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Topical Paromomycin with or without Gentamicin for Cutaneous Leishmaniasis


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BACKGROUND

There is a need for a simple and efficacious treatment for cutaneous leishmaniasis with an acceptable side-effect profile.

METHODS

We conducted a randomized, vehicle-controlled phase 3 trial of topical treatments containing 15% paromomycin, with and without 0.5% gentamicin, for cutaneous leishmaniasis caused by Leishmania major in Tunisia. We randomly assigned 375 patients with one to five ulcerative lesions from cutaneous leishmaniasis to receive a cream containing 15% paromomycin–0.5% gentamicin (called WR 279,396), 15% paromomycin alone, or vehicle control (with the same base as the other two creams but containing neither paromomycin nor gentamicin). Each lesion was treated once daily for 20 days. The primary end point was the cure of the index lesion. Cure was defined as at least 50% reduction in the size of the index lesion by 42 days, complete reepithelialization by 98 days, and absence of relapse by the end of the trial (168 days). Any withdrawal from the trial was considered a treatment failure.

RESULTS

The rate of cure of the index lesion was 81% (95% confidence interval [CI], 73 to 87) for paromomycin–gentamicin, 82% (95% CI, 74 to 87) for paromomycin alone, and 58% (95% CI, 50 to 67) for vehicle control (P<0.001 for each treatment group vs. the vehicle-control group). Cure of the index lesion was accompanied by cure of all other lesions except in five patients, one in each of the paromomycin groups and three in the vehicle-control group. Each lesion was treated once daily for 20 days. The primary end point was the cure of the index lesion. Cure was defined as at least 50% reduction in the size of the index lesion by 42 days, complete reepithelialization by 98 days, and absence of relapse by the end of the trial (168 days). Any withdrawal from the trial was considered a treatment failure.

CONCLUSIONS

This trial provides evidence of the efficacy of paromomycin–gentamicin and paromomycin alone for ulcerative L. major disease. (Funded by the Department of the Army; ClinicalTrials.gov number, NCT00606580.)
Leishmania, a genus of Trypanosomatacid protozoa, is endemic in 98 countries or territories worldwide, with infection transmitted by the bite of a female sand fly. The estimated yearly incidence of leishmaniasis is 1.5 million cases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis. Cutaneous leishmaniasis results from the parasitization of skin macrophages and generally is manifested as a papule that enlarges to a nodule that often ulcerates during a period of 1 to 3 months. Cutaneous leishmaniasis species cause cutaneous leishmaniasis. There are at least five species, including Leishmania major, on the Eurasian and African continents and seven species in the Americas. Cutaneous leishmaniasis resolves without treatment in a few months to several years, with the period depending on the infecting species. For L. major, there was resolution of single lesions by 45 days of follow-up in approximately 20% to 45% of untreated patients in Tunisia or Iran, and approximately 70% had resolution of single lesions by 105 days. Despite these findings, cutaneous leishmaniasis can cause substantial morbidity owing to the continued presence of a skin ulcer and the psychological effect of disfigurement.

The World Health Organization (WHO) disability weight for cutaneous leishmaniasis is 0.023 (on a scale of 0 to 1, with increasing values reflecting more severe disease). This weight is greater than that for malaria-induced anemia (0.012) and similar to that for hookworm-induced anemia (0.024).

A variety of therapies for cutaneous leishmaniasis exist and have been reviewed (19 in a systematic review by González and colleagues alone). Clearly, there remains a need for a treatment that is simple and efficacious with an acceptable side-effect profile.

One nonsystemic treatment is the topical application of paromomycin-containing creams. Paromomycin is an antibacterial aminoglycoside that for unknown reasons also has efficacy against leishmaniasis. The dose that is required to produce a 50% reduction in parasite load in in vitro samples is generally between 1 and 40 µg per milliliter. The maximum serum concentration is 23 µg per milliliter after intramuscular administration. Intramuscular paromomycin cured visceral leishmaniasis in 95% of patients in India but was less successful in those with cutaneous leishmaniasis. To increase the amount of paromomycin that is delivered to the skin and parasites in these often-necrotic cutaneous lesions, El-On and colleagues created a formulation containing 15% paromomycin in white soft paraffin that also contained 12% methylbenzethonium chloride. This formulation was more effective than no treatment for L. major infection in Israel and more effective than vehicle-control treatment for infection with L. mexicana and L. braziliensis in Guatemala. However, cost and “severe irritancy and intolerance” associated with the use of 12% methylbenzethonium chloride, as seen in up to 75% of patients, has resulted in the infrequent use of this formulation. A cream containing 15% paromomycin in white soft paraffin plus 10% urea was sponsored by the WHO but was no more effective than vehicle control against L. major infection in Iran and Tunisia.

We developed a cream containing 15% paromomycin sulfate plus 0.5% gentamicin sulfate in a complex base (called WR 279,396) to aid drug penetration. Gentamicin was included in the formulation on the basis of efficacy studies in mice showing that gentamicin augmented the rate of cure and decreased the rate of relapse, as compared with paromomycin alone, particularly for species causing cutaneous leishmaniasis in the Americas. Although gentamicin is not directly cytocidal to leishmania parasites, it may attenuate them, facilitating immunologic mechanisms of eradication through helper T-cell immunity. In addition, combination products may impart broad-range antibacterial activity against secondary bacterial infections that could otherwise delay wound healing, and such products may avert the development of resistance. In a previous phase 2 study, WR 279,396 was more effective than vehicle control against L. major infection at the same site in Tunisia at which the WHO formulation was ineffective. Here, we report the results of our phase 3 randomized, double-blind, vehicle-controlled, parallel-group study in which we compared WR 279,396 (15% paromomycin–0.5% gentamicin cream) with paromomycin alone (15% paromomycin cream) and with vehicle-control cream against cutaneous leishmaniasis caused by L. major in Tunisia.
METHODS

STUDY DESIGN AND CONDUCT
From January 2008 through July 2011, we conducted the study at medical clinics in or near the area of Sidi Bouzid, Tunisia, in which the species is endemic. The protocol was approved by the ethics committee of the Institut Pasteur de Tunis and by the Human Research Protections Office, U.S. Army Medical Research and Materiel Command, and is available with the full text of this article at NEJM.org. The study was sponsored by the Office of the Surgeon General, Department of the Army, and is considered a phase 3 study by the Food and Drug Administration under Investigational New Drug application 50098. All patients or their legal representatives provided written informed consent, and children under the age of 18 years also provided assent. The study was conducted in accordance with the protocol, and all the authors vouch for the completeness and accuracy of the data and analyses. Study drugs were purchased by the sponsor. A patent for the topical formulation of WR 279,396 is owned by the U.S. Army. (For details about contributions of the authors, see the Supplementary Appendix, available at NEJM.org.)

STUDY TREATMENTS
The three types of cream (paromomycin–gentamicin, paromomycin alone, and vehicle control) were manufactured by Teva Pharmaceuticals in accordance with Good Manufacturing Practices. For each patient, all lesions (i.e., the index lesion plus nonindex lesions) were treated topically once daily for 20 days by a member of the study staff who documented the study treatment. (For details of the application procedures, see the Supplementary Appendix.)

STUDY PATIENTS
Inclusion criteria included an age of 5 to 65 years; overall good health besides cutaneous leishmaniasis; if female, absence of pregnancy and lactation; and the presence of five or fewer lesions, with an index lesion that was ulcerative, measured 1 to 5 cm in diameter, and was confirmed to contain leishmania by means of culture or microscopical examination of lesion material. Exclusion criteria included clinically significant lymphadenopathy or mucosal involvement, against which a topical agent would not be expected to be effective. (See the Supplementary Appendix and the protocol for all eligibility criteria.)

END POINTS

Efficacy
We assessed efficacy by measuring the area of the cutaneous leishmaniasis lesion at baseline and at 20 days (completion of treatment), 28 days, 42 days, 49 days, 98 days, and 168 days (end of the study). For each lesion, protocol-specified measures of response were as follows: initial clinical improvement (reduction in the area of the index lesion by 50 to 99% at 42 days, as compared with baseline), initial clinical cure (complete reepithelialization [no ulcer present] at 42 days, or initial clinical improvement followed by 100% reepithelialization by 98 days), relapse (initial clinical improvement or cure followed by an increase in lesion size or reulceration by 168 days), and final clinical cure (initial clinical cure without relapse). The protocol-specified primary efficacy end point was the final clinical cure of an index lesion.

The protocol-specified definition of treatment failure was an absence of initial clinical improvement or cure or an absence of final clinical cure. In addition, withdrawal of the patient before the end of the study because either the patient or the investigator thought the lesion was unlikely to have a response constituted treatment failure, as did loss to follow-up.

Safety
The safety end points were adverse events and application-site reactions (i.e., patients’ assessment of pain and irritation or clinicians’ assessment of erythema, edema, and vesicles). Safety end points were assessed daily during therapy. Renal toxic effects and ototoxic effects from aminoglycoside exposure were ascertained by means of serum creatinine measurements at the end of therapy (at 20 days) and patients’ daily reports of tinnitus and vertigo.

STATISTICAL ANALYSIS
The sample size of 375 patients was based on estimated rates of final clinical cure of 94% in the paromomycin–gentamicin group and 71% in
the vehicle-control group, as shown in a previous study. On the basis of these rates, a sample size of 125 patients in each of these two groups provided a statistical power of 99% to detect a significant difference in the rates of final clinical cure rates (94% vs. 71%). For powering the study, it was postulated that the cure rate in the paromomycin group would be between the rates in the paromomycin–gentamicin group and the vehicle-control group.

The modified intention-to-treat population consisted of patients who received at least one dose of study treatment. We tested two hypotheses using a fixed testing-sequence procedure with an overall two-sided alpha level of 0.05 or less. The first null hypothesis was that there was no difference in the final clinical cure rate between paromomycin–gentamicin and vehicle control and there was no significant difference in the rate of a final clinical cure between the paromomycin group and the vehicle-control group. The second null hypothesis was that there was no significant difference in the rate of a final clinical cure between the paromomycin–gentamicin group and the paromomycin group. A two-sided uncorrected chi-square analysis was used to test these hypotheses. Thus, the study was designed to determine whether paromomycin–gentamicin or paromomycin alone was superior to vehicle control and whether the combination product (paromomy-
cin–gentamicin) was superior to paromomycin alone (analytic details are provided in the Supplementary Appendix).

## RESULTS

### PATIENTS

Of the 1432 screened patients, 383 were randomly assigned to receive a study treatment. A total of 375 received an investigational product and constituted the modified intention-to-treat population (Fig. 1). Patient characteristics are shown in Table 1 and in the Supplementary Appendix. Approximately half the patients were male and adult. Patients had 1 to 5 lesions, and 58% had more than one lesion. The lesion size was significantly larger in the paromomycin–gentamicin group than in the other two groups. A total of 797 lesions were treated. Essentially all lesions were ulcerative. The majority of lesions were on the lower limbs, followed by the upper limbs, head, and torso. We determined the parasite species of 78% of the index lesions; all were L. major.

### TREATMENT COMPLIANCE

Of the 375 patients, 371 (99%) completed all 20 days of treatment. One patient receiving paromomycin alone and 3 receiving vehicle control withdrew consent before completing all 20 days of study cream administration.

### EFFICACY

The rate of final clinical cure of the index lesion, the primary efficacy end point, was 81% (95% confidence interval [CI], 73 to 87) with paromomycin–gentamicin, 82% (95% CI, 74 to 87) with paromomycin alone, and 58% (95% CI, 50 to 67) with vehicle control (P<0.001 for each active-drug group vs. the vehicle-control group) (Table 2). Paromomycin–gentamicin and paromomycin alone performed equally well in this study.

The primary reason for failure was an absence of initial improvement by 42 days, which occurred with the vehicle control approximately twice as often as in each active-drug group (Table 2). The next most common reason for failure was relapse, which generally consisted of enlargement by 49 days after initial improvement at 42 days. More patients had a relapse in each active-drug group than in the vehicle-control group (Table 2). There were eight withdrawals before a protocol-specified evaluation time point because the investigator or patient perceived an absence of response. Seven other patients withdrew for other reasons, did not complete therapy, or were lost to follow-up (Fig. 1 and Table 2).

Although the protocol-specified primary end point was cure of the index lesion, the absence of a response in any lesion constitutes treatment failure for the patient. Therefore, the rate of cure of all lesions is of clinical interest. One patient in each of the active-drug groups and three patients in the vehicle group had a nonindex lesion that was not cured (Table 2). Therefore, 80% of patients receiving paromomycin–gentamicin and 81% of those receiving paromomycin alone had all lesions cured, as compared with 56% of patients receiving vehicle control (P<0.001 for both comparisons).

Although the protocol-specified criteria for cure (improvement at an early time point of 42 days, cure at an intermediate time point of 98 days, and no relapse by 168 days) are in accord with

### Table 1. Baseline Characteristics of the Patients.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Paromomycin–Gentamicin (N = 125)</th>
<th>Paromomycin Alone (N = 125)</th>
<th>Vehicle Control (N = 125)</th>
<th>All Patients (N = 375)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex — no. (%)</td>
<td>56 (45)</td>
<td>68 (54)</td>
<td>69 (55)</td>
<td>193 (51)</td>
</tr>
<tr>
<td>Age — yr</td>
<td>23±16</td>
<td>25±16</td>
<td>23±15</td>
<td>24±16</td>
</tr>
<tr>
<td>Age &gt;17 yr — no. (%)</td>
<td>63 (50)</td>
<td>66 (53)</td>
<td>61 (49)</td>
<td>190 (51)</td>
</tr>
<tr>
<td>Total no. of lesions in group</td>
<td>243</td>
<td>272</td>
<td>282</td>
<td>797</td>
</tr>
<tr>
<td>Area of all lesion ulcers per patient — mm²</td>
<td>126±121</td>
<td>90±75</td>
<td>98±112</td>
<td>105±105</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD. There were no significant differences among groups.
clinical practice, some clinicians may expect a treatment to provide a cure at an early time point. The percentage of all lesions that were 100% healed at 42 days were 79%, 83%, and 57% in the paromomycin–gentamicin, paromomycin, and vehicle-control groups, respectively (P<0.001 for each active-drug treatment group vs. the vehicle-control group) (Table 2). The typical response of a lesion to treatment is shown in Figure 2.

The mean area of ulceration of the index lesion increased between 1 day and 20 days (the end of therapy) in the paromomycin–gentamicin group and in the paromomycin group but not in the vehicle-control group, with no withdrawals or treatment interruptions because of this reaction. By 28 days, however, the size of the mean lesion in the active-drug groups had decreased to approximately baseline levels.

**Table 2. Efficacy Outcomes in the Modified Intention-to-Treat Population.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Paromomycin–Gentamicin (N = 125)</th>
<th>Paromomycin Alone (N = 125)</th>
<th>Vehicle Control (N = 125)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final clinical cure of index lesion (primary end point) — no. (%)‡</td>
<td>101 (81)</td>
<td>102 (82)</td>
<td>73 (58)</td>
</tr>
<tr>
<td>Final clinical cure of index lesion but not all nonindex lesions — no.†</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lack of final clinical cure of index lesion — no.</td>
<td>24</td>
<td>23</td>
<td>52</td>
</tr>
<tr>
<td>Index lesion not cured — no.</td>
<td>23</td>
<td>22</td>
<td>48</td>
</tr>
<tr>
<td>No improvement at 42 days — no.</td>
<td>19</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Relapse — no.</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Improvement at 42 days but enlargement at 49 days — no.</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Reepithelialization at 42 days but enlargement at 49 days — no.‡</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Withdrawal by investigator or patient owing to disease progression — no.§</td>
<td>0</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Withdrawal by patient for other reason — no.</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Incomplete treatment or lost to follow-up — no.</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total reepithelialization of ulcerated lesions at 42 days — no./total no. of ulcerated lesions at baseline (%)§</td>
<td>190/240 (79)</td>
<td>218/262 (83)</td>
<td>160/279 (57)</td>
</tr>
</tbody>
</table>

* The absolute difference in the rates of the primary end point was 23 percentage points (95% confidence interval [CI], 11 to 34; P<0.001) between paromomycin–gentamicin and vehicle control, 24 percentage points (95% CI, 12 to 34; P<0.001) between paromomycin alone and vehicle control, and –1 percentage point (95% CI, –10 to 9; P = 0.87 by a two-sided uncorrected chi-square test) between paromomycin–gentamicin and paromomycin alone.
† In cases in which a final clinical cure occurred in the index lesion but not all nonindex lesions, all nonulcerated lesions were cured, including the one nodular lesion.
‡ Of the eight patients who were withdrawn owing to disease progression, the patient in the paromomycin group was withdrawn by the investigator because of lymphangitis (suspected disease dissemination). The remaining seven patients were in the vehicle-control group: three were withdrawn by the investigator because the index lesion had increased in size by 28 days; one had index-lesion enlargement by a factor of 1.6 by 20 days, accompanied by development of three new lesions; two had index-lesion inflammation by 49 days; and one withdrew consent at 28 days because the index lesion had not changed in size. The effect of the lack of definitive end-point determination with respect to patients lost to follow-up was explored (see the Supplementary Appendix).
§ Complete reepithelialization occurred in significantly more lesions in either active group than in the vehicle-control group (P<0.001 for each comparison, by a two-sided uncorrected chi-square test).

**SAFETY**

All adverse events that were deemed by the investigators as at least possibly related to a study treatment were reactions of mild or moderate severity at the application site. Adverse events that occurred in at least 1% of patients in any group are shown in Table 3. Erythema and skin irritation were present in all groups; the latter was attributed to the dressing. Minute vesicles were significantly more frequent in the active-drug groups than in the vehicle-control group. Superinfection (clinical signs of secondary bacterial infection of cutaneous leishmaniasis lesions) was significantly more common in the vehicle-control group than the active groups. One serious adverse event — acute poststreptococcal glomerulonephritis — occurred in the paromomycin–gentamicin group and was considered to be unrelated to the study treatment.
There were no cases of clinically significant tinnitus or vertigo or changes in serum creatinine levels between screening and the evaluation at 20 days, and no patient was removed from the study owing to an adverse event. There were no deaths.

**Discussion**

This trial showed that either of two creams containing 15% paromomycin, one with and one without 0.5% gentamicin, was superior in efficacy to a vehicle-control cream for treating ulcerative cutaneous leishmaniasis caused by *L. major* in Tunisia. There was no advantage in the addition of gentamicin observed in this study. The index lesion was cured in 101 of 125 patients (81%) receiving paromomycin–gentamicin, in 102 of 125 patients (82%) receiving paromomycin alone, and in 73 of 125 patients (58%) receiving vehicle control (*P*<0.001 for each paromomycin group vs. the vehicle-control group).

Our phase 3 study was conducted on the basis of successful results of a previous phase 2 study conducted in both Tunisia and Paris. A comparison of the results of these two studies is provided in the Supplementary Appendix.

Paromomycin–gentamicin and paromomycin alone, when administered to leishmania-induced ulcers for 20 days, resulted in systemic paromomycin exposure that was less than 10% of the exposure from a standard regimen of intramuscular paromomycin. Thus, as expected, there were no instances of clinically meaningful renal toxic effects or ototoxic effects in this trial. The formation of minute vesicles was more frequent in the active-drug groups than in the vehicle-control group. Clinicians and patients should anticipate that when either active cream is used, a transient inflammatory reaction involving lesion enlargement and vesicle formation may occur during treatment. However, no patient requested to withdraw or had treatment suspended because of this inflammatory reaction; there were none.

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**Figure 2. Response of a Typical Lesion to Treatment with Paromomycin–Gentamicin.**

On the first day of the study, the ulceronodular facial lesion was 24.0 mm by 19.7 mm in area (Panel A). By 20 days, at the end of therapy, the lesion had visibly flattened (Panel B) and measured 2.1 mm by 1.1 mm in area. There was complete reepithelialization of the lesion by 42 days (Panel C), and the lesion had not relapsed by 157 days (Panel D).
no problems with compliance. We postulate that the inflammatory reaction aids ulcer resolution. Superinfection was more common with vehicle control than with paromomycin, which we attributed to an absence of local antibacterial effect of the aminoglycosides. All superinfections were treated with oral antibiotics and resolved.

This trial provides evidence that either paromomycin formulation in the present hydrophilic vehicle control is an effective treatment for ulcerative \textit{L. major} disease in Tunisia. Early treatment of \textit{L. major} cutaneous leishmaniasis with a cream is simpler than the current treatment options for cutaneous leishmaniasis and follows the recent WHO recommendations for the treatment of \textit{L. major} cutaneous leishmaniasis.\cite{1}

The similarity in the natural cure rate for \textit{L. major} infection in such geographically separate areas as Tunisia\textsuperscript{2} and Iran\textsuperscript{3} suggests that either cream would be effective treatment for ulcerative \textit{L. major} in general, although studies are needed to address this expectation. The efficacy of these creams against nonulcerative disease or disease caused by other species of leishmania remains to be fully investigated. In this regard, we note that in animals, paromomycin–gentamicin and paromomycin alone were equally effective against \textit{L. major}, a result that predicted the findings of our study, but paromomycin–gentamicin was more effective than paromomycin alone against American species such as \textit{L. panamensis} and \textit{L. amazonensis}.

For all cases of cutaneous leishmaniasis, the therapeutic index for the two paromomycin creams is enhanced by their acceptable side-effect profile, as compared with alternative interventions.

Table 3. Adverse Events Occurring in More Than 1% of Patients in Any Study Group.

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Paromomycin–Gentamicin (N = 125)</th>
<th>Paromomycin Alone (N = 125)</th>
<th>Vehicle Control (N = 125)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild number (percent)</td>
<td>Mild number (percent)</td>
<td>Mild number (percent)</td>
</tr>
<tr>
<td>Application site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>6 (5)</td>
<td>7 (6)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Vesicles</td>
<td>26 (21)</td>
<td>27 (22)</td>
<td>9 (7)</td>
</tr>
<tr>
<td>Edema</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Pain</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>5 (4)</td>
<td>2 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Paronychia</td>
<td>2 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Superinfecion</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>0</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Oropharyngeal pain</td>
<td>4 (3)</td>
<td>3 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>3 (2)</td>
<td>2 (2)</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

The opinions or assertions expressed in this article are the views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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