Materials and Methods

Study Design and Population

We retrospectively examined a dedicated database of consecutive lesions, identifying those with atypical dermoscopy diagnoses mimicking MM, with a complete set of RCM images and histopathological diagnoses at the Department of Dermatology of Modena, Italy, between January 2010 and December 2016. Atypical melanocytic lesions were considered those with at least 1 feature from the revisited 7-point checklist, without any other dermoscopy patterns typical of other skin neoplasms [23].

All lesions were initially assessed with clinical investigations, dermoscopy and RCM imaging, and had confirmation of histopathology diagnosis. Study exclusion criteria were all lesions of the face [24]. Images of the remaining 1484 lesions were then retrospectively analyzed with evaluators blinded to histopathological outcome. RCM diagnoses were made based on RCM features which had already been associated with BCCs, seborrheic keratoses (SebKs), dermatofibromas (DFs) and squamous cell carcinomas (SCCs), or which had been considered "nonspecific" when RCM diagnostic features could not be clearly identified [25]. Lesions with melanocytic RCM-positive MM features, according to key RCM features of melanocytic lesions [26, 27], were excluded. A total of 178 lesions were without melanocytic diagnosis at RCM and included in the study (Fig. 1). All investigations were conducted according to the Declaration of Helsinki principles, with respect to human subjects in biomedical research, and was approved by the Ethics Committee of Modena (protocol No. 169/17).

Instruments

Dermoscopy and RCM images stored in a dedicated database had been previously prospectively acquired with the DermLite Photo System (DermLite Photo 3Gen LLC, San Juan Capistrano, CA, USA) and confocal laser microscopy (VivaScope 1500®, MAVIG GmbH, Munich, Germany), respectively. Instruments and acquisition procedures have been previously described [27, 28].

Dermoscopic Evaluation

Dermoscopic images were evaluated for inclusion criteria by two experts (F.F., V.D.M.), blinded to histopathology diagnoses. Selected dermoscopic images were classified according to the revisited 7-point checklist, including atypical pigment network, blue-whitish veil,

atypical vascularization, peripheral streaks, regressive structures, irregular blotches or atypical globules [23].

RCM Evaluations

Each lesion had at least 3 VivaBlock® mosaic images captured at 3 different standardized levels (epidermal layer, dermoepidermal junction and upper dermis). RCM features considered indicative of BCC included mild keratinocyte atypia, streaming epidermis, cords connected to the epidermis, dark silhouettes, peritumoral clefts, ulceration/erosion, tumor island size and location (epidermic or dermic), branch-like structures in tumor island, peripheral palisading, vascular morphology (linear or coiled vessels) and diameter, collagen surrounding tumor islands, solar elastosis and inflammatory infiltrates [19–22, 29].

RCM features indicative of SebKs included the presence of a regular epidermal pattern, keratin-filled invaginations, epidermal projections, corneal pseudocysts, packed round to polymorphous dermal papillae, a mixed vascular pattern, cords or bulbous projections [30].

Features indicative of DFs were the presence of dense bright dermal papillary rings and thickened refractile collagen bundles [31], and those indicative of SCCs were the disruption of the stratum corneum, severe cellular pleomorphism, atypical cells and architectural disarray of the epidermis [32].

Histopathology Evaluation

Histopathology was performed prospectively for all lesions using hematoxylin-eosin staining. All lesions were evaluated by a histopathologist specialized in cutaneous diseases.

Statistical Analysis

Descriptive statistics and complete case analysis was used for all comparisons between groups. Pearson's χ^2 test and Fisher's exact test were used to reveal associations between variables and groups.

A hierarchical cluster analysis was performed to identify potential homogeneous subgroups of BCC lesions. All variables were included to clustering. To determine the optimal number (k) of clusters, the Calinski and Harabasz stopping method was used [33]: the largest pseudo-F value indicates the most distinct clustering. After selecting the optimal number of clusters, the cluster characteristics were analyzed with the χ^2 test. All statistical analyses were performed using STATA® software version 14 (StataCorp 2015; Stata Statistical Software: Release 14; StataCorp LP, College Station, TX, USA), and $p < 0.05$ was considered statistically significant.