Correlation of Dermoscopy With In Vivo Reflectance Confocal Microscopy of Streaks in Melanocytic Lesions

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Objective: To analyze dermoscopically identified streaks by direct correlation with features visualized on reflectance confocal microscopy (RCM).

Design: We evaluated by RCM melanocytic lesions showing peripheral streaks on dermoscopy. A digital camera connected to the RCM computer enabled direct analysis of the streaks. The lesions were excised and histopathologically analyzed.

Setting: Dermatology clinic specializing in pigmented lesions.

Patients: The study population comprised 7 patients with melanocytic lesions, including 2 melanomas, 4 dysplastic nevi, and 1 compound nevus with spitzoid features.

Results: In 6 of the cases, peripheral streaks were visualized on RCM as confluent aggregates composed of bright, ill-demarcated cells. These aggregates were contiguous with the bright central part of the lesion and appeared to be curving around dermal papillae. Of the 6 lesions, 3 with elongated aggregates visualized on RCM harbored peripheral, elongated nests on histopathologic examination, and 2 with shorter, more ill-defined peripheral aggregates visualized on RCM had smaller, more poorly formed peripheral nests on histopathologic examination. The seventh lesion showed few peripheral streaks on dermoscopy; however, corresponding features visualized on RCM showed discrete, dense, round nests aligned in proximity. We did not recognize in the present series distinguishing characteristics on RCM that could differentiate between the peripheral streaks of malignant melanoma and nevi.

Conclusions: Direct dermoscopy-RCM correlation is a feasible method to study streaks and may help to improve the classification methods used in dermoscopy. Additional studies with larger series are needed to confirm our findings and may help elucidate the morphologic and biological nature of peripheral streaks.

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Detection and removal of malignant melanoma (MM) at an early stage offers the greatest hope for cure of this lethal cancer.1 Dermoscopy is a noninvasive technique that allows for the visualization of subsurface structures by rendering the corneal layer translucent. With appropriate interpretation by an experienced examiner, dermoscopy increases the clinician's accuracy for MM diagnosis compared with clinical examination alone.2,3 Peripheral streaks are dermoscopic structures that are worrisome for MM.4 Peripheral streaks were found to have a greater than 95% specificity for MM and a sensitivity of 18% to 23%.5 The importance of streaks is underscored by the fact that the key dermoscopic algorithms include streaks as one of their criteria to identify MM.6 Peripheral streaks can also be found in benign lesions; streaks are considered the hallmark of Spitz nevus, in which case they usually show symmetric distribution at the periphery of the lesion, and they can be seen infrequently in dysplastic nevus (DN).7,8 However, in clinical practice, melanocytic nevi can be vexing and present with peripheral streaks that are not symmetric, morphologically mimicking those seen in MM.

A more profound investigation of dermoscopic structures such as streaks could affect clinical practice by identifying subtle differences, or alternatively, recognizing the limitations of dermoscopy in differentiating MM and benign nevi. To that end, reflectance confocal microscopy (RCM) could be a useful tool. Reflectance confocal microscopy is a noninvasive imaging technique that allows for in vivo visual-
ization of microscopic structures and cellular details of the epidermis and superficial dermis at near histopathologic resolution.\textsuperscript{9,10} Melanin provides strong contrast to RCM images by backscattering light, causing pigmented structures to appear bright.\textsuperscript{9} Similar to dermoscopy, RCM allows for imaging of melanocytic lesions in vivo and in the en face plane,\textsuperscript{11,13} enabling direct correlation with dermoscopic images.\textsuperscript{14} The aim of the present study was to analyze dermoscopically identified streaks using direct correlation with features visualized on RCM.

**METHODS**

**PATIENTS**

Seven patients, each contributing 1 melanocytic lesion, were enrolled in the study after obtaining written consent. The research protocol was approved by the institutional review board at the Memorial Sloan-Kettering Cancer Center, New York, NY. All lesions showed peripheral streaks (as defined in the following subsection) on dermoscopy. The study included pigmented melanocytic lesions of the trunk and limbs only. Lesions on the face and palmoplantar skin were excluded because their dermoscopic patterns are distinct. Two of the cases (cases 1 and 2) were previously reported in an article describing the technique of direct dermoscopy-RCM correlation.\textsuperscript{14} The present study builds on the findings of the previous article,\textsuperscript{14} with more in-depth analysis of a specific dermoscopic structure, namely streaks.

**DERMOSCOPIC EVALUATION AND DEFINITIONS**

The clinical and dermoscopic features were documented using the Fuji S1 SLR digital camera (FujiFilm, Tokyo, Japan) and 60-mm Macro Nikkor lens (Nikon Corp, Tokyo, Japan) with an Epillum dermoscopy attachment (Canfield Imaging Systems Inc, Fairfield, NJ).\textsuperscript{14} The dermoscopic global patterns and specific structures were based on the previously described pattern analysis method.\textsuperscript{9,15} The term peripheral streaks used in the present study encompasses several morphologic structures, including radial streaming, pseudopods, and irregular extensions.\textsuperscript{9,17}

**RCM EVALUATION**

A commercially available RCM (Vivascope 1500; Lucid Inc, Rochester, NY), which has been described previously,\textsuperscript{9,15} was used. Reflectance confocal microscopy acquires horizontal tissue images with a 500-\textmu m by 500-\textmu m field of view (individual image). An automated stepper was used to scan 4 \times 4 mm sections, producing a “mosaic image.” The global pattern on dermoscopy was correlated with the mosaic image at the level of the dermoepidermal junction. In addition, an RCM automated stepper that obtains sequentially deeper individual images from the corneal layer to the superficial dermis (Z-axis stack) was used for RCM analysis of the dermoscopically identified streaks.

The method of precise dermoscopy-RCM correlation was used as previously described.\textsuperscript{14}

**HISTOPATHOLOGIC ANALYSIS**

All lesions were excised because of suspicious clinical and dermoscopic features and histopathologically analyzed. Excised tissue was fixed in formalin, processed routinely, and embedded in paraffin by usual methods for vertical sectioning of skin specimens. Vertical sections were stained with hematoxylin-eosin. Immunohistochemical stains, including A103 and HMB45, were ordered as necessary for routine dermopathological evaluation. Diagnoses were retrieved from the hospital information system, and the slides were retrieved from the pathology archive. The slides were then evaluated by the study dermatopathologist (M.G.) and scanned for findings that appeared to be the best correlates of RCM structures under analysis. This is an indirect correlation of RCM with histopathologic findings, since spatial localization on histopathologic examination of structures seen on RCM cannot be accurately achieved at present.

**RESULTS**

The Table summarizes the clinical data, dermoscopic and RCM features, histopathologic findings, and diagnosis for the studied lesions. The lesions consisted of 2 early invasive MMs, 4 DN, and 1 compound melanocytic nevus with architectural disorder and moderate atypia with spitzoid features. The latter was not a classic Spitz nevus, but because the superficial portion of the lesion showed spitzoid morphology and since both dermoscopy and RCM are used to evaluate the superficial portion of a lesion, it was designated a spitzoid lesion (SL). Of the 7 lesions, 6 were reported by the patients or identified by dermatologists, using serial digital photographs, to be new or changed lesions (Table).

Four lesions (2 MMs and 2 DNs) had a multicomponent dermoscopic global pattern (Table). The RCM mosaic image at the dermoepidermal junction correlated well with dermoscopic global pattern and showed an irregular architecture with heterogeneous brightness in all lesions (Figure 1). The RCM mosaic images at the dermoepidermal junction can be grouped into the following descriptive categories: (1) 3 of the lesions (cases 1, 3, and 7) showed a predominant meshwork pattern created by a grid of bright cords of pigmented basal cells and melanocytes (Figure 1A2, C2, and G2), and (2) 4 of the lesions (cases 2 and 4-6) harbored an interlacing pattern consisting of cords of heterogeneously bright cells that intersect and branch in a more disarrayed fashion than in the meshwork pattern (Figure 1B2, D2, E2, and F2). Two lesions—SL (case 2) and MM (case 1, at 1 pole)—showed dermoscopically a periphery almost entirely composed of streaks (Figure 1A1 and B1). On the RCM mosaic, both lesions showed well-demarcated circumferential, peripheral, bright aggregates, contiguous with the center of the lesion (Figure 1A2 and B2). In all the other lesions, the peripheral streaks on dermoscopy and corresponding structures on the RCM mosaic were seen only focally.

All lesions showed streaks with bulbous tips on dermoscopy, and in 5 of the lesions (cases 1, 2, and 5-7), the streaks were curved in shape (Table). Direct correlation of dermoscopy with RCM mosaic images enabled the identification of the RCM structures (Figure 2) that corresponded with dermoscopically identified streaks (Figure 1, white arrows). In 6 of the cases (cases 1-6), the peripheral streaks were visualized on RCM as confluent aggregates of bright cells (Figure 2A-F). The ag-
peripheral nests with variable discohesion on histopathology; RCM, reflectance confocal microscopy; RGP, reticular-globular pattern; SP, starburst pattern.

Figure 3

Table. Clinical Data, Dermoscopy, RCM, and Histopathologic Analysis of the Studied Lesions

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex/Age/ y/ Location/Size/History</th>
<th>Histopathologic Diagnosis</th>
<th>Dermoscopic Global Pattern, Peripheral Streak Morphology</th>
<th>RCM Analysis of Streaks</th>
<th>Histopathologic Analysis of the Periphery of Lesions at High Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/49/thigh/7 mm/ growing lesion</td>
<td>Melanoma with spitzoid features, Breslow thickness, 0.55 mm, Clark level 2</td>
<td>MCP (with SP area), curved and linear streaks with bulbous tips</td>
<td>Elongated, dense, bright aggregates of dendritic cells with poor cellular demarcations; aggregates curve around the DP</td>
<td>Curved or round plump nests with variable discohesion and clefting, occasionally merging. The nests are composed of large atypical spindle and epithelioid melanocytes with indistinct cell borders and abundant dusty pink cytoplasm.</td>
</tr>
<tr>
<td>2</td>
<td>M/14/dorsal foot/ 5 mm/growing lesion</td>
<td>Compound melanocytic nevus with moderate atypia and superficial spitzoid morphology</td>
<td>SP, curved streaks with bulbous tips</td>
<td>Elongated, dense, bright aggregates of cells with poor cellular demarcations; aggregates curve around the DP</td>
<td>Elongated, curved or round and sometimes merging, plump nests with variable discohesion and clefting composed of large atypical spindle and epithelioid melanocytes with indistinct cell borders and abundant dusty pink cytoplasm.</td>
</tr>
<tr>
<td>3</td>
<td>M/34/upper abdomen/ 4 mm/new lesion</td>
<td>Compound dysplastic nevus with moderate to severe atypia, bordering on early melanoma</td>
<td>MCP, linear streaks with bulbous tips</td>
<td>Elongated, dense, bright aggregates of mostly fusiform cells with poor cellular demarcations, curving around the DP</td>
<td>Elongated curved nests with variable discohesion and clefting, composed of large atypical spindle and epithelioid melanocytes with indistinct cell borders and abundant dusty pink cytoplasm.</td>
</tr>
<tr>
<td>4</td>
<td>F/43/leg/7 mm/lesion noted by physician</td>
<td>Melanoma, Breslow thickness, 0.4 mm, Clark level 2</td>
<td>MCP, linear streaks with bulbous tips</td>
<td>Short, ill-defined, bright aggregates of cells with poorly defined cellular demarcations; aggregates appear to curve around the DP and overlie dermal collagen</td>
<td>Poorly to well-formed, small to medium-sized nests, with variable discohesion and clefting, and individual atypical melanocytes fusing along DEJ in area with ill-formed rete. Large, atypical, spindle and epithelioid melanocytes with indistinct cell borders and moderate amount of dusty amorphophilic cytoplasm.</td>
</tr>
<tr>
<td>5</td>
<td>M/49/shoulder/2 mm/ lesion changed in color</td>
<td>Compound dysplastic nevus with slight atypia</td>
<td>MCP, curved streaks with bulbous tips</td>
<td>Short, ill-defined, bright aggregates of fusiform cells with poor cellular demarcations; aggregates curve around the DP</td>
<td>Small, round and curved, elongated nests along edges of rete. Atypical, round and spindled, small to medium-sized melanocytes with poorly defined cellular borders and small to moderate amount of pink to dusty pink cytoplasm.</td>
</tr>
<tr>
<td>6</td>
<td>F/37/forearm/2 mm/ new lesion</td>
<td>Nevus with architectural disorder and moderate atypia, inflamed</td>
<td>GHP, curved streaks with bulbous tips (peripheral globules are also seen)</td>
<td>Elongated, dense, bright aggregates of mostly fusiform cells with poor cellular demarcations; aggregates curve around DP, which contain numerous melanophages</td>
<td>N/A*</td>
</tr>
<tr>
<td>7</td>
<td>M/37/chest/3.5 mm/ patient noted new lesion</td>
<td>Compound dysplastic nevus with moderate atypia</td>
<td>RGP, closely aligned globules appearing as curved streaks</td>
<td>Round, dense, bright cellular nests located on the mesh, with single bright cells between the nests</td>
<td>Discrete, medium-sized nests with minimal discohesion located on sides and tips of rete, composed of medium-sized cells with a moderate amount of dusty pink to amorphophilic cytoplasm.</td>
</tr>
</tbody>
</table>

Abbreviations: DEJ, dermoepidermal junction; DP, dermal papillae; GHP, globular-homogeneous pattern; MCP, multicomponent pattern; NA, not available; RCM, reflectance confocal microscopy; RGP, reticular-globular pattern; SP, starburst pattern.

*Routine histopathologic diagnosis was performed at the Memorial Sloan-Kettering Cancer Center, but original slides were not available for further analysis at the time of study.

The peripheral aggregates correlating with peripheral streaks were mostly composed of cell aggregates with ill-defined cellular demarcations and heterogeneous brightness; in some cases there were visible nuclei or dendritic structures, confirming the presence of melanocytes in these nests. The peripheral aggregates were contiguous with the bright central part of the lesion (Figure 1A2-F2 and Figure 3A). The peripheral aggregates seen on RCM correlated with peripheral nests with variable discohesion on histopathologic analysis (Table). In 1 case (case 2), there were also dermoscopic structures that were initially perceived as peripheral globules, but RCM analysis of these structures showed confluent aggregates of bright cells that were identical to the RCM appearance of the streaks in other cases (not shown).

In 6 cases (cases 1-6), the peripheral aggregates appeared on RCM to be curving around the dermal papillae (Figure 2). Three of the lesions (cases 1-3) harbored pe-
Peripheral, elongated, curved nests on histopathologic examination (Figure 3B and D). These lesions showed elongated, dense, bright aggregates on RCM (Figure 3A and C). Two lesions with shorter, more ill-defined aggregates on RCM (cases 4-5) had smaller, more poorly formed peripheral nests on histopathologic examination.

Dermoscopic analysis of case 7 showed a few peripheral streaks (Figure 1G, white arrow) and numerous peripheral globules. However, corresponding RCM showed that the structures perceived as confluent streaks on dermoscopy were in fact discrete, dense, round nests aligned in proximity (Figure 2G). The peripheral globules seen on dermoscopy correlated with more widely spaced dense nests on RCM. The nests seen on RCM correlated on histopathologic examination with medium-sized round nests with minimal discohesion (Table, case 7).

**COMMENT**

Peripheral streaks are dermoscopic structures that prompt suspicion for MM, particularly when asymmetrically distributed. The presence of streaks is used as one of the dermoscopic criteria for the diagnosis of MM. In a study of 37 in situ MMs, irregular extensions were seen in 62% and pseudopods in 54% of the lesions. Pseudopods were found to be more frequent with increasing diameter of the MM. Peripheral streaks are also a common characteristic of Spitz nevi. In most Spitz nevi, the streaks are circumferentially distributed in a regular pattern. However, up to 25% of Spitz nevi are atypical with asymmetric distribution of streaks on dermoscopy, making differentiation from MM, which also have an irregular...
distribution of streaks, difficult. Streaks can be also seen in DN and were observed in up to 23%. Streaks, and in particular pseudopods, can at times be difficult to differentiate from peripheral globules. Such distinction may be important because peripheral globules were hypothesized to represent an active growth phase of DN, while the biological significance of streaks is yet unknown.

A handful of publications have mentioned, but did not focus on, RCM or histopathologic features of dermoscopic streaks, whereas the aim of the present study was to analyze dermoscopically identified streaks using direct RCM–indirect histopathologic correlation. The direct dermoscopy-RCM analysis of streaks was possible due to recent advances in instrumentation. In addition, our study is unique in that we included streaks across various melanocytic lesions, including MM and nevi.

In the present study, 2 lesions—SL (case 2) and MM (case 1, at 1 pole)—showed a periphery almost entirely composed of streaks. The SL appeared on initial analysis to harbor peripheral globules in addition to peripheral streaks. However, in this case, RCM showed that both streaks and globules correlated with similar confluent peripheral cellular aggregates contiguous with the center of the lesion. A retrospective closer analysis of the dermoscopic image of this case, prompted by the RCM findings, found that the structures appearing as peripheral globules were actually connected via fine extensions to the center of the lesion, confirming that this lesion harbored a true starburst pattern composed of streaks. On dermoscopy, 1 pole of the MM (case 1) showed circumferential peripheral streaks. On direct correlation of dermoscopy with RCM, these bright cellular aggregates were
elongated, broad, and curved. Our observations are in agreement with the study by Pellacani et al\textsuperscript{21} that observed well-demarcated refractile cellular aggregates at the periphery of Spitz nevi. Histopathologic analysis of both lesions (cases 1 and 2) identified spitzoid features. Taken together, these findings suggest that circumferential, peripheral, bright aggregates contiguous with the center of the lesion are the RCM attribute of dermoscopic starburst pattern and histopathologic spitzoid morphology.

Our analysis of peripheral dermoscopic streaks in 3 of the 4 DN lesions (cases 3, 5, and 6) showed well-demarcated, oval to round cellular aggregates on RCM that were contiguous with the center of the lesion. Although similar to the findings in the SL and MM, these aggregates were seen in DN only focally. In another DN (case 7), there were multiple globules throughout the lesion on dermoscopic analysis in addition to structures that appeared as peripheral streaks, which prompted the inclusion of this case in the study. However, RCM showed that these streak-like structures were in fact composed of discrete nests of melanocytes, identical to the ones perceived on dermoscopy as obvious globules but packed closely together at the lesion’s periphery with proliferation of single melanocytes between the nests. These nests were entirely different from the peripheral aggregates seen on RCM in all other lesions, and notably, such dense, round nests are considered more compatible with a benign melanocytic pattern on RCM.\textsuperscript{13} A retrospective closer look at these streak-like structures on the dermoscopic image showed that they appear as a string of indistinct beads (Figure 1G1). This case underscores the added value RCM has in refining the classification of dermoscopic structures. For all the other cases (cases 1-6), the RCM correlates of dermoscopically identified streaks showed strikingly similar patterns in the nevi and early MM. All featured bright aggregates of cells with poor cellular demarcation that were contiguous with the central part of

\textbf{Figure 3.} Case 1. The reflectance confocal microscopy (RCM) mosaic (1.5 × 1.5 mm) shows bright rete meshwork at the center (CTR) of the lesion (A), contiguous with a peripheral streak (A, white outlined square). On histopathologic examination (hematoxylin-eosin, original magnification ×40), the center (CTR) of the lesion shows pigmented keratinocytes and a proliferation of single and nested melanocytes (B, asterisks) along the edges and tips of rete ridges. The periphery (P) of the lesion shows an elongated melanocytic nest (B, arrows). On RCM, the streak appears as a bright aggregate (C, yellow asterisks) that is sharply demarcated and curves around the dermal papillae (C, white asterisks). On higher magnification (hematoxylin-eosin, original magnification ×200), the curved, elongated peripheral nest seen on histopathologic examination (D, arrows) is composed of large, atypical melanocytes with indistinct cell borders.

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Peripheral streaks were previously correlated on histopathologic examination with discrete or confluent junctional nests of pigmented, spindle-shaped melanocytes; the nests were hypothesized to form a tubular arrangement in the horizontal plane. However, it has been recognized that dermoscopic-histopathologic correlation of specific structures is complicated by the different planes of view, with dermoscopy viewing the en face plane and histopathologic analysis viewing the vertical plane (hence our term indirect correlation). A recent study of 2 MMs using transverse sectioning described the histopathologic features of peripheral streaks as neoplastic cells distributed in confluent junctional nests. We were able to identify on histopathologic examination elongated, curved nests of melanocytes at the periphery of 3 of the lesions (cases 1-3) that showed elongated, dense, bright peripheral aggregates on RCM. These findings are in line with previous reports and suggest that dermoscopic streaks can be visualized on histopathologic analysis. Notably cases 1 and 2 showed circumferential streaks (starburst pattern), thus having a higher probability of capturing the longitudinal axis of a peripheral streak on histopathologic examination. In contrast, we identified smaller, more poorly formed peripheral nests in 2 cases (cases 4 and 5), and on RCM, these cases also showed shorter, more ill-defined peripheral aggregates (compared with cases 1-3). Although the horizontal section would be the more optimal histopathologic correlate for RCM analysis, it is not practical as long as the gold standard for diagnosis is histopathologic analysis of vertical sections. We therefore suggest that the RCM–vertical histopathologic correlation, although indirect, may indeed be a useful tool for understanding the 3-dimensional morphology of dermoscopic structures.

For MM and even for nevi that are increasing in diameter, peripheral streaks might represent the “tumor front,” since radial spread may require the tumor to infiltrate and alter surrounding epidermal and/or dermal architecture. Although the vertical growth phase of MM has been studied extensively, there is a paucity of studies regarding the radial growth phase. The evaluation of streaks via RCM, which views the en face plane, provides a unique opportunity to evaluate the horizontal growth of melanocytic lesions. An interesting RCM finding is that the peripheral cellular aggregates that compose the streaks appear to contour from rete ridge to rete ridge around the dermal papillae rather than uniformly infiltrating the entire basal layer of the epidermis including the suprapapillary plates or obliterating the dermal papillae. On histopathologic analysis, this corresponds with the proliferation of melanocytes at the sides and tips of the rete ridges rather than over the suprapapillary plates. This curving pattern may account for the pseudopod shape of streaks that is often seen on dermoscopy. One could hypothesize that since the dermal papillae contain essential vasculature, the tumor curves around, “respecting” this array so as not to disrupt vascular supply. The basement membrane has been shown to serve as a platform that guides the tumor spread pattern. Further, exposed and cryptic domains in stromal protein were shown to guide MM cell migration. The pattern of growth observed by RCM in early MM and in nevi with streaks and the possible interaction with underlying matrix warrant further evaluation.

In conclusion, direct dermoscopy-RCM correlation is a feasible method to study specific dermoscopic structures. Reflectance confocal microscopy allows for a high-resolution analysis of dermoscopic structures, which may help to improve the classification methods used in dermoscopy. Peripheral streaks seen on dermoscopy appear as confluent, bright cellular aggregates on RCM. Interestingly, we did not recognize in the present series of cases distinguishing characteristics on RCM that could differentiate between the peripheral streaks of MM and nevi. Additional studies with larger series are needed to confirm our findings and may help elucidate the morphologic and biological nature of peripheral streaks.

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Author Contributions: Dr Scope had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Scope and Marghoob. Acquisition of data: Scope, Gill, and Benveuto-Andrade. Analysis and interpretation of data: Scope, Gill, Benveuto-Andrade, Halpern, Gonzalez, and Marghoob. Drafting of the manuscript: Scope, Gill, Benveuto-Andrade, and Gonzalez. Critical revision of the manuscript for important intellectual content: Gill, Halpern, Gonzalez, and Marghoob. Administrative, technical, and material support: Gill and Benveuto-Andrade. Study supervision: Halpern and Marghoob.
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REFERENCES


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Correction

Error in Byline and Author Contributions. In the Study by Scope et al titled “Correlation of Dermoscopy With In Vivo Reflectance Confocal Microscopy of Streaks in Melanocytic Lesions,” published in the June 2007 issue of the Archives (2007;143[6]:727-734), the last name of the third author in the byline on page 727 and in the Author Contributions on page 733 was misspelled. The author’s name should have read as follows: Cristiane Benvenuto-Andrade, MD.