A Blanching Technique for Intradermal Injection of the Hyaluronic Acid Belotero

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Summary: With the proliferation of dermal fillers in the aesthetic workplace have come instructions from various manufacturers regarding dermal placement. Determination of injection needle location in the dermis has in large part been based on physician expertise, product and needle familiarity, and patient-specific skin characteristics. An understanding of the precise depth of dermal structures may help practitioners improve injection specificity. Unlike other dermal fillers that suggest intradermal and deep dermal injection planes, a new hyaluronic acid with a cohesive polydensified matrix may be more appropriate for the superficial dermis because of its structure and its high degree of integration into the dermis. To that end, the authors designed a small study to quantify the depth of the superficial dermis by means of ultrasound and histology. Using ultrasound resources, the authors determined the depths of the epidermis, the dermis, and the reticular dermis in the buttoks of six patients; the authors then extrapolated the depth of the superficial reticular dermis. Histologic studies of two of the patients showed full integration of the product in the reticular dermis. Following determination of injection depths and filler integration, the authors describe a technique (“blanching”) for injection of the cohesive polydensified matrix hyaluronic acid into the superficial dermis. At this time, blanching is appropriate only for injection of the cohesive polydensified matrix hyaluronic acid known as Belotero Balance in the United States, although it may have applications for other hyaluronic acid products outside of the United States. (Plast. Reconstr. Surg. 132: 59S, 2013.)

Aatomically, the dermis is divided into two clearly distinguished parts: the papillary dermis and the reticular dermis. The papillary dermis, located between the basal layer of the epidermis and the reticular dermis, has a different fibrillar structure from the reticular dermis. In the papillary dermis, the collagen and elastic fibers appear as a delicate branching network, roughly perpendicular to the basal membrane of the epidermis. By contrast, the reticular dermis, beneath the papillary dermis, is divided into the superficial, mid, and deep reticular dermis. The fibrillar structure of the reticular dermis consists mostly of thicker fibers of collagen and elastin parallel to the basal membrane of the epidermis. Figure 1 shows the structural arrangement of the epidermis, papillary dermis, and reticular dermis.

Information issued by manufacturers of injectable hyaluronic acid gels often includes instructions that these hyaluronic acid agents must be

Disclosure: Dr. Micheels has been compensated for presentations about products manufactured by Merz Aesthetics at educational meetings in the European Community. He is also a consultant for Merz, ANtēs, IBSA Pharma, Q-Med Switzerland, and Allergan. Other than his educational presentations, he has nothing to disclose that would represent a conflict of interest. Dr. Sarazin and Dr. Besse have no financial interests to disclose. Dr. Sundaram serves as a consultant and/or clinical investigator for Allergan, ANtēs, CosmoFrance, Ipsen, Medicis/Valeant, Mentor/Johnson & Johnson, Merz, and Q-Med/Galderma. Dr. Flynn is a consultant for Merz and Allergan; he has received research support from Merz Aesthetics. The authors were assisted in development of this article by David J. Howell, Ph.D. (San Francisco), who was compensated by Merz Aesthetics for his contributions.
placed intradermally or subdermally. Intradermal injection techniques are especially pertinent for less reticulated gels and/or gels with lower concentrations of hyaluronic acid, which are often intended to treat fine wrinkles and thin skin areas (e.g., crow’s feet). Both because of the instructions from the manufacturers and an inherent wish on the part of the physician to remediate dermal deficiencies as fully as possible, an understanding of the papillary dermis and reticular dermis is clearly relevant to the placement of dermal fillers.

Even with a comprehensive understanding of the dermis, however, recent advancements in hyaluronic acid technology have introduced new dermal fillers that bring their unique characteristics. The cohesive polydensified matrix hyaluronic acid known commercially as Belotero Balance [manufactured by Anteis, Geneva, Switzerland, and distributed by Merz Pharmaceuticals in Switzerland, Germany, Austria, Italy, and Russia (called Belotero Basic outside of the United States, Belotero Balance in the United States, distributed by Merz Aesthetics, Greensboro, N.C.)], for example, is manufactured in ways that differ from the hyaluronic acids known commercially as Juvéderm (Allergan, Inc., Irvine, Calif.) and Restylane (Medicis Pharmaceutical Corp., Scottsdale, Ariz.). Specifically, Belotero is produced using two cycles of cross-linking with butanediol diglycidyl ether, rather than one cycle of cross-linking. The result is a dermal filler gel with zones of greater and lesser density. In contrast to the varying particulate sizes found in Belotero (cohesive polydensified matrix hyaluronic acid), the nonanimal stabilized hyaluronic acid Restylane is a sieved (particulate) hyaluronic acid gel, in which particles of a uniform size are created. Juvéderm is composed of a three-dimensional matrix of microscopically visible particles and long and short hyaluronic acid molecules, cross-linked with butanediol diglycidyl ether to increase resistance to degradation. Highly magnified images of cohesive polydensified matrix hyaluronic acid, nonanimal stabilized hyaluronic acid, and three-dimensional matrix show a cohesive, homogenous appearance of cohesive polydensified matrix hyaluronic acid not found in images of nonanimal stabilized hyaluronic acid and three-dimensional matrix gels.

Generally speaking, hyaluronic acid gels designed to correct average and deep wrinkles

Fig. 1. Illustration of structures in the papillary and reticular dermis. (Used with permission of Gory Details Illustration. © 2013 A. B. Hernandez.)
are injected subdermally. Flynn et al. have shown that the hyaluronic acid Belotero provides a high degree of integration into the dermis, and thus allows for true intradermal integration. Other fillers, when injected into the dermis, appear to form large pools or clumps, with the products migrating subdermally.

Some physicians have questioned the ability to inject hyaluronic acids intradermally, but it is possible with the right products and with precision in such a narrow space. To help better understand the depth of the various dermal layers and to help address the challenges of intradermal injection, investigators designed the study reported here.

**STUDY OBJECTIVE**

The purposes of this pilot study were three-fold: (1) to quantify the depth of the superficial dermis for injection with the cohesive polydensified matrix hyaluronic acid; (2) to show the histologic distribution of cohesive polydensified matrix hyaluronic acid when injected into this space; and (3) to investigate whether a “blanching” technique for injection of the hyaluronic acid with cohesive polydensified matrix would successfully address wrinkles in the superficial dermis of the face, taking advantage of the unique properties of cohesive polydensified matrix hyaluronic acid in such a confined space. (Editor’s note: In a study published in 2011, Flynn et al. reported early efforts to ascertain dermal integration and histology of monophasic and biphasic hyaluronic acid fillers. The data provided a broad-based introduction to several dermal fillers. In contrast, this study focuses only on quantification of depth of superficial injection and injection techniques for cohesive polydensified matrix hyaluronic acid.)

**PATIENTS AND METHODS**

**Ultrasound Measurements of Injected Hyaluronic Acid**

Investigators used a General Electric logiQ E9 ultrasound instrument with a hockey stick L8 18i probe (GE Healthcare, Little Chalfont, United Kingdom) set at 17 MHz. To better view the image, investigators placed a SonarAid (Geistlich Pharma, Wolhusen, Switzerland) transducer above the injected buttocks. Three 0.2-ml papules of hyaluronic acid—one of which was cohesive polydensified matrix hyaluronic acid gel—were created, using an injection technique similar to the one deployed in allergic testing, creating an intradermal wheal. With six patients injected, a total of eight samples were available for measurement. To visualize injection of the hyaluronic acid by means of ultrasound, the transducer was placed immediately over the needle and the material was then injected using a 30-gauge, ½-inch needle affixed to the syringe.

Care was taken to insert the needle as tangentially into the skin as possible (at an angle of approximately 10 degrees) to guarantee intradermal placement of cohesive polydensified matrix hyaluronic acid. Figure 2 is an image of an injection of the cohesive polydensified matrix hyaluronic acid at the angle noted, taken during a clinical treatment and thus without the transducer used in ultrasound measurement.

**Histologic Studies of Injected Hyaluronic Acid**

For the histologic tests in the buttock skin area, investigators created intradermal papules in two patients by a single injection of 0.2 ml of cohesive polydensified matrix hyaluronic acid in

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**Fig. 2.** Injection of the cohesive polydensified matrix hyaluronic acid gel at the angle noted, taken during a clinical treatment and thus without the transducer. (Photograph courtesy of Patrick Micheels, M.D. Used with permission.)
each patient. One milliliter of lidocaine without epinephrine was injected around the papules so that the biopsy specimens could be taken from the area without excessive pain at 3 minutes after lidocaine injection. No lidocaine was injected directly into the papules. Four-millimeter round punches were used for the biopsies; specimens were fixed in formalin and embedded in paraffin. Slides were stained with colloidal iron, Alcian blue, and hematoxylin and eosin. Images and measurements were obtained with an Axio Scope A1 microscope and an AxioCamMRc camera (Carl Zeiss, Oberkochen, Germany).

RESULTS

Clinical Observations

Following the creation of the intradermal papules, the skin manifested an orange peel appearance. The cohesive polydensified matrix hyaluronic acid injection was painless over two-thirds of the volume injected. The last third of the injection process with cohesive polydensified matrix hyaluronic acid was associated with greater sensitivity, according to reports from the subjects.

Depth-of-Needle Considerations

Based on several different ultrasonic images and measurements, we were able to confirm two positional findings. The first is that the angle of penetration is very close to the one mentioned in theory (≤10 to 12 degrees). On the sagittal view, the angle at which the needle penetrates was on average 166.8 degrees or 13.2 degrees, depending on the side of the needle considered (i.e., toward the epidermis or facing away from the skin’s surface), with extremes of 169 degrees (or 11 degrees) to 165 degrees (or 15 degrees).

The second finding is that the depth of needle tip penetration into the dermis varies considerably, from 0.083 mm (83 μm) to 0.4 mm (400 μm), with an average depth penetration value calculated at 0.205 mm (205.5 μm). This means that, according to our calculations, the needle tip is well within the upper third of the reticular dermis, specifically, the tip is in the superficial reticular dermis.

Ultrasound Findings of Injected Hyaluronic Acid

The average thickness of the epidermis in the injected buttocks was 0.37 mm (371.5 μm), with a range from 0.34 mm (345 μm) to 0.41 mm (413 μm). In the buttocks of both patients, the reticular dermis was, on average, 1.74 mm thick (1747 μm), with a range from 1.55 mm (1550 μm) to 1.84 mm (1840 μm). By extrapolation, the superficial reticular dermis (one-third of the total depth of the reticular dermis) is of average thickness in the population injected: 0.57 mm (570 μm), with a range from 0.516 mm (516.6 μm) to 0.61 mm (613.3 μm). If we add an average epidermal thickness of 0.1 mm (100 μm), this gives us an average depth between the superficial corneal layer and the one-third superficial and one-third mid reticular dermal junction of 0.67 mm (670 μm), with a range from 0.61 mm (617 μm) to 0.71 mm (713 μm). Table 1 shows ultrasound measurements (in microns) of epidermis, dermis, superficial dermis, needle penetration, and angle of needle penetration for the cohesive polydensified matrix hyaluronic acid in all six patients. Table 2 shows measurements of the thickness and estimated volume of the papules for cohesive polydensified matrix hyaluronic acid in all six patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Height of Epidermis (μm)</th>
<th>Height of Total Dermis (μm)</th>
<th>Height of Superficial Dermis (⅓ of total dermis) (μm)</th>
<th>Depth of the Needle (CPM HA) (μm)</th>
<th>Angle of Needle Penetration (CPM HA) (degrees)</th>
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</thead>
<tbody>
<tr>
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<td>345</td>
<td>1800</td>
<td>600.0</td>
<td>−344</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>385</td>
<td>1890</td>
<td>606.6</td>
<td>−400</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>413</td>
<td>1840</td>
<td>613.3</td>
<td>−084</td>
<td>12</td>
</tr>
<tr>
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<td>355</td>
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<tr>
<td>5</td>
<td>364</td>
<td>1550</td>
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<td>−083</td>
<td>11</td>
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<tr>
<td>6</td>
<td>367</td>
<td>1780</td>
<td>593.3</td>
<td>−200</td>
<td>14</td>
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</tbody>
</table>

CPM HA, cohesive polydensified matrix hyaluronic acid.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Thickness of the Papule (μm)</th>
<th>Estimated Volume of the Papule (μm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>477</td>
<td>617</td>
</tr>
<tr>
<td>2</td>
<td>413</td>
<td>332</td>
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<tr>
<td>3</td>
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<tr>
<td>6</td>
<td>333</td>
<td>442</td>
</tr>
</tbody>
</table>
estimated volume of the papules (in microns) for the cohesive polydensified matrix hyaluronic acid.

The appearance on sonography of the cohesive polydensified matrix hyaluronic acid implant was very similar to the appearance of the neighboring, noninjected dermis. The papule itself was oblong in shape, with an obtuse angle in relation to noninjected skin. On the sonographic images, the cohesive polydensified matrix hyaluronic acid manifests as minimal change within the dermis. Figure 3 shows ultrasound images of the cohesive polydensified matrix hyaluronic acid.

**Histologic Findings of Injected Hyaluronic Acid**

Using multiple histologic samples taken from the buttocks of two volunteer patients, histologic investigators, not unlike the ultrasound investigators, determined the thickness of the epidermis and the thickness of the papillary and reticular dermis. Total skin thickness in the two subjects’ biopsy specimens varied from 5 to 7 mm, with an average of 6.56 mm. In particular, in the epidermis, the thickness varied from 0.10 to 0.15 mm in the two biopsy specimens. The average thickness of the epidermis for these two subjects was 0.12 mm (120 μm). In the papillary dermis, the thickness varied from 0.14 to 0.3 mm, with an average thickness of 0.22 mm (221 μm). Total reticular dermis thickness varied from 4.1 to 6 mm, with an average thickness of the reticular dermis of 5.16 mm (5162 μm). These findings lead us to believe that, in these two subjects, the thickness of the superficial reticular dermis of the buttocks was, on average, 1.7208 mm (1720.8 μm). Table 3 shows the histologic measurements (in millimeters) of the cohesive polydensified matrix hyaluronic acid implants in a 42-year-old male patient and a 61-year-old female patient.

Whatever the dye used (hematoxylin and eosin, Alcian blue, or colloidal iron), the cohesive polydensified matrix hyaluronic acid was distributed consistently across the full thickness of the reticular dermis. In Figure 4, the cohesive polydensified matrix hyaluronic acid occupies the full thickness of the reticular dermis; it is evenly distributed, with mild nonspecific inflammation consisting of a few lymphocytes around the capillaries.

**Blanching Technique for Injection into Fine Wrinkles**

The blanching technique described here was presented to European physicians in 2011 and

| Table 3. Histologic Measurements of the Cohesive Polydensified Matrix Hyaluronic Acid in Two Patients |
|---------------------------------|---------------------------------|---------------------------------|
|                                 | Patient 1 (mm) *                | Patient 2 (mm) †                |
| Total thickness                 | 7.00                           | 7.00                           |
| Epidermis                       | 0.11                           | 0.12                           |
| Papillary dermis                | 0.25                           | 0.14                           |
| Reticular dermis                | 6.00                           | 5.50                           |

*Patient 1 was a 42-yr-old man.  †Patient 2 was a 61-yr-old woman.
has been mentioned in recent articles in the clinical literature.²¹¹ The position used to perform the blanching technique is also notably similar to the technique of multiple punctures used to inject the American bovine collagen Zyderm I (Inamed Corp., Santa Barbara, Calif.) in the not-too-distant past.¹² The blanching technique has been deployed by the lead author (P.M.) of this Supplement article in more than 800 patients over a period of 7 years, with no consequential adverse events. The reason for the nomenclature is simple: during treatment, the clinician will observe blanching of the injected area. (In the authors’ opinion, blanching is caused not by vasoconstriction but instead by the transparent appearance of the gel as a result of its proximity to the skin’s surface. Other reasons for blanching could include tissue distention and blood displacement.) The blanching is short-lived. In our clinical practice, the blanched countenance has disappeared in less than 10 minutes. Figure 5 shows immediate blanching following injection with the cohesive polydensified matrix hyaluronic acid, followed by rapid dissolution of the discoloration.

To carry out the blanching technique, only 30-gauge, ½-inch long needles should be used. Placement of the needle is almost parallel to the skin, so that the angle of penetration of the needle does not exceed 12 degrees. The diameter of the needles used in this study was 0.3 mm (300 μm).

To ensure superficial, or intradermal, placement of material, investigators found it advisable to visualize the outline of the needle. This was accomplished by “tenting” the needle within the skin and verifying that the outline of the needle was visible to the injector. The bevel may face upward or downward in this blanching technique. However, positioning the bevel downward for thin skin may limit overly superficial placement. The bevel can be placed upward for thicker skin.

Instead of uninterrupted retrograde injection, multiple punctures are injected very close to each other, leading to deposition of tiny aliquots. Injection this close to the surface of the skin creates a tiny bead, or papule. Once the first papule has been made, the needle must next be inserted into the perimeter of the first papule to create the second one. Multiple punctures continue just under the surface until the wrinkle has been completely removed with the cohesive polydensified matrix hyaluronic acid. Postinjection molding of the area should be completed to ensure a smooth final correction. Figure 6 shows the angle and the repetition of tiny aliquots of the cohesive polydensified matrix hyaluronic acid gel.

Figures 7 through 10 are representative results of patients who have been treated with the cohesive polydensified matrix hyaluronic acid by

![Image](image-url)
means of the blanching technique for correction of their facial fine lines and wrinkles. Correction has been noted past 1 year in one of the patients (Fig. 10) and up to almost 2 years in another patient (Fig. 8).

Note that as this article goes to press, the blanching technique is suitable only for injection of the cohesive polydensified matrix hyaluronic acid Belotero Balance in the United States. In addition, although the blanching is a short-lived and benign effect with superficial injection, it is troubling for deeper particulate injections, perhaps even indicating arterial embolization.

**DISCUSSION**

Reports in the literature support the belief that hyaluronic acid gels present some risk of creating the appearance of the Tyndall effect.\textsuperscript{13–15} To limit the presence of the Tyndall effect, some physicians have injected hyaluronic acids such as nonanimal stabilized hyaluronic acid and threedimensional matrix more deeply, into the subdermal layer. By contrast, in our clinical experience with superficial injection of the cohesive polydensified matrix hyaluronic acid, we have observed no Tyndall effect. In 2011, Flynn and colleagues showed improved dermal integration of Belotero compared with Restylane and Juvederm.\textsuperscript{5}
were no discrete optically isolated pools of hyaluronic acid when Belotero was used. Thus, the article demonstrated that, when the biphasic product was used, large areas of only hyaluronic acid existed within the hypodermis, allowing light to be refracted and, as a result, accounting for the beading and Tyndall effect particularly seen with these products. A recently published retrospective study of 300 patients supports the absence of the Tyndall effect in use of cohesive polydensified matrix hyaluronic acid.16 (The article is also discussed in the article “A Multicenter Study of the Safety and Effectiveness of Hyaluronic Acid with a Cohesive Polydensified Matrix for Treatment of Nasolabial Folds in Subjects with Fitzpatrick Skin Types IV, V, and VI” elsewhere in this Supplement.)

The cohesive polydensified matrix hyaluronic acid may not present a risk for the Tyndall effect because of its design and viscoelastic properties.8,17,18 Average molecular weights of the hyaluronic acid fragments deployed to make the various hyaluronic acid products differ. Although the molecular weight of the hyaluronic acid fragments of nonanimal stabilized hyaluronic acid and three-dimensional matrix are fairly close (200 to 300 kDa), the molecular weight of the hyaluronic acid fragments of the cohesive polydensified matrix hyaluronic acid is considerably higher (approximately 800 kDa).19 Perhaps because of these properties or because of other properties not yet well characterized in the literature, the cohesive polydensified matrix hyaluronic acid gel appears to be a good candidate for intradermal injection, without undue concern about unevenness. From a histologic perspective, the differences in appearance of the hyaluronic acid gels in the dermis may depend in part on the methods of the hyaluronic acid gel’s reticulation (i.e., its cross-linked arrangement of hyaluronic acid molecules). Reticulation is proprietary for each of the three manufacturers.

On ultrasound imaging, the cohesive polydensified matrix hyaluronic acid gel was isoechogenic compared with the surrounding dermal tissue. This leads us to suppose that the product contains few, if any, dense particles. In addition, even though the cohesive polydensified matrix hyaluronic acid gel has particles of varying sizes, it presents as a very homogenous structure. The gel was distributed over the full thickness of the dermis and did not produce acute angles at the injected skin/noninfected skin junction, either transversely or sagittally. The absence of dense particles supports the argument that this cohesive gel is appropriate for use in the superficial reticular dermis. Similarly, its efficacy in addressing fine lines and wrinkles provides further support for the use of the cohesive polydensified matrix hyaluronic acid gel in the aesthetic marketplace. A recent split-face comparison of the monophasic cohesive polydensified matrix

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**Fig. 9.** A 49-year-old white man received 2 ml of the cohesive polydensified matrix hyaluronic acid gel in the superficial dermis for treatment of glabellar lines, forehead, and nasolabial folds area. The posttreatment photograph was taken at 4 weeks after injection. (Photographs courtesy of Patrick Micheels, M.D. Used with permission.)
hyaluronic acid gel to an available biphasic hyaluronic acid gel showed “greater improvement” in the endpoint of “evenness” of skin injected with the cohesive polydensified matrix hyaluronic acid gel. This may be attributable to the minimal presence of hyaluronidase within the superficial reticular dermis. However, we have been unable to confirm this explanation either from the clinical literature or from conversations with colleagues. In our study, the pain reported by subjects in the third of the injection process with cohesive polydensified matrix hyaluronic acid may have been caused by distention of the nerve endings of the skin after injection of cohesive polydensified matrix hyaluronic acid gel.

In general, the histologic samples were consistent with the ultrasound findings. The cohesive polydensified matrix hyaluronic acid appears to integrate evenly into the dermal tissue.

CONCLUSIONS

To achieve a true superficial intradermal injection, the needle must be placed correctly. The narrow depth is the reason why the blanching technique can only be used with 30-gauge ½-inch or finer needles. The physician must use a very close multipuncture approach for intradermal placement, rather than the conventional retrograde approach associated with hyaluronic acid injection into the mid and deep dermis. Because of its particular properties of even distribution and low G', the cohesive polydensified matrix hyaluronic acid gel is suitable for very superficial intradermal injection using the blanching technique described in this article, with little to no risk of visible product or the Tyndall effect—a phenomenon sometimes observed with hyaluronic acid gels currently available in the U.S. market when they are injected too superficially (i.e., into the superficial reticular dermis). In our clinical practice, very little of the cohesive polydensified matrix hyaluronic acid gel is injected into each area treated. The low volume per wrinkle means that many areas may be treated, resulting in effectiveness and high patient satisfaction levels.

**Fig. 10.** A 31-year-old East Indian man received 0.25 ml per side of the cohesive polydensified matrix hyaluronic acid gel in the superficial dermis of the nasolabial folds. The posttreatment photograph was taken at 15 months after injection, without any touchup after initial injection. (Photographs courtesy of Patrick Micheels, M.D. Used with permission.)

**PATIENT CONSENT**

Patients provided written consent for use of their images.
ACKNOWLEDGMENTS

The authors express considerable appreciation to the MedImage Institute of Radiology and the Laboratoire d’Histopathologie Viollier-Weintraub (Geneva, Switzerland) for materials and equipment and to the volunteer subjects who agreed to be a part of the study. In addition, the authors sincerely appreciate the editorial contributions of David J. Howell, Ph.D. (San Francisco, Calif.), in support of development and submission of the manuscript. Figures 1 through 6 were illustrated by Alexandra B. Hernandez of Gory Details Illustration.

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