0	50.0 to 100.0
Time between fentanyl dose and baseline VAS score, h	Mean (SD)	1.5 (0.55)	2.2 (2.20)
	Median (range)	1.4 (0.8 to 2.4)	1.6 (1.0 to 8.4)
	IQR	1.2 to 1.8	1.2 to 1.9

IQR, Interquartile range.
*Race categories are not mutually exclusive; subjects could identify with more than one category.

annemergmed.com). For the black widow spider study medication patients and the placebo patients, respectively, pruritus was reported in 62.1% and 54.8%, arthralgia in 24.1% and 19.4%, and rash in 13.8% and 25.8%.

LIMITATIONS

It is likely that some of our patients did not have latrodectism because there are no pathognomonic signs, symptoms, or tests to establish the diagnosis unequivocally. This misclassification produces bias toward the null hypothesis (no effect of antivenom). Furthermore, placebo effect affects all research involving the treatment of pain. The effect can be large and generally also biases toward the null hypothesis. The external validity of our results is unknown because our study was performed in academic centers with trained investigators, had few pediatric patients, and enrolled only patients presenting within 24 hours of symptom onset. Use of antivenom in other settings may reveal additional efficacy or safety issues that we were unable to detect in a small study. We were also unable to compare the experimental antivenom to Antivenin (*Latrodectus mactans*) because it is in short supply and the amount available from the manufacturer was not sufficient for the trial. Another limitation is sample size, which may allow spurious results to be statistically significant according to chance. Finally, we studied pain, which is only part of the latrodectism syndrome.

DISCUSSION

We found that administration of an investigational black widow spider antivenom was followed by fewer treatment failures than placebo treatment. Secondary endpoints also supported efficacy. No patient developed symptoms consistent with an acute allergic reaction. These results support the feasibility of Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ as a treatment for latrodectism.

The patients enrolled in our study appeared similar to those in previous reports, with a predominance of men aged 30 to 40 years.[4,6] These results extend our phase 2 randomized trial assessing the practicality of 3 potential outcome measures. That study also found a reduced rate of treatment failure in the antivenom group, with 3 of 13 patients (23%) in the treatment group and 7 of 11 (64%)

Table 2. Baseline clinical findings in subjects receiving antivenom or placebo.

Signs and Symptoms	BWS AV	Placebo
Local discomfort, diaphoresis at the bite site, or both, No. (%)	26 (89.7)	31 (100)
Migrated pain from bite site to larger muscle groups, No. (%)	27 (93.1)	29 (93.5)
Muscle cramps or muscle pain, No. (%)	28 (96.6)	30 (96.8)
Chest, abdominal, back, neck, or limb pains not attributable to other causes, No. (%)	27 (93.1)	28 (90.3)
Fatigue, No. (%)	13 (44.8)	21 (67.7)
Weakness, No. (%)	17 (58.6)	23 (74.2)
Other symptoms, No. (%)	8 (27.6)	8 (25.8)
Bite site with localized area of mild erythema with localized diaphoresis and piloerection, No. (%)	24 (82.8)	25 (80.6)
Generalized diaphoresis, No. (%)	13 (44.8)	15 (48.4)
Conjunctival erythema, No. (%)	6 (20.7)	5 (16.1)
Periorbital edema, No. (%)	4 (13.8)	6 (19.4)
Pulse rate, mean (SD), beats/min	70.2 (19.16)	75.1 (14.11)
Respiration, mean (SD), breaths/min	17.7 (3.05)	17.8 (4.59)
Systolic blood pressure, mean (SD), mm Hg	136.5 (24.77)	139.8 (24.83)
Diastolic blood pressure, mean (SD), mm Hg	79.6 (17.11)	82.7 (17.71)
Temperature, mean (SD), °F/°C	97.8 (1.70)/36.6 (0.95)	97.9 (0.93)/36.6 (0.51)
Generalized diaphoresis, No. (%)	9 (31.0)	10 (32.3)
Nausea, No. (%)	8 (27.6)	12 (38.7)
Vomiting, No. (%)	1 (3.4)	2 (6.5)

in the placebo group.[6] However, the difference was not statistically significant (P=.06).

Our results differ from those of a randomized trial of Australian redback spider (*L hasseltii*) antivenom, which concluded that antivenom was ineffective against a similar venom.[16] Previous research has demonstrated that redback spider antivenom effectively neutralized the effect of black widow spider venom.[16,17] Both antivenoms contain antibody (Fab$_2$) derived from horse serum, with studies showing efficacy in an animal model before each lot release. The primary endpoint in that trial was clinically significant reduction in the severity of pain 2 hours after the study treatment, using the verbal rating scale. There are several differences between studies that could account for the

Table 3. Primary and secondary efficacy endpoints in subjects receiving study drug.

Endpoint	BWS AV N=29	Placebo N=31	Difference
Primary			
Treatment failure, % (95% CI)	51.7 (32.5 to 70.6)	77.4 (58.9 to 90.4)	−25.7 (−49.1 to −2.3)*
Secondary, time, min			
Pain intensity difference model estimate (95% CI)			
0 to 30	21.2 (13.1 to 29.3)	10.3 (2.4 to 18.1)	10.9 (−12.7 to 34.5)
0 to 60	34.6 (26.5 to 42.7)	19.8 (12.0 to 27.6)	14.8 (−8.8 to 38.4)
0 to 90	36.0 (27.9 to 44.1)	20.8 (12.9 to 28.6)	15.2 (−8.4 to 38.8)
0 to 120	38.7 (30.6 to 46.8)	19.1 (11.2 to 26.9)	19.7 (−3.9 to 43.2)
0 to 150	40.5 (32.4 to 48.6)	19.4 (11.5 to 27.2)	21.1 (−2.5 to 44.7)
Summed pain intensity difference, mean (SD)			
0 to 150	4,521 (4,297.4)	2,387 (3,230.2)	2,133 (176.9 to 4,089.6)

*P=.019.

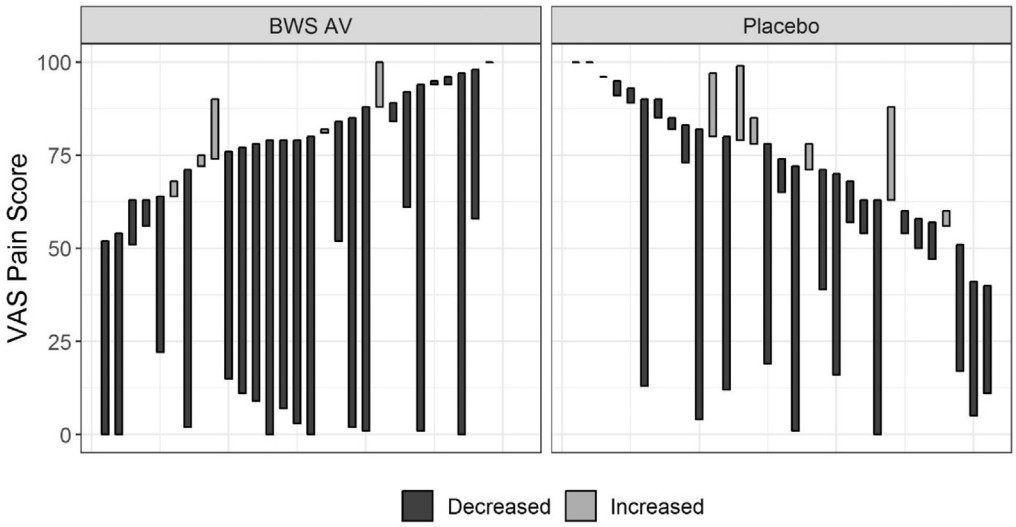

Figure 3. Individual subject changes in VAS score by treatment group. Baseline and final measured VAS score for each patient (BWS AV n=29; placebo n=31).

contrasting outcomes. First, "clinically significant reduction in pain" in the study by Isbister et al[16] was determined with an unvalidated scoring system. Other important differences include a lower pain threshold for enrollment. For example, Isbister et al[16] included patients with lower pain scores, thereby reducing the opportunity for efficacy and biasing toward no effect. Indeed, one patient who received antivenom was enrolled with an initial pain score of zero; thus, a decrease in pain score was impossible. Finally, despite these and other differences, the study approached statistical significance even though it failed to reach its planned sample size.

Nearly half of the antivenom-treated patients met our definition of treatment failure. Potential explanations include that the antivenom was ineffective or the dose insufficient. This seems unlikely because an ineffective antivenom would be expected to produce more failures and less reduction in pain score. An insufficient dose would be expected to reduce pain initially, which would then rebound, and this occurred much more commonly in the placebo group. A more likely explanation may be misclassification. Patients may have been incorrectly classified as having a black widow spider bite because there is no proven test to diagnose latrodectism. Therefore, the clinical judgment of investigators experienced

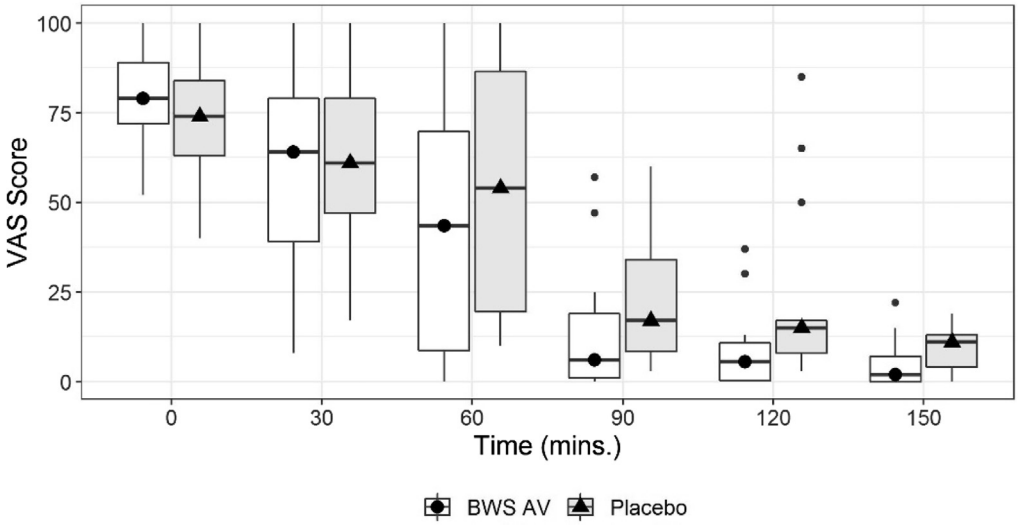

Figure 4. VAS score over time by treatment group. VAS scores are shown from baseline through the 150-minute treatment period. VAS scores for all subjects (BWS AV n=29; placebo n=31) are included through the 60-minute point. The number of observations decreases at 90, 120, and 150 minutes as subjects failed treatment and collection of VAS scores was discontinued.

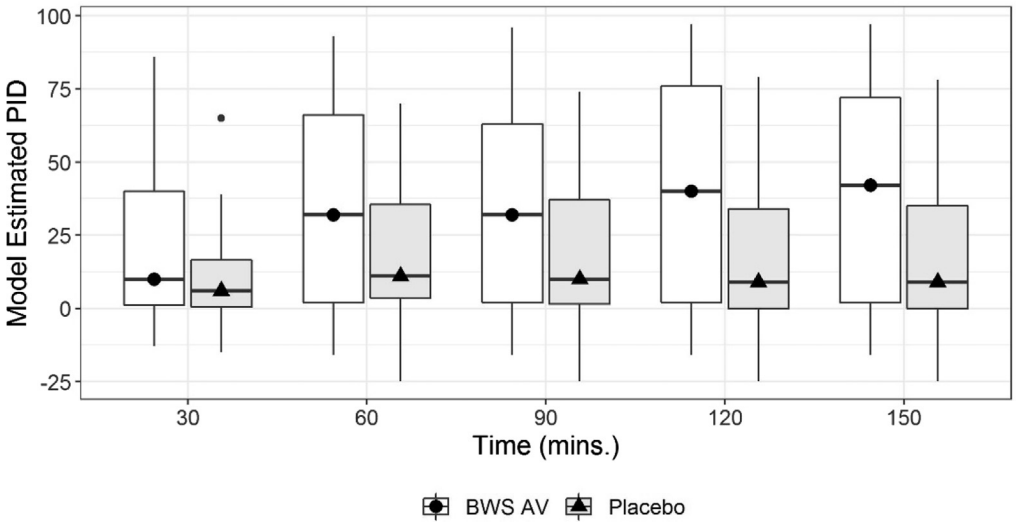

Figure 5. Pain intensity difference scores over time by treatment group. Pain intensity difference over time by treatment group is shown from baseline through the 150-minute treatment period. The difference between group medians widened over time (BWS AV n=29; placebo n=31). *PID*, Pain intensity difference.

in the treatment of black widow spider envenomation was used to establish the diagnosis.

Unfortunately, a clinical syndrome dominated by pain means that patients with waxing and waning pain syndromes (eg, abdominal pain of unclear cause, ovarian torsion, muscle cramping of unclear cause) may be enrolled. A patient with a different pain syndrome will not be expected to respond because the antivenom does not interfere with pain receptors; rather, it binds and removes latrotoxin, thereby preventing its effect on the postsynaptic neuronal membrane. Misclassification produces bias toward the null (against antivenom effectiveness) and therefore would increase the number of apparent treatment failures.[18,19]

In contrast, we expected to observe a placebo effect, but the extent of improvement in the placebo group was surprising. Potential explanations of this effect include a strong placebo effect, delayed onset of an analgesic effect from fentanyl administered before the study drug, spontaneous resolution of pain, or another cause of the pain (misdiagnosis of latrodectism), which then spontaneously remitted. To ensure that patients were truly envenomated, we attempted to quantitate α-latrotoxin in the blood, but were unable to reliably measure serum concentrations. To date, there is no known method to unequivocally establish *L mactans* envenomation. However, this inability has the beneficial effect of realistically simulating clinical practice in which the clinician must rely on clinical diagnosis.

Another measure to help characterize the clinical relevance of a finding is effect size. One measure of effect size is the number needed to treat to prevent one treatment failure. In our study, 1 treatment failure was prevented for every 3.9 patients treated (2.4 patients in our previous study).[6] The Oxford League table of analgesics in acute pain reports that in randomized, placebo-controlled, double-blind, single-dose studies of patients with moderate to severe pain, the number needed to treat for meperidine 100 mg intramuscularly was 2.9, morphine 10 mg intramuscularly was 2.9, and ketorolac 30 mg intramuscularly was 3.4 to reduce pain 50% for 4 to 6 hours.[20]

Our finding of no acute allergic events reported is promising, but, combined with those in our previous randomized trial, only 42 patients have received the investigational antivenom; therefore, the safety experience is insufficient to calculate adverse event rates.

In conclusion, the efficacy of Antivenin *Latrodectus* (Black Widow) Equine Immune F(ab′)$_2$ is supported by a reduced rate of treatment failure and improved pain intensity difference and summed pain intensity difference scores in the antivenom group.

Supervising editor: Henry E. Wang, MD, MS. Specific detailed information about possible conflict of interest for individual editors is available at https://www.annemergmed.com/editors.

Author affiliations: From the Rocky Mountain Poison and Drug Center, Denver Health and Hospital Authority, Denver, CO (Dart, Heard, Burnham, Anderson); Loma Linda University, Loma Linda, CA (Bush, Lo); the Department of Emergency Medicine, University of Colorado School of Medicine, Aurora, CO (Heard); the Department of Emergency Medicine, Louisiana State University Health Sciences Center, Shreveport, LA (Arnold); the Department of Emergency Medicine, University of California–Davis, Sacramento, CA (Sutter); the Department of Clinical Emergency Medicine, University of California–San Francisco, Fresno, CA (Campagne); the Division of Medical Toxicology and Departments

of Emergency Medicine and Pediatrics, University of Virginia School of Medicine, Charlottesville, VA (Holstege); the University of New Mexico Health Sciences Center and New Mexico Poison and Information Center, Albuquerque, NM (Seifert); the Department of Emergency Medicine, Maricopa Integrated Health System, Phoenix, AZ (Quan); the Department of Emergency Medicine, Texas Tech University Health Sciences Center, El Paso, TX (Borron); the Department of Emergency Medicine, University of Florida College of Medicine, Gainesville, FL (Meurer); Rare Disease Therapeutics, Inc., Franklin, TN (McNally); and the Instituto Bioclon S.A. de C.V., Tlalpan, Mexico (Garcia-Ubbelohde). Dr. Bush is currently affiliated with the Brody School of Medicine, East Carolina University, Greenville, NC. Ms. Anderson is currently affiliated with CPC Clinical Research, Aurora, CO.

Author contributions: RCD, JM, and WG-U designed the trial. RCD obtained research funding and supervised the conduct of the trial and data collection. RIB provided statistical analysis. RCD and VEA drafted the article, and all authors contributed to its revision. RCD takes responsibility for the paper as a whole.

All authors attest to meeting the four ICMJE.org authorship criteria: (1) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND (2) Drafting the work or revising it critically for important intellectual content; AND (3) Final approval of the version to be published; AND (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding and support: By *Annals* policy, all authors are required to disclose any and all commercial, financial, and other relationships in any way related to the subject of this article as per ICMJE conflict of interest guidelines (see www.icmje.org). Financial support for conduct of the trial was provided to the coordinating clinical site (Rocky Mountain Poison and Drug Center [RMPDC]) by Rare Disease Therapeutics, Nashville, TN; and Instituto Bioclon S.A. de C.V., Tlalpan, Mexico. Dr. Dart, Mr. Burnham, and Ms. Anderson were employed by RMPDC and received standard salary throughout the duration of the conduct of the trial. Drs. McNally and Garcia-Ubbelohde are employed by Instituto Bioclon S.A. de C.V. and Rare Disease Therapeutics, Inc., respectively, and received standard salary throughout the duration of the conduct of the trial. Drs. Bush, Heard, Arnold, Sutter, Campagne, Holstege, Seifert, Lo, Quan, Borron, and Meurer received funds from RMPDC to cover administration and procedural costs associated with the conduct of the trial at their respective investigative sites. No RMPDC employee received direct compensation for his or her role in this trial.

Publication dates: Received for publication February 19, 2018. Revisions received July 10, 2018; December 14, 2018, and January 26, 2019. Accepted for publication February 5, 2019.

Presented at the North American Congress of Clinical Toxicology annual meeting, September 2016, Boston, MA.

Trial registration number: NCT00657540

The trial sponsor did not have possession of the research data and did not participate in collection, management, or analysis of data.

REFERENCES

1. Gummin DD, Mowry JB, Spyker DA, et al. 2016 Annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 34th annual report. *Clin Toxicol*. 2017;55:1072-1252.
2. Maretic Z. Latrodectism: variations in clinical manifestations provoked by *Latrodectus* species of spiders. *Toxicon*. 1983;21:457-466.
3. Pneumatikos IA, Galiatsou E, Goe D, et al. Acute fatal toxic myocarditis after black widow spider envenomation. *Ann Emerg Med*. 2003;41:158.
4. Clark RF, Wethern-Kestner S, Vance MV, et al. Clinical presentation and treatment of black widow spider envenomation: a review of 163 cases. *Ann Emerg Med*. 1992;21:782-787.
5. Murphy CM, Hong JJ, Beuhler MC. Anaphylaxis with *Latrodectus* antivenin resulting in cardiac arrest. *J Med Toxicol*. 2011;7:317-321.
6. Dart RC, Bogdan G, Heard K, et al. A randomized, double-blind, placebo-controlled trial of a highly purified equine F(ab)$_2$ antibody black widow spider antivenom. *Ann Emerg Med*. 2013;61:458-467.
7. Bioclon Instituto. *Investigator's Brochure: Analatro [Antivenin Latrodectus (Black Widow) Equine Immune F(ab)$_2$]*. Mexico City, Mexico: Instituto Bioclon; 2011:1-18.
8. Food and Drug Administration Center for Drugs and Evaluation Research. Guidance for industry: analgesic indications: developing drug and biological products 2014. Available at: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm384691.pdf. Accessed March 4, 2019.
9. Gallagher EJ, Bijur PE, Latimer C, et al. Reliability and validity of a visual analog scale for acute abdominal pain in the ED. *Am J Emerg Med*. 2002;20:287-290.
10. Gallagher EJ, Liebman M, Bijur PE. Prospective validation of clinically important changes in pain severity measured on a visual analog scale. *Ann Emerg Med*. 2001;38:633-638.
11. Todd KH, Funk KG, Funk JP, et al. Clinical significance of reported changes in pain severity. *Ann Emerg Med*. 1996;27:485-489.
12. Bijur PE, Latimer CT, Gallagher EJ. Validation of a verbally administered numerical rating scale of acute pain for use in the emergency department. *Acad Emerg Med*. 2003;10:390-392.
13. Myrvik MP, Drendel AL, Brandow AM, et al. A comparison of pain assessment measures in pediatric sickle cell disease: visual analog scale versus numeric rating scale. *J Pediatr Hematol Oncol*. 2015;37:190-194.
14. Harris PA, Taylor R, Thielke R, et al. Research Electronic Data Capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381.
15. Medical Dictionary for Regulatory Activities. Available at: https://www.meddra.org. Accessed March 14, 2019.
16. Isbister GK, Page CB, Buckley NA, et al. Randomized controlled trial of intravenous antivenom versus placebo for latrodectism: the second Redback Antivenom Evaluation (RAVE-II) study. *Ann Emerg Med*. 2014;64:620-628.e622.
17. Graudins A, Padula M, Broady K, et al. Red-back spider (*Latrodectus hasselti*) antivenom prevents the toxicity of widow spider venoms. *Ann Emerg Med*. 2001;37:154-160.
18. Flegal KM, Brownie C, Haas JD. The effects of exposure misclassification on estimates of relative risk. *Am J Epidemiol*. 1986;123:736-751.
19. Wacholder S, Hartge P, Lubin JH, et al. Non-differential misclassification and bias towards the null: a clarification. *Int J Occup Environ Med*. 1995;52:557-558.
20. League Oxford. Oxford League table of analgesics in acute pain. Available at: http://www.bandolier.org.uk/booth/painpag/Acutrev/Analgesics/Leagtab.html. Accessed March 4, 2019.