Nodular skin lesions: correlation of reflectance confocal microscopy and optical coherence tomography features

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Abstract

Background Nodular lesions have common clinical appearance but different prognoses. Differential diagnosis between melanoma (MM), basal cell carcinoma (BCC) and dermal naevus (DN) poses a challenge in clinical practice. Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) are promising non-invasive imaging techniques, potentially able to decrease redundant biopsies. RCM allows in vivo visualization of skin down to the papillary dermis at almost histological resolution, while OCT, particularly dynamic OCT (D-OCT), provides images deeper within the dermis and reveals the vascular pattern.

Objectives To identify correlating features observed with RCM and OCT associated with the different nodular lesion diagnoses.

Methods We retrospectively assessed 68 nodular lesions (30 MM, 20 BCC and 18 DN) with RCM and subsequently OCT. At the end of the study, evaluations were matched with histopathological diagnosis and statistical analysis was performed.

Results In MM, 57% (17/30) evidenced both cerebriform nests at RCM and icicle-shaped structures at OCT, with higher average Breslow index. In 80% of BCCs with basaloid islands at RCM, OCT showed ovoid structures. More than half of DN (56%) showed hyporeflective nests at OCT and either dense nests or dense and sparse nests at RCM.

Conclusions The combined use of RCM and OCT offers a better understanding of the morphological architecture of nodular lesions, correlating RCM parameters with OCT and vice versa, assisting in turn with early differential diagnosis of malignant and benign nodular lesions. The correlation between icicle-shaped structures and cerebriform nests in MM and their association with Breslow index requires future research.

Introduction

Nodular lesions have a common clinical appearance but very different prognostic implications. The differential diagnosis between malignant and benign nodular lesions is a challenge in everyday clinical practice. The malignant melanoma (MM) nodular variant expresses a vertical phase of growth and often has relatively high Breslow indexes,¹² so a timely correct diagnosis is crucial for correct lesion management. Conversely, non-melanoma skin cancer (NMSC) rarely impacts on patient mortality and is most frequently observed in the form of a basal cell carcinoma (BCC), a locally aggressive, low-grade neoplasia, prevalent among sun-damaged elders.³⁴ Nodular lesions therefore include MM, BCC and benign lesions, such as dermal naevi (DN).

The efficacy of dermoscopy to distinguish between MM, BCC and DN with a nodular outward appearance, as compared to flat lesions, is limited.⁵⁶ As a result, unnecessary biopsy, misdiagnosis or surgical excision of benign nodules is frequent.
Reflectance confocal microscopy (RCM), a non-invasive imaging technique that enables in vivo horizontal scanning of skin lesions at nearly histological resolution, has been proven to assist in routine evaluation of diagnostically challenging nodular lesions. However, given RCM’s limited imaging depth (200 μm), it’s diagnostic performance can be compromised by the hyperkeratosis shown by many protruding nodules and the presence of ulceration in aggressive lesions.

| Table 1 | Reflectance confocal microscopy (RCM) definition of morphological criterion and vascularization |
|---------|---------------------------------------------------------------------------------------------|
| **Morphological criterion** | **Definition** |
| Pagetoid cells | Solitary and bright cells, found within the epidermis, with round or plump oval nuclei, abundant pale cytoplasm and a tendency towards spreading upward. Found in several cancerous conditions, melanoma in situ among them |
| Atypical cells at DEJ | Large and roundish or pleomorphic melanocytes with hyperreflective cytoplasm and dark nucleus and irregular in size, shape and reflectivity. Found within the basal layer |
| Erosion/Ulceration | Dark and sharply demarcated areas with irregular outlines, containing cellular debris and amorphous material |
| Dense nests | Roundish/void clusters of reflecting melanocytes, compactly arranged in sharp margined structures within the dermis |
| Dense and sparse nests | Roundish/void, sharp margined dermal clusters of reflecting melanocytes, not tightly aggregated and with visible internal cellular contours |
| Basaloid islands | Tightly packed aggregates with peripheral palisading and lobulated shape, outlined by a dark halo (clefing) |
| Cerebriform nests | Confluent amorphous aggregates of hyporeflective cells with ill-defined borders, granular cytoplasm and lacking evident nuclei. Fine hyporeflective ‘fissures’ give them a brain-like appearance |
| **Vascularisation** | |
| Inside tumour | Dilated vessels running within tumoural nests. Seen in intradermal benign naevi (DN) |
| Centre on tumour | Telangiectatic horizontal vessels, often enlarged and branched, located superficially in the dermis and between tumour islands. The slow flow of blood sometimes allows visualization of white blood cells as small round bright structures hugging the lumen wall. Typically found in basal cell carcinoma (BCC) |
| Chaotic | Enlarged tortuous and polymorphous vessels, with an irregular and chaotic distribution. Indicator of prominent neovascularization in melanoma |

| Table 2 | Optical coherence tomography (OCT) definition of morphological criterion and vascularization |
|---------|---------------------------------------------------------------------------------------------|
| **Morphological criterion** | **Definition** |
| Icicle-shaped structures | Hyperreflective, more or less large vertical structures, resembling icicles with their peak in the reticular dermis. They are dense infiltrates of lymphocytes and cancerous cells and are a striking, exclusive architectural attribute of MM |
| Ovoid structures | Hyperreflective round or ovoid structures appearing as dark islands in the dermis, either connected to the epidermis or solitary and well demarcated. Typical of BCC |
| Hyporeflective nests | Well defined dark nests of naevus cells in the papillary or superficial reticular dermis of DN |
| Vessel shape | |
| Dot | Small red points corresponding to vertically oriented vessels |
| Blob | Larger red globules with round or oval shape corresponding to vertically oriented vessels |
| Coiled | Spiraliform or convoluted red lines or circles |
| Line | Fine linear red structures |
| Curved | Comma-like red structures |
| Serpiginous | Tortuous red structures |
| Vessel pattern | |
| Mottled | Dots, blobs or coil-shaped vessels assembled in limited and sharply defined areas |
| Mesh | Linear vessels arranged in a reticular and intertwined disposition |
| Branching (arborising) | Linear, curved or serpiginous vessels branching with a progressive thinning in subsequent vascular ramifications |
| Branching (bulging) | Linear, curved or serpiginous vessels with swollen protrusions originating from the main trunk |
| Vessel distribution | |
| Regular | Homogenously organized vessels with the same pattern throughout the OCT image |
| Irregular | Vessels arranged in a chaotic fashion, with different shapes and patterns randomly coexisting |
| Melanoma n = 30 | Pagetoid cells (RCM) | Erosion/ulceration (RCM) | Atypical cells DEJ (RCM) |
|-----------------|-------------------------|---------------------------|------------------------|
|                 | Absent | Focalised | Abundant | 26 (86.7) | P-value | Absent | Present | 21 (70.0) | P-value | Absent | Focalised | Abundant | 26 (86.7) | P-value |
|                 | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % |
| Epidermal thickness (OCT) | Normal | 0 | 0 | 0 | 3 | 11.5 | 0.886 | 2 | 22.2 | 1 | 4.8 | 0.200 | 0 | 0 | 0 | 0 | 3 | 11.5 | 0.886 |
| | Thickenened | 0 | 0 | 0 | 0 | 3 | 11.5 | 0 | 0 | 0 | 3 | 14.3 | 0 | 0 | 0 | 0 | 3 | 11.5 |
| | Thinned | 1 | 100 | 3 | 100 | 20 | 76.9 | 7 | 77.8 | 17 | 81 | 1 | 100 | 3 | 100 | 20 | 76.9 |
| Hyperkeratosis/ crust (OCT) | Absent | 1 | 100 | 3 | 100 | 22 | 84.6 | 0.701 | 8 | 88.9 | 18 | 85.7 | 0.815 | 1 | 100 | 3 | 100 | 22 | 84.6 | 0.701 |
| | Present | 0 | 0 | 0 | 0 | 4 | 15.4 | 1 | 11.1 | 3 | 14.3 | 0 | 0 | 0 | 0 | 4 | 15.4 |
| Icicle-shaped structures (OCT) | Absent | 0 | 0 | 0 | 0 | 10 | 38.5 | 0.315 | 4 | 44.4 | 6 | 28.6 | 0.398 | 0 | 0 | 1 | 33.3 | 9 | 34.6 | 0.771 |
| | Present | 1 | 100 | 3 | 100 | 16 | 61.5 | 4 | 44.4 | 15 | 71.4 | 1 | 100 | 2 | 66.7 | 17 | 65.4 |
| Vessel shape type (OCT) | Not evaluable | 0 | 0 | 0 | 0 | 1 | 3.8 | 0.528 | 0 | 0 | 0 | 0 | 0.596 | 0 | 0 | 0 | 0 | 1 | 3.8 | 0.392 |
| | Dot | 0 | 100 | 2 | 66.7 | 5 | 19.2 | 3 | 33.3 | 6 | 28.6 | 1 | 100 | 3 | 100 | 4 | 15.4 |
| | Blob | 0 | 0 | 1 | 33.3 | 1 | 3.8 | 0 | 0 | 2 | 9.5 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| | Coiled | 0 | 0 | 0 | 0 | 2 | 7.7 | 0 | 0 | 2 | 9.5 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| | Line | 0 | 0 | 0 | 0 | 2 | 7.7 | 1 | 11.1 | 1 | 4.8 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| | Curved | 0 | 0 | 0 | 0 | 3 | 11.5 | 1 | 11.1 | 2 | 9.5 | 0 | 0 | 0 | 0 | 3 | 11.5 |
| | Serpiginous | 0 | 0 | 0 | 0 | 12 | 46.2 | 4 | 44.4 | 8 | 38.1 | 0 | 0 | 0 | 0 | 12 | 46.2 |
| Vessel pattern type (OCT) | Not evaluable | 0 | 0 | 0 | 0 | 1 | 3.8 | 0.545 | 1 | 11.1 | 0 | 0 | 0.185 | 0 | 0 | 0 | 0 | 1 | 3.8 | 0.545 |
| | Mottled | 1 | 100 | 3 | 100 | 8 | 30.8 | 2 | 22.2 | 10 | 47.6 | 1 | 100 | 3 | 100 | 8 | 30.8 |
| | Mesh | 0 | 0 | 0 | 0 | 3 | 11.5 | 2 | 22.2 | 1 | 4.8 | 0 | 0 | 0 | 0 | 3 | 11.5 |
| | Branching (arborising) | 0 | 0 | 0 | 0 | 13 | 50 | 4 | 44.4 | 8 | 38.1 | 0 | 0 | 0 | 0 | 12 | 46.2 |
| | Branching (bulging) | 0 | 0 | 0 | 0 | 2 | 7.7 | 0 | 0 | 2 | 9.5 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| Vessel distribution (OCT) | Regular | 1 | 100 | 3 | 100 | 2 | 7.7 | <0.001 | 2 | 22.2 | 4 | 19 | 0.842 | 1 | 100 | 3 | 100 | 2 | 7.7 | <0.001 |
| | Irregular | 0 | 0 | 0 | 0 | 24 | 92.3 | 7 | 77.8 | 17 | 81 | 0 | 0 | 0 | 0 | 24 | 92.3 |

**Melanoma n = 30**

| Tumour architecture (RCM) | Dense nests | Dense/Sparse nests | Cereblrifom nests | P-value | Insiders tumour | Centre tumour | Chaotic tumour | P-value |
|---------------------------|-------------|-------------------|------------------|---------|----------------|---------------|---------------|---------|
| Insiders tumour | 3 (10.0) | N | % | N | % | N | % | N | % | N | % | N | % | N | % |
| Epidermal thickness (OCT) | Normal | 1 | 25 | 0 | 0 | 2 | 11 | 0.685 | 0 | 0 | 0 | 0 | 3 | 12 | 0.041 |
| | Thickenened | 0 | 0 | 1 | 12.5 | 2 | 11 | 0 | 0 | 1 | 100 | 2 | 7.7 | 3 | 100 | 21 | 80.8 |
| | Thinned | 3 | 75 | 7 | 87.5 | 14 | 78 | 0 | 0 | 0 | 0 | 24 | 92.3 | 7 | 77.8 | 17 | 81 |
| Hyperkeratosis/ crust (OCT) | Absent | 3 | 75 | 8 | 100 | 15 | 83 | 0.392 | 2 | 66.7 | 0 | 0 | 24 | 92.3 | 0.016 |
| | Present | 1 | 25 | 0 | 0 | 3 | 17 | 0 | 0 | 1 | 100 | 2 | 7.7 | 1 | 33.3 | 1 | 100 | 2 | 7.7 |
| Icicle-shaped structures (OCT) | Absent | 4 | 100 | 5 | 62.5 | 1 | 5.6 | <0.001 | 1 | 33.3 | 0 | 0 | 9 | 35 | 0.771 |
| | Present | 0 | 0 | 3 | 37.5 | 17 | 94 | 2 | 66.7 | 1 | 100 | 8 | 65 | 2 | 66.7 | 1 | 100 | 8 | 65 |
| Vessel shape type (OCT) | Not evaluable | 0 | 0 | 0 | 0 | 1 | 5.6 | 0.075 | 0 | 0 | 0 | 0 | 1 | 3.8 | 0.539 |
| | Dot | 1 | 25 | 4 | 50 | 3 | 17 | 1 | 33.3 | 0 | 0 | 7 | 27 | 0 | 0 | 0 | 0 | 7 | 27 |
| | Blob | 0 | 0 | 1 | 12.5 | 1 | 5.6 | 0 | 0 | 0 | 0 | 2 | 7.7 | 0 | 0 | 2 | 7.7 |
| | Coiled | 0 | 0 | 0 | 0 | 7.7 | 2 | 11 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| | Line | 2 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 7.7 | 0 | 0 | 0 | 0 | 2 | 7.7 |
| | Curved | 0 | 0 | 1 | 12.5 | 2 | 11 | 0 | 0 | 1 | 100 | 2 | 7.7 | 0 | 0 | 1 | 100 | 2 | 7.7 |
| | Serpiginous | 1 | 25 | 2 | 25 | 9 | 30 | 0 | 0 | 0 | 0 | 10 | 39 | 0 | 0 | 0 | 0 | 10 | 39 |
Additional diagnostic information may be obtained using optical coherence tomography (OCT), a non-invasive imaging tool that produces horizontal (enface) and transversal images with higher penetration depth (up to 2 mm).\textsuperscript{13,14} In addition to structural images of the skin, the use of the dynamic OCT (D-OCT) technique allows the visualization of vascular structures.\textsuperscript{15–17} However, OCT images have an inferior resolution compared to RCM scans, which does not enable visualization of the individual cells but of architectural changes only\textsuperscript{18,19} and the frequent presence of projection artefacts, caused by interference with the signal from the tissue directly below vessels or by the blood flow in the vessel, especially in deeper layers, can interfere with image clarity.\textsuperscript{20}

In vivo OCT images of human skin show a strong scattering in tissues with a few layers and some optical inhomogeneities and, most importantly, enable the visualization of architectural changes only, not of single cells.\textsuperscript{18} Differential diagnosis between MM and DN using OCT alone is extremely difficult, since the resolution is not high enough to define single cells.\textsuperscript{19,21}

The use of both RCM and OCT provides clinicians with two complementary sets of data,\textsuperscript{22,23} which can assist in differential diagnosis of nodular lesions for correct identification of MM, BCC and DN, decreasing the frequency of unnecessary excisions. As there are no studies of the correlation between the features observed with these two innovative imaging techniques, the current study aims to identify correlating features observed with RCM and OCT associated with different nodular lesion diagnoses.

**Materials and methods**

A retrospective selection of nodular lesions, defined as palpable lesions superficially located with a diameter \(> 0.5 \text{ cm}\), and not including subcutaneous originating lesions,\textsuperscript{12} regardless of histological diagnosis, was performed. A dedicated database at the Department of Dermatology, University of Modena and Reggio Emilia, was searched for registrations between January 2014 and December 2018. Inclusion criteria for the current study required a complete set of images (clinical, dermoscopic, RCM, OCT and histological). The study was approved by the local Ethics Committee (Committee Prot. No. 458/2018/OSS/AOUIMO), and the investigation was conducted in accordance with the Declaration of Helsinki.

Two dermatologists (F.G., S.M.), blinded to histopathological diagnosis and dermoscopic images, independently assessed all RCM, and subsequently all OCT images for the selected lesions. Where discrepancies arose, a third dermatologist was consulted (F.F.). Assessors were not requested to perform a diagnosis, but to identify the presence or absence of key morphological features associated with MM, BCC and DN, as identified from research of literature, outlined below.

The evaluated area is \(8 \times 8 \text{ mm}\) with RCM and \(6 \times 6 \text{ mm}\) with OCT; the entire lesions were included in the acquired area, and we subsequently evaluated possible correlations among different parameters from a statistical analysis.

**RCM assessment**

Reflectance confocal microscopy assessment included the evaluation of a complete set of at least three Viva-Block images (Vivascope1500; Mavig, GmbH, Munich, Germany) of the epidermal layer, dermo-epidermal junction (DEJ) and upper dermis, according to predefined RCM criteria (Table 1). The presence and distribution of pagetoid cells, ulceration, atypical cells in the DEJ, the presence of dense nests, dense and sparse nests,\textsuperscript{7} basa-loid islands\textsuperscript{24} or cerebriform nests\textsuperscript{2} and the type of vascularization were evaluated.

**D-OCT assessment**

All lesions were assessed with the VivoSight\textsuperscript{®} D-OCT (Michelson Diagnostics Ltd, Maidstone Kent, UK) according to

| Melanoma \(n = 30\) | Tumour architecture (RCM) | Vascularization (RCM) |
|----------------------|----------------|---------------------|
|                      | Dense nests 4 (13.3) | Dense/Sparse Nests 8 (26.73) | Cerebriform Nests 18 (60.0) |
|                      | \(N\) | % | \(N\) | % | \(N\) | % |
| Vessel pattern type (OCT) | Not evaluable | 0 | 0 | 0 | 1 | 5.6 | 0.184 |
|                      | Mottled | 1 | 25 | 5 | 62.5 | 6 | 33 |
|                      | Mesh | 2 | 50 | 0 | 0 | 1 | 5.6 |
|                      | Branching (arborising) | 1 | 25 | 3 | 37.5 | 8 | 44 |
|                      | Branching (bulging) | 0 | 0 | 0 | 0 | 2 | 11 |
| Vessel distribution (OCT) | Regular | 0 | 0 | 4 | 50 | 2 | 11 | 0.041 |
|                      | Irregular | 4 | 100 | 4 | 50 | 16 | 89 |
|                      | Inside tumour 3 (10.0) | 0 | 0 | 0 | 1 | 3.8 | 0.691 |
|                      | Centre tumour 1 (3.3) | 1 | 33.3 | 0 | 0 | 11 | 42 |
|                      | Chaotic 26 (86.7) | 0 | 0 | 0 | 0 | 3 | 12 |

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Table 4  Basal cell carcinoma (BCC) results: correlation of RCM and OCT parameters

| BCC n = 20 | Pagetoid cells (RCM) | Erosion/Ulceration (RCM) | Tumour architecture (RCM) | Vascularization (RCM) |
|------------|----------------------|--------------------------|--------------------------|----------------------|
|            | Absent               | Focalised                | Abundant                 | Absent               | Present               | Basaloid islands     | Centre tumour         | Chaotic 1 (50)       | P-value |
| Basal       | 17 (85.0)            | 2 (10.0)                 | 2 (10.0)                 | 12 (60.0)            | 8 (40)               | 20 (100)             | 19 (95.0)             | 1 (5.0)             | 0.292  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                  | P-value  |
| N          | 3 17.6               | 2 (10.0)                 | 12 (70.6)                | 8 66.7               | 6 75                 | 14 (70)              | 13 (68.4)             | 1 0.054             |          |
| %          | 100                  | 50                       | 100                      | 100                  | 100                  | 100                  | 100                   | 100                 |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Hyperkeratosis/crust (OCT) | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 17 (100)            | 1 (100)                  | 2 (100)                  | 12 (100)            | 8 (100)             | 20 (100)             | 19 (100)             | 1 (100)             | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                  | P-value  |
| N          | 2 11.8               | 1 (100)                  | 1 50                     | 3 25                 | 1 12.5               | 4 20                  | 4 21.1                | 0 0                  | 0.054  |
| %          | 100                  | 100                      | 50                       | 12.5                 | 12.5                 | 20                    | 20                    | 0                    |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Ovoid       | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 17 (100)            | 0 (0)                    | 2 (100)                  | 12 (100)            | 8 (100)             | 20 (100)             | 19 (100)             | 1 (100)             | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                  | P-value  |
| N          | 2 11.8               | 0 0                      | 1 50                     | 3 25                 | 1 12.5               | 4 20                  | 4 21.1                | 0 0                  | 0.054  |
| %          | 100                  | 0                       | 50                       | 12.5                 | 12.5                 | 20                    | 20                    | 0                    |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Icicle-shaped structures (OCT) | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Absent | Present | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 17 (100)            | 1 (100)                  | 2 (100)                  | 13 108.3             | 8 100               | 20 (100)             | 19 (100)             | 1 (100)             | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                  | P-value  |
| N          | 0 0 0               | 1 100                    | 0 0                      | 0 0                   | 0 0                   | 0 0                   | 0 0                    | 0 0                  | 0.054  |
| %          | 0                    | 100                      | 0                        | 0                    | 0                    | 0                    | 0                      | 0                    |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Vessel shape type (OCT) | Dot | Blob | Coiled | Line | Curved | Serpiginous | Not evaluable | Mottled | Mesh | Branching (arborising) | Branching (bulging) | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 2 11.8               | 1 (100)                  | 1 50                     | 5 47.1               | 0 0                  | 1 50                 | 3 17.6                 | 3 17.6               | 1 (100)            | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                   | No                  | P-value  |
| N          | 1 50                 | 0 0                      | 1 50                     | 5 47.1               | 0 0                  | 1 50                 | 3 17.6                 | 3 17.6               | 1 (100)            | 0.054  |
| %          | 50                   | 0                        | 50                       | 47.1                 | 0                    | 50                   | 17.6                   | 17.6                 | 100                 |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Vessel pattern type (OCT) | Not evaluable | Mottled | Mesh | Branching (arborising) | Branching (bulging) | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 3 17.6               | 1 100                    | 1 50                     | 6 58.8               | 0 0                  | 1 50                 | 3 17.6                 | 3 17.6               | 1 (100)            | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                   | No                  | P-value  |
| N          | 1 50                 | 0 0                      | 1 50                     | 6 58.8               | 0 0                  | 1 50                 | 3 17.6                 | 3 17.6               | 1 (100)            | 0.054  |
| %          | 50                   | 0                        | 50                       | 58.8                 | 0                    | 50                   | 17.6                   | 17.6                 | 100                 |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Vessel pattern distribution (OCT) | Regular | Irregular | Regular | Irregular | Regular | Irregular | Regular | Irregular | Regular | Irregular | Basaloid islands | Centre tumour | Chaotic 1 (50) | P-value |
| Basal       | 17 100               | 0 0                      | 2 100                    | 12 100               | 7 87.5               | 0.209                | 19 95                 | 18 94.7             | 1 (100)            | 0.054  |
| n          | No                   | No                       | No                       | No                   | No                   | No                   | No                    | No                   | No                  | P-value  |
| N          | 0 0 0               | 1 100                    | 0 0                      | 0 0                  | 1 12.5               | 1 (5)                | 1 5                    | 1 5                  | 1 (100)            | 0.054  |
| %          | 0                    | 100                      | 0                        | 0                    | 12.5                 | 100                  | 100                   | 100                  | 100                 |          |
| P-value     | 0.054                | 0.054                    | 0.054                    | 0.054                | 0.054                | 0.054                | 0.054                 | 0.054               |          |
| Naevi               | Pageoid cells (RCM) | Erosion/Ulceration (RCM) | Atypical cells DEJ (RCM) | Tumour architecture (RCM) | Vascularization (RCM) |
|--------------------|---------------------|--------------------------|--------------------------|---------------------------|----------------------|
|                    | Absent | Focalised | P-value | Absent | Focalised | P-value | Absent | Focalised | P-value | Absent | Focalised | P-value | Absent | Focalised | P-value | Absent | Focalised | P-value |
| n = 18             | N %     | N %       | P-value  | N %     | N %       | P-value  | N %     | N %       | P-value  | N %     | N %       | P-value  | N %     | N %       | P-value  | N %     | N %       | P-value  |
| Epidermal thickness (OCT) | Normal | 0 0 0 0 | 0.396 | 0 0 0 0 | 0.021 | 0 0 0 0 | 0.180 | 0 0 0 0 | 0.090 | 0 0 0 0 | 0.004 |
|                    | Thickened | 13 86.7 2 66.7 | 15 88.2 0 0 | 14 87.5 1 50 | 7 70 8 100 | 15 93.8 0 0 |
|                    | Thinned | 2 13.3 1 33.3 | 2 11.8 1 100 | 2 12.5 1 50 | 3 30 0 0 | 1 6.3 1 100 1 100 |
| Hyperkeratosis /crust (OCT) | Absent | 15 100 3 100 | 17 100 1 100 | 16 100 2 100 | 0.025 | 10 100 8 100 | 16 100 1 100 1 100 |
|                    | Present | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Hypore-reflective nests (OCT) | Absent | 7 46.7 1 33.3 | 0.671 | 7 41.2 1 100 | 0.250 | 7 43.8 1 50 | 0.867 | 6 60 2 25 | 0.138 | 6 37.5 1 100 1 100 0.245 |
|                    | Present | 8 53.3 2 66.7 | 10 58.8 0 0 | 9 56.3 1 50 | 4 40 6 75 | 10 62.5 0 0 |
| Icicle-shaped structures (OCT) | Absent | 15 100 3 100 | 17 100 1 100 | 16 100 2 100 | 10 100 8 100 | 16 100 1 100 1 100 |
|                    | Present | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Vessel shape type (OCT) | Not evaluable | 0 0 0 0 | 0.615 | 0 0 0 0 | 0.912 | 0 0 0 0 | 0.771 | 0 0 0 0 | 0.691 | 0 0 0 0 | 0.455 |
|                    | Dot | 9 60 3 100 | 11 64.7 1 100 | 10 62.5 2 100 | 7 70 5 62.5 | 11 68.8 1 100 0 0 |
|                    | Blob | 1 6.7 0 0 | 1 5.9 0 0 | 1 6.3 0 0 | 1 10 0 0 | 1 6.3 0 0 0 0 |
|                    | Coiled | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 0 0 |
|                    | Line | 3 20 0 0 | 3 17.6 0 0 | 3 18.8 0 0 | 1 10 2 25 | 2 12.5 0 0 1 100 |
|                    | Curved | 2 13.3 0 0 | 2 11.8 0 0 | 2 12.5 0 0 | 1 10 1 12.5 | 2 12.5 0 0 0 0 |
|                    | Serpiginous | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Vessel pattern type (OCT) | Not evaluable | 0 0 0 0 | 0 0 0 0 | 0.467 | 0 0 0 0 | 0.596 | 0 0 0 0 | 0.737 | 0 0 0 0 | 0.282 |
|                    | Mottled | 10 66.7 2 66.7 | 11 64.7 1 100 | 11 68.8 1 50 | 7 70 5 62.5 | 11 68.8 1 100 0 0 |
|                    | Mesh | 5 33.3 1 33.3 | 6 35.3 0 0 | 5 31.3 1 50 | 3 30 3 37.5 | 5 31.3 0 0 1 100 |
|                    | Branching (arborising) | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
|                    | Branching (bulging) | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
| Vessel distribution (OCT) | Regular | 15 100 3 100 | 17 100 1 100 | 16 100 2 100 | 10 100 8 100 | 16 100 1 100 1 100 |
|                    | Irregular | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 | 0 0 0 0 |
predefined OCT criteria\textsuperscript{13,14,21,25} (Table 2). A “multi-slice” area (6 × 6 mm) is created by the device and includes 120 cross-sectional/transversal OCT images. Images (6 mm wide) have an optical resolution of 7.5 μm in the lateral direction and 5 μm in the axial direction.

Vessel morphology was assessed and classified on enface D-OCT images captured by the VivoSight. The enface D-OCT image comprises a standard (structural) OCT data at the assessors’ selected depth, with a red overlay representing vessels; the intensity of the red colour corresponds to the strength of the D-OCT signal. All lesions were examined at a depth of 300 μm, previously proven to enable the most comprehensive appraisal of morphological features.\textsuperscript{20} Lesion vascular shape and pattern were assessed according to Ulrich’s classification.\textsuperscript{26}

**Statistics**

All RCM features were correlated with OCT parameters according to Pearson’s chi-square test, or in the cases of <5 observations, the Fisher exact test was used. Statistical analysis was performed using STATA\textsuperscript{®} software version 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.). For all tests, a $P < 0.05$ was considered statistically significant.

**Results**

A total of 68 nodular lesions met inclusion criteria. Selected lesions, according to histopathological assessment, included 30 MMs, 20 BCCs and 18 DN. The mean Breslow index of included MMs was 1.90 mm (0.25–5.5, ±1.4).

**Correlation of RCM and OCT features: MM**

All RCM and OCT features identified for MMs were correlated and are outlined in Table 3. Pagetoid cells were found to be significantly associated with an irregular distribution of vessels ($P < 0.001$). Twenty-six of the 30 lesions showed an abundant distribution of pagetoid cells throughout the RCM image. Atypical cells at the DEJ were correlated with a thinning of the epidermis (24/29; NS) and significantly correlated with the presence of an irregular distribution of vessels ($P < 0.001$). A chaotic vascular network at RCM was significantly associated with a thinning of the epidermis ($P = 0.041$). Additionally, a serpiginous vessel shape in OCT was identified in almost half of the lesions with abundant pagetoid cells (46.2%; NS). Cerebriform nests were observed in 18 MMs (60.0%) of which 17 (94.4%) also exhibited icicle-shaped structures ($P < 0.001$).

Furthermore, a greater average Breslow thickness for MMs with both icicle-shaped structures (OCT) and cerebriform nests (RCM) compared to the average Breslow thickness of all MM lesions was observed (2.4 mm [0.25–5.5, ±1.5] vs 1.90 mm [0.25–5.5, ±1.4]; $P < 0.05$).

Most MMs (26/30; 86.7%) expressed a chaotic vascularization at RCM and were predominately associated at OCT with serpiginous (10) or dotted shape (7) vessels.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Melanoma. With enface OCT (a), a serpiginous vessel shape, with an arborizing vessel pattern and an irregular distribution in the frame of observation was frequently observed. In RCM (b), pagetoid cells spreading in the epidermal layer with numerous cerebriform nest (white asterisk) and chaotic vessels (red arrows) (c) were often observed. In OCT transversal section (d), we observed icicle-shaped structures (white square) and cerebriform nests (white asterisk) and vessels (red arrows) (e).
Correlation of RCM and OCT features: BCC

The correlation between BCC RCM and OCT features is outlined in Table 4. The absence of pagetoid cells in RCM was confirmed and was significantly associated with the presence of ovoid structures (NS) and a regularly arranged vascular distribution in OCT ($P < 0.001$). Furthermore, in 80% of the BCC lesions with basaloid islands identified at RCM, ovoid structures were observed in OCT. Most BCC lesions featured vessels centred on tumour (95.0%) at RCM and a linear or curved vessel shape (76.5%) and branching arborizing vessel pattern (58.8%) at OCT.

Correlation of RCM and OCT features: DN

Reflectance confocal microscopy and OCT feature correlations for DN is outlined in Table 5. The absence at RCM of pagetoid cells, erosion or ulceration and atypical cells was associated with the observation of epidermal thickening in OCT ($P = 0.021$, $P = 0.396$ and $P = 0.18$, respectively). Either mottled or mesh vessel patterns were observed at OCT for all DN, organized in a regular distribution (100%). Hyporeflective nests at OCT were observed in more than half of DN (10/18; 56%) and were associated with dense and sparse nests observed in RCM.

Discussion

RCM has been proven to be a useful tool in the diagnosis of MM and BCC, with diagnostic sensitivity and specificity of 96.5% and 94.1% for MM$^{12}$ and 97.1%, and 78.95% for BCCs. The main limitations of RCM technology include its reduced penetration depth and loss of resolution in cases of ulceration or hyperkeratosis, making its application in nodular lesions less effective.$^{12}$ OCT, a novel non-invasive diagnostic tool, allows the visualization of deeper skin structures at lower image resolution compared to RCM.$^{13}$ However, differential diagnosis between MM and DN with OCT alone seems difficult, due to the resolution of OCT not enabling the definition of single cells.$^{21,27}$ Another limitation of D-OCT is the...
frequent presence of projection artefacts, caused by interference with the signal from the tissue directly below vessels or by the blood flow in the vessel, especially in deeper layers. However, artefacts are easily recognizable, and D-OCT remains one of the most relevant techniques for the visualization of the global vessel distribution and vascular architecture in cutaneous lesions. In fact, despite these limitations, initial studies suggest that OCT assists in improving the diagnostic accuracy of various skin lesions. While several OCT morphological features have yet to be associated with particular diagnoses, some parameters have been established as hallmarks of MM (marked architecture disarray, icicle-shaped structures). Ferrante di Ruffano et al. in their latest review were unable to establish OCT vascular criteria for the differential diagnosis of MM and BCC.

Lesions with atypical cells at the DEJ observed at RCM also had a thinned epidermis in OCT. This association reflects the growing behaviour of a tumour mass located in the dermis that pushes up and erodes the superficial layers of the skin.

Of particular interest, results highlight that almost all (17 out of 18) MMs with cerebriform nests at RCM also showed icicle-shaped structures at OCT (Fig. 1). Gambichler et al. suggest that the icicle structure is an expression of the vertical infiltration phase by dermal infiltrates of melanocytes. The authors of the current study hypothesize that this feature could also be justified by some type of “shadow cone” effect produced by cerebriform nests in the underlying portion of the OCT image (Fig. 2). This observation supports the hypothesis of a correspondence between morphological features from both techniques: icicle-shaped structures at OCT could imply the presence of cerebriform nests at RCM and vice versa. In fact in OCT transversal section, we observed both icicle-shaped structures and cerebriform nests. Furthermore, the subgroup analysis of MMs with coexisting cerebriform nests and icicle-shaped structures showed a statistically significant higher average Breslow thickness for these lesions. This finding may imply that MMs with both these morphological features at RCM and OCT are thicker and more advanced lesions.

Additionally, a third of MMs revealed an association between a chaotic distribution of vessels in RCM and a serpiginous vessel shape in OCT. In the study by Carvalho et al., the authors reported the presence of dotted vessels in all lesions, including lesions with high Breslow indexes, and depending upon thickness, the dotted vessels were also associated with other vessel types. In the current study including nodular and mostly thick MM lesions, serpiginous and dotted vessels were mainly observed. The association of the chaotic distribution of vessels observed in RCM with irregular distribution of vessels observed in OCT is hypothesized to be an expression of the uncontrollable angiogenesis typical of a growing malignant mass.

In the current study, BCC tumour architecture was represented by the presence of basoloid islands in RCM and ovoid structures in OCT in 80% of BCC lesions. In RCM, the vascularization was characterized by vessels centred on tumour mass,
with vascular structures circling basaloid islands. In OCT, more than half of BCCs exhibited linear and curved vessels, arranged in an arborizing pattern.\(^5\) A linear and curved shape in OCT may be due to the arrangement of vessels circling basaloid islands as observed in RCM (Fig. 3). In the current study, most BCC lesions exhibited linear and curved vessels. Interestingly, Themstrup et al., in their study of nodular BCCs with OCT, described that at a depth of 300 μm there was a prevalence of serpiginous vessels, whereas in the current study only 1 lesion was characterized by serpiginous vessels.\(^5\)**

In most BCCs RCM showed a vascularization with vessels centered on the tumour mass, that circling the islands, while vessels were regularly distributed throughout the frame of observation in OCT. This feature remarkably differs from vascularization in MM, which had an irregular distribution of vessels.

In DN, RCM showed an absence of both atypical cells at the DEJ and a pagetoid growth pattern\(^7\) and were correlated with typical hyporeflective nests, a thickened epidermis and no ulceration in OCT. Vascularization observed in OCT was commonly represented by dotted vessels with a mottled, regular pattern (Fig. 4). This spectrum of features is well representative of a lesion without an aggressive growth and a benign attitude: these lesions do not need neovascularization and have an orderly cytoarchitecture.

The current retrospective study is not without limitations. The small lesion cohort makes statistically significant outcomes difficult to achieve, and the authors were often constrained to report simple trends and observations. However, the trends warrant further research, especially into the presence of icicle-shaped structures in OCT and cerebriform nests in RCM and its correlation with thicker, more advanced nodular MM lesions. Other outcomes not supported by previous literature also need investigation in larger lesion cohort studies.

The combination of in vivo evaluation of nodular lesions with both RCM and OCT seems to be able to offer a more complete understanding of the morphological architecture of nodular lesions, appreciating the expression of RCM parameters in OCT and vice versa, assisting in turn with early differential diagnosis of malignant and benign nodular lesions. The correlation between icicle-shaped structures and cerebriform nests in MM and their association with Breslow index requires future research.

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