Intralesional Antimony for Single Lesions of Bolivian Cutaneous Leishmaniasis

Jaime Soto,1 Ernesto Rojas,2 Miguel Guzman,2 Aleida Verduguez,2 Winne Nena,3 Maria Maldonado,3 Mary Cruz,2 Lineth Gracia,2 Darsi Villarroel,4 Isidoro Alavi,4 Julia Toledo,5 and Jonathan Berman6

1FUNDERMA (Fundación Nacional de Dermatología), Santa Cruz, 2Universidad Mayor de San Simón, Cochabamba, 3Hospital Local and 4Hospital Regional Chapare, Chipiriri, Bolivia; 5FADER-CIBIC, Bogota, Colombia; and 6AB Foundation, North Bethesda, Maryland

Background. Cutaneous leishmaniasis is an ultimately self-curing disease for which systemic therapy with pentavalent antimony (Sb) is effective but with side effects. We evaluated 2 local treatments, intralesional (IL) Sb and cryotherapy, for single lesions due to Bolivian Leishmania (v.) braziliensis in a placebo-controlled study.

Methods. Patients were randomized between IL Sb (650 µg/mm² of lesion area on days 1, 3, and 5), cryotherapy (days 1 and 14), and placebo cream (daily for 20 days) in a 3:2:3 allocation. Lesion area was measured prior to therapy, and at 1, 3, and 6 months after therapy. The criteria for lesion cure were as follows: not doubling in size at 1 month, at least 50% diminution in size at 3 months, and complete reepithelialization at 6 months. Local adverse effects were recorded.

Results. Cure rates were 21 of 30 (70%; 95% confidence interval [CI], 52%–83%) for IL Sb, 4 of 20 (20%; 95% CI, 8%–42%) for cryotherapy, and 5 of 30 (17%; 95% CI, 7%–34%) for placebo cream (P < .001 for IL Sb vs each other group). IL Sb adverse events were limited to injection site pain, with a mean value of 1.0 (mild).

Conclusions. The comparative cure rate, small amount of drug administered, and tolerance data for IL Sb suggest that if local therapy for single L. braziliensis lesions is chosen, this treatment is attractive. Given the difficulties of performing placebo-controlled trials in the New World, the combined placebo and cryotherapy cure rate (18%; 95% CI, 10%–31%) is likely to become the standard against which future interventions for L. braziliensis are compared.

Clinical Trials Registration. NCT01300975.

Keywords. cutaneous leishmaniasis; Bolivia; L. braziliensis; intralesional antimony; placebo.

Cutaneous leishmaniasis (CL) in the New World (NW) is present from the Texas–Mexico border down through South America to the level of the Tropic of Capricorn. New World CL generally presents as a papule that enlarges and ulcerates over 1–3 months [1]. Lesions can develop in anybody who intrudes into an endemic region and gets bitten by an infected sand fly. In recent years, industrialized nations have become more aware to the problem owing to increasing numbers of imported cases either in military personnel or travelers. The primary species causing NW CL are diverse, primarily L. (v.) braziliensis, L. (v.) panamensis, L. (v.) guyanensis, L. (v.) peruviana, L. (l.) mexicana, and L. (l.) amazonensis. [The subgenus designation v. (viannia) or l. (leishmania) is often omitted, thus, for example, L. (v.) braziliensis = L. braziliensis.] Because “members of the L. braziliensis complex are the species most frequently associated with human disease in the New World, especially L. braziliensis, [and] this species also has the widest geographic distribution in the Americas” [2], L. braziliensis is the species of most widespread clinical concern as well as the species that causes approximately 85% of disease in Bolivia [3].

The natural history of Leishmania infection depends on the ability of the host to mount an effective T helper cell 1 (Th1) response to the intramacrophage parasite. Th1 responses are generally present in routine CL, which self-heals in 3–15 months [1]. The specific

Received 2 December 2012; accepted 22 January 2013; electronically published 6 February 2013.
Correspondence: Jaime Soto, MD, FUNDERMA, Calle Ayacucho 416, Of 101, Santa Cruz, Bolivia (jaime.soto@infoleis.com).
Clinical Infectious Diseases 2013;56(9):1255–60
© The Author 2013. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.
DOI: 10.1093/cid/cit049
infected species causing CL determines where in this wide range of time periods self-cure is likely to occur. Over the 6 months during which patients expect to be cured and during which clinical trials are performed, there are considerable data on the placebo cure rate for *L. panamensis* but much less for *L. braziliensis*. For *L. panamensis*, placebo rates vary from 0% (0/11 [4]) to 37% (17/46 [5]) and 38% (9/24 [6]). For *L. braziliensis*, we are only able to find data from Guatemala, for which 8% (2/25) of cases were cured [7]. The natural history of *L. braziliensis* in South America appears to be not yet reported.

Classic treatment for CL is pentavalent antimony (Glucantime or Pentostam) administered parenterally at a dose of 20 mg/kg/day for 20 consecutive days. A large study with time or Pentostam) administered parenterally at a dose of 15 mL for a 60-kg person and which must be administered daily for 3 weeks; routine mild-moderate clinical toxicity (local pain at the injection sites, myalgias and arthralgias, gastrointestinal complaints, liver enzyme elevations, pancreatic enzyme elevations); and rare but potentially mortal cardiac arrhythmias [10]. The oral agent miltefosine, for which the per-protocol cure rate in Palos Blancos, Bolivia, was 15 of 16 (94%) [9], is approximately as effective as antimony in Bolivia. Nevertheless, gastrointestinal side effects are well recognized and strict female reproductive contraception is required.

New World CL is a disease that ultimately self-cures, with the important caveat that *L. braziliensis* and other members of the *L. viannia* subgenus uncommonly disseminate to the mucous membranes of the nose and mouth, causing mucosal disease that does not self-cure. New World CL is treated to speed lesion cure and to prevent mucosal disease for disseminating species such as *L. braziliensis*. Whether systemic therapy or local therapy should be used to treat CL is presently undecided. Factors favoring systemic therapy are high rates of lesion cure and theoretical elimination of subclinical disseminated parasites. Factors favoring local therapy are adverse effects of systemic therapy and theoretical elimination of subclinical parasites by immune processes once the bulk of parasites in the cutaneous lesion are killed. The presence of only 1 lesion, which increases the feasibility of local measures, is an important factor favoring local treatment.

Intralesional injection of pentavalent antimony (IL Sb) has been used for *L. major* from Iran with a modest cure rate (56%) [11]. However, this therapeutic approach has been used for *L. braziliensis* from Brazil with the higher cure rate of 80% [12]. Because intralesional Sb injection is the local therapy with the best reported cure rate for South American *L. braziliensis* disease, the primary purpose of the present trial was to evaluate IL Sb for Bolivian CL.

Topical paromomycin is a leading local treatment in the Old World. The ability of topical formulations of paromomycin to cure depends on penetration of this divalent cation into the lesion and thus depends on the precise formula of the cream in which the paromomycin is suspended. Other than the combination of paromomycin plus methylbenzethonium chloride in Vaseline, which had minimal efficacy in Colombia [13], patented paromomycin-containing creams are under investigation but cannot be obtained except from pharmaceutical companies. Cryotherapy has been used as sole therapy for *L. major* from Iran; the cure rate was modest (57%–58%) [11, 14]. Secondary aims were to evaluate a locally formulated topical paromomycin cream and cryotherapy.

Finally, all CL trials inherently involve a comparison to the natural rate of cure, whether documented within the study or assumed based on historical data. The fourth arm of our study was placebo treatment with an emollient cream.

### METHODS AND PATIENTS

#### Study Design

The original design was a 4-arm, open-label comparison of IL Sb, cryotherapy, topical paromomycin cream, and placebo cream for the treatment of small, single lesions due to *L. braziliensis* in Bolivia.

When local formulation of topical paromomycin cream was unsuccessful, that arm was deleted and the final design was a comparison of IL Sb, cryotherapy, and placebo cream. Patients were assigned to the 3 groups via a randomized deck of cards in the ratio 3:2:3. The sample size, based on feasibility of accrual over 6 months, was adequate to differentiate putative cure rates of 80% (IL Sb group [12]) vs 10% (placebo group [7]).

#### Patients

Patients in the Chapare province, Bolivia, catchment area were identified and, after signing informed consent and meeting entrance criteria, were treated at the Hospital Local, Chipiriri, Bolivia. Patients were enrolled between May 2011 and January 2012.

The eligibility criteria were male or female sex; ≥12 years of age; 1 ulcerative lesion ≤30 mm in largest diameter, thus with a total lesion area of ≤900 mm²; parasitological diagnosis by visualization in the direct smear or biopsy, or culture from a lesion aspirate; no specific or putatively specific antileishmanial therapy (Sb, pentamidine, amphotericin B, miltefosine, imidazoles, allopurinol) in the last 3 months; no mucosal lesions in the nose and mouth by physical examination; and no history of concomitant diseases including immunosuppression that would be likely to interact, either positively or negatively,
with IL Sb treatment. Parasites were speciated by polymerase chain reaction [15].

**Interventions**

Intralesional Sb (N-methylglucamine [Glucantime Rhodia Laboratories, France]; 81 mg/mL) was administered on each of days 1, 3, and 5 as per Oliveira-Neto et al [12] and Layegh et al [14]. A small button of Xylocaine was applied by means of a thin needle at the 4 cardinal points of the lesion. Sb was then administered via a small-gauge (23 g) needle at each cardinal point, with the needle being moved in all directions to infiltrate the whole lesion. The amount injected was 650 µg (0.008 µL)/mm² of lesion area.

Cryotherapy was performed as per Asilian et al [11] and Layegh et al [14]. Liquid nitrogen was sprayed using a CryAc device (Brymill Co) for 5–20 seconds until the lesion and 1–2 mm of surrounding normal tissue appeared frozen. Cryotherapy was performed on days 1 and 14. Postoperative care included daily cleansing with an antiseptic solution and cream for 1 week following each cryotherapy application.

**Placebo**

An emollient cream compounded by the Facultad de Farmacia, Universidad Mayor de San Simón (Cochabamba, Bolivia), was used as the lesion size prior to therapy. The size of the lesion after fusidic acid/dicloxacillin treatment was used as the lesion size prior to experimental treatment.

**Outcome Parameters and Analysis**

Patients were followed for a total of 6 months after the end of treatment and seen thrice during that time: at 1 month, 3 months, and 6 months after the end of therapy.

**Efficacy**

The endpoint parameter was reduction in lesion size. Lesion size was defined as the area of the lesion ulcer, and was computed as maximum ulcer width × maximum ulcer length. Lesion size was measured at study entrance, then at 1 month, 3 months, and 6 months after the end of therapy. The change in lesion size was calculated by expressing lesion sizes after therapy as a percentage of the lesion size prior to therapy.

The criteria for failure were doubling of lesion size by 1 month after therapy, <50% diminution in lesion size at 3 months after therapy, relapse (substantial enlargement after previous diminution), and not achieving a lesion size of 0 mm² at 6 months after therapy. Any lesion that did not fail was considered to be cured. Thus, for a patient to be cured, the lesion could not have doubled soon after therapy (1 month), failed to make substantial progress toward healing (at least 50% resolution by 3 months), relapsed, or failed to completely reepithelialize at 6 months.

**Adverse Effects**

Local adverse effects were assessed during treatment when treatments were applied by study personnel: days 1, 3, and 5 for the IL Sb group; days 1 and 14 for the cryotherapy group; and 1–2 days per each of the 3 weeks of therapy for the placebo cream group.

Patients were evaluated for local pain, itching, irritation demonstrated by erythema and/or edema, and vesicles/bulla. Each adverse effect was graded on a 0–3 scale: 0, absent; 1, mild (present but treatment not required); 2, moderate (present and needed specific treatment); 3, severe (present with such intensity that antileishmanial therapy had to be stopped).

Grade 2 adverse effects were treated as follows: pain, ibuprofen 400 mg–2–3 times a day for 1–3 days; itching, loratadine 10 mg for 2–4 days plus hydrocortisone 1% cream once a day for 1–3 days plus emollient cream twice a day for 1–4 days; irritation (erythema and/or edema), ice for 3–5 minutes for 1–3 times a day plus hydrocortisone 1% cream once a day for 1–3 days; vesicles/bulla, hydrocortisone 1% cream once a day for 1–3 days plus emollient cream twice a day for 1–4 days.

Systemic adverse effects were addressed for the first 5 IL Sb patients. When there were no abnormalities in electrocardiographic results, transaminase levels, complete blood count, and creatinine levels in those patients, per protocol, such investigations were not performed for subsequent IL Sb patients.

Categorical variables (number of patients cured, number of patients with lesions at specified body sites) were compared by the χ² test or Fisher exact test. Continuous variables (age and lesion size at entrance, adverse event grades, Sb dosages) were compared by Student t test.

**Ethical Review**

The study was approved by the Comité de Bioética de la Facultad de Medicina, Universidad Mayor de San Simón, Cochabamba, Bolivia.

**RESULTS**

Patient characteristics are shown in Table 1. Patients had a mean age of 29 years and a mean lesion size of 218 mm². The
lesion was predominately on the lower limb, and 86% of speci-
ated parasites were *L. braziliensis*.

### Treatment and Study Compliance

IL Sb and cryotherapy treatments were administered by study
staff, and the targeted number of administrations was achieved
for all patients. The proportion of patients who were apparent-
ly superinfected and received treatment with fusidic acid alone
or with dicloxacillin reflected the proportion of patients ran-
domized to the treatment groups. Four patients in the IL Sb
group, 2 patients in the cryotherapy group, and 3 patients in
the placebo cream group were treated with fusidic acid. Of
these patients, 2 (1 in the IL Sb group and 1 in the placebo
cream group) also required dicloxacillin. Compliance with
follow-up was excellent: only 3 of 80 patients (1 patient in
each of the 3 treatment groups) were lost by the 6-month
follow-up.

### Efficacy

The cure rates per experimental group were 70% (52%–83%)
for IL Sb, 20% (8%–42%) for cryotherapy, and 17% (7%–34%)
for placebo cream (Table 1). The IL Sb cure rate was statisti-
cally larger than the cure rates for either the cryotherapy
group or the placebo cream group (*P* ≤ .001).

For all groups, the reason for failure was predominately lack
of sufficient improvement in the lesion size at 3 months.

---

**Table 1. Study Treatment Data**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IL Sb</th>
<th>Cryotherapy</th>
<th>Placebo Cream</th>
<th>All Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients</strong></td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td><strong>Enrollment parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion size, mm², mean [SD]</td>
<td>259 [191]</td>
<td>205 [118]</td>
<td>188 [145]</td>
<td>218 [160]</td>
</tr>
<tr>
<td>Lesion location, No. (%)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arms/hand</td>
<td>3 (10%)</td>
<td>6 (30%)</td>
<td>8 (27%)</td>
<td>17 (21%)</td>
</tr>
<tr>
<td>Head/neck</td>
<td>3 (10%)</td>
<td>2 (10%)</td>
<td>6 (20%)</td>
<td>11 (14%)</td>
</tr>
<tr>
<td>Chest/back</td>
<td>2 (7%)</td>
<td>1 (5%)</td>
<td>0 (0%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Leg</td>
<td>22 (73%)</td>
<td>11 (55%)</td>
<td>16 (53%)</td>
<td>49 (61%)</td>
</tr>
<tr>
<td>Speciesa,*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. braz</em>:1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. braz</em>:7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. braz</em>:14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. guy</em>:1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. lain</em>:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. guy</em>:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. amaz</em>:2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Efficacy parameters, No. of patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure</td>
<td>21</td>
<td>4</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>1 mo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3 mo&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>Failure</td>
<td>8</td>
<td>15</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>1 mo</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>3 mo</td>
<td>3</td>
<td>9</td>
<td>17</td>
<td>29</td>
</tr>
<tr>
<td>Relapse</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><strong>ITT cure rate (95% CI)&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>70% (52%–83%)</td>
<td>20% (8%–42%)</td>
<td>17% (7%–34%)</td>
<td>38% (28%–48%)</td>
</tr>
<tr>
<td><strong>Adverse event score, mean [SD]&lt;sup&gt;d&lt;/sup&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>1 [0.74]</td>
<td>1.8 [0.44]</td>
<td>0.33 [0.18]</td>
<td>0.82 [0.85]</td>
</tr>
<tr>
<td>Itching</td>
<td>0.07 [0.25]</td>
<td>0.1 [0.31]</td>
<td>0.73 [0.91]</td>
<td>0.33 [0.67]</td>
</tr>
<tr>
<td>Irritation (erythema/edema)</td>
<td>0.17 [0.46]</td>
<td>1.6 [0.68]</td>
<td>0.4 [0.5]</td>
<td>0.62 [0.79]</td>
</tr>
<tr>
<td>Vesicles/bullae</td>
<td>0 [0]</td>
<td>1.2 [0.41]</td>
<td>0 [0]</td>
<td>0.3 [0.56]</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; IL Sb, intralesional systemic therapy with pentavalent antimony; ITT, intent-to-treat; SD, standard deviation.

<sup>a</sup> *L. braz/amaz/guy/lain* = *Leishmania braziliensis/guyanensis/amazonensis/lainsonii*.

<sup>b</sup> “Cure” at 1 or 3 months signifies lesions that were 100% reepithelialized at that time and were later shown not to relapse at 6 months.

<sup>c</sup> IL Sb vs cryotherapy or cream (*P* < .001).

<sup>d</sup> Values are on a scale of 0–3 (see Methods).

* *P* > .05 for all groups.
Twenty-nine of 47 failures (62%) were declared at the 3-month follow-up, whereas 16 of 47 failures (34%) occurred at 1 month. Failure criteria were lenient in this trial, in that only 50% diminution in lesion size was required at 3 months. However, 28 of the 30 cured lesions would also be declared cured if the stricter criterion of complete lesion reepithelialization at 3 months was employed. One ultimately cured lesion in the placebo cream group was 19% of its original size at 3 months and then completely reepithelialized at 6 months; one ultimately cured lesion in the IL Sb group was 46% of its original size at 3 months before completely reepithelializing at 6 months.

For the IL Sb group, the mean total amount of Sb administered to patients over the 3 injections was 503 mg (SD, 372 mg). There was no statistical difference in the amounts for the 21 cures (453 mg [SD, 278 mg]) vs the amounts for the 9 non-cures (618 mg [SD, 535 mg]; \( P = .61 \)). Because the mean weight of the IL Sb patients was 65 kg, had the patients been treated with the standard course of antimony at 20 mg/kg/day for 20 days, the patients would have received a mean dose of 26 000 mg. The intralesionally administered dose was therefore 2% of the dose that would have been administered intramuscularly.

Of the 9 patients determined to be superinfected upon entrance, 4 were cured (44%), a cure rate similar to that for the 71 nonsuperinfected patients, of whom 26 were cured (37%).

**Adverse Effects**

No patient experienced grade 3 (severe) side effects such that therapy had to be stopped even transiently (Table 1). As expected, cryotherapy was more painful than topical application of cream. Cryotherapy was also significantly more painful than IL Sb injection (\( P \leq .001 \)). Also as expected, cryotherapy created more irritation (erythema and/or edema) and more vesicles/bullae than either topical application of cream or IL Sb injection (\( P \leq .001 \)). IL Sb was more painful (\( P \leq .001 \)) but showed a trend toward less irritation (\( P = .06 \)) compared to cream application.

**DISCUSSION**

Intralesional injection of pentavalent antimony cured 70% (52%–83%) of single lesions due to Bolivian *L. braziliensis* in a controlled trial in which the placebo cure rate was 17% (95% CI, 7%–34%). This cure rate for Andean *L. braziliensis* compares well to the 80% cure rate found for *L. braziliensis* in Brazil [12] and establishes a 70%–80% cure rate for South American *L. braziliensis* generally.

Cutaneous leishmaniasis is an ultimately self-curing disease, but CL chemotherapy studies generally lack a placebo group, which makes it uncertain if the drug cure rate found in a particular trial is an improvement upon the unknown placebo cure rate. For South American *L. braziliensis*, drug cure rates might be compared to the 8% placebo rate in Guatemala, except that the biology of Guatemalan and South American *L. braziliensis* disease differs (mucosal disease is not seen in Guatemala), and the Guatemalan data are 20 years old. The inability to formulate a paromomycin cream that could be administered to patients caused our planned placebo group of 20 patients to be expanded to 30 subjects. The similarity of the 20% cure rate in the 20 cryotherapy patients to the cure rate in placebo patients may permit the data from the placebo cream and the “pseudo-placebo” cryotherapy group to be pooled. For the placebo and cryotherapy patients combined, 9 patients cured and 41 patients failed, for a cure rate of 18%. With the relatively large number of 50 “placebo” patients, the standard deviation is small (5%). Given the difficulties in performing placebo-controlled trials of CL in the New World and the lack of any data since 2004, these data are likely to be the standard of comparison for future interventions vs South American *L. braziliensis*.

For IL Sb, the primary adverse effect was injection-related pain, which had an average value of 1.0 on a 3-point scale where 1.0 signifies “mild (present but treatment for pain not required).” Because the total amount of Sb to be injected was approximately 2% of the amount needed to treat via the intramuscular route, systemic adverse events and laboratory abnormalities were not anticipated, or seen when investigated in the initial 5 patients.

Whether local therapy is appropriate for potentially disseminating NW CL is a complex issue. The answer will partially depend on the ability to follow patients for extended periods of time to rule out lymphatic and mucosal metastasis [16]. Another consideration is the number of Bolivian CL patients who meet present entrance criteria. Twenty-two of 45 patients in a 2004 study [8] met the criteria for single, <900 mm\(^2\) lesions. Eighty-four percent of patients in a 2010 epidemiological survey had single lesions (J. Soto, unpublished data). The present study can be considered proof-of-concept that if a decision is made to treat single Bolivian CL lesions with local therapy, 3 intralesional injections of Sb over 1 week is attractive: the cure rate is far higher than that of placebo; the small amount of drug administered is inexpensive; the route of administration obviates systemic side effects; 3 visits to medical facilities within 1 week is not inconvenient; and the adverse effect of local pain is well tolerated.

**Notes**

**Financial support.** This work was supported by the AB Foundation.

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
References