Dermoscopic-pathologic correlation of flat nevi with *ex vivo* dermoscopy with derm dotting

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In daily practice, most pathology labs process skin biopsy specimens without access to the clinical and/or dermoscopic images. In the evaluation of skin tumors, this information can be crucial to process and diagnose these lesions correctly. ***Ex vivo*** dermoscopy (EVD) was first described as a valuable tool in dermatopathology in 2007. Scope (1) and co-workers described the added value of ***ex vivo*** dermoscopy (EVD) for the evaluation of skin lesions by guiding macroscopy. They reported that EVD can reduce errors by aiding the selection of areas in which to perform step sectioning, can help in the diagnosis of ambiguous lesions and in the evaluation of section margins.


Marc Haspeslagh

In general, EVD images are broadly similar to IVD images and most of the structures and colors observed on IVD can also be recognized on EVD images (2). In some cases, EVD may reveal increased blue and white color and a loss of red. Because of the high quality of EVD images, they are very useful to visualize small lesions, to detect focal alterations in skin tumors, to evaluate margins and to guide tissue sectioning. Especially EVD images of pigmented lesions provide a sharp and detailed picture, allowing an excellent orientation and a guided cutting of the lesions. The advantage of EVD in the histopathological diagnosis of skin lesions was confirmed by others, but the need remained for a practical method of marking and targeting the suspicious margins and focal alterations seen on the EVD. In answer to this, derm dotting was introduced in 2013 (3), a method whereby these focal or suspected alterations are marked with nail varnish. The use of EVD with DD as new processing method was scientifically validated and proved to be a reliable method to visualize small lesions, to detect focal alterations in skin tumors, to evaluate section margins and to guide tissue sectioning (4).

EVD and adapted macro sectioning contribute to a better understanding of the specific architecture of melanocytic lesions. In our experience EVD and DD especially allow to better understand and classify the heterogeneous group of flat nevi. Besides the Miescher and Unna nevi, flat nevi form a large heterogeneous group. Many of these lesions are wrongly called dysplastic nevi although they are benign and carry no proven risk for melanomatous degeneration. With this

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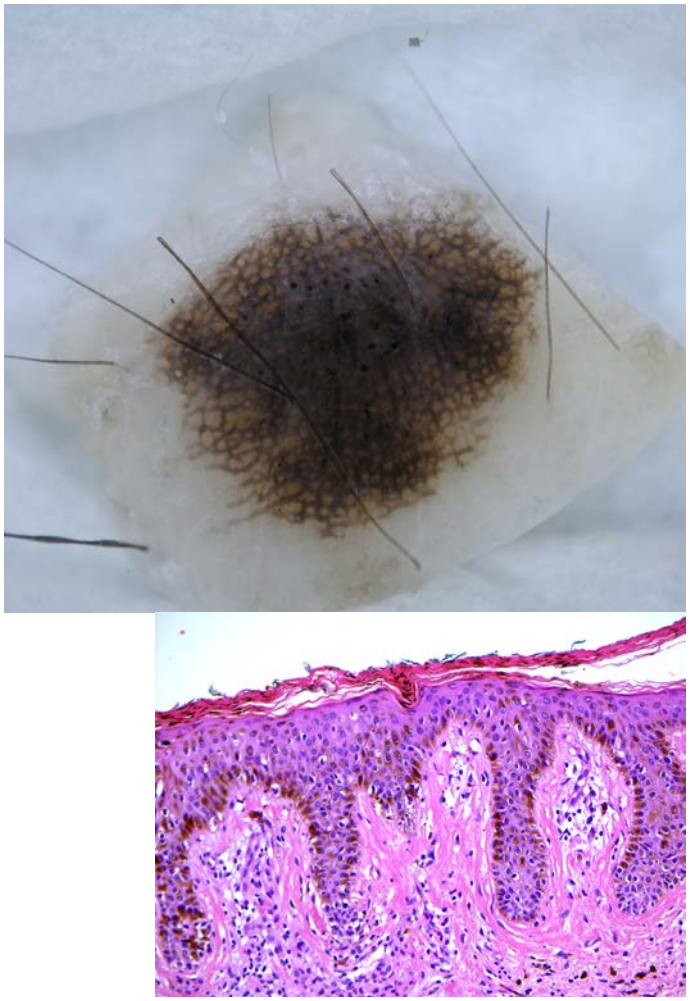
Figure 1:

Flat hyperpigmented nevus with broad central blotch corresponding to pigmented parakeratosis and peripheral reticular network corresponding to pigmented melanocytes arranged in nests or solitary units along elongated rete ridges.



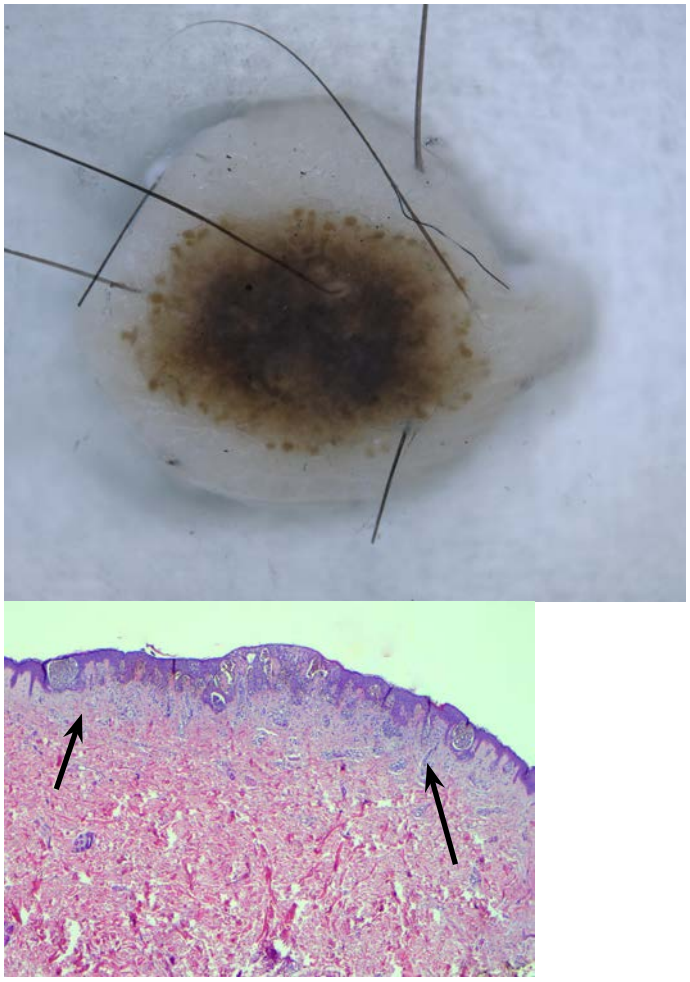
dermoscopic-pathologic approach we try to subclassify this mixed group of flat nevi. Some of these subtypes are already recognized as clinicopathologic entities; others are new or not yet well-defined. Some of these specific clinicopathological subtypes are very difficult to diagnose without dermoscopic information. The goal of this approach is to find the histopathologic correlate of the atypical clinical presentation or the reason for excision.

A well-described subtype are the hypermelanotic flat nevi (**Figure 1**). This common flat nevus is darkly pigmented and often considered atypical leading to excision. In its typical form it is a reticular nevus with variable central hyperpigmentation. The central brown-black homogeneous area, the so-called black lamella, is formed by melanin in the horny layer (pigmented parakeratosis). The hyperpigmentation can also be patchy. The nevus is skin type-dependent as it is mainly seen in skin type 3 or 4. It is also called black nevus. This nevus was described by Cohen in 1997 (5) and probably corresponds to what Clark (6) called 'the new nevus of midlife'. These lesions are mainly localized on the trunk and extremities with female predominance.

Another known subtype are the 'actively growing nevi' (**Figure 2**). It is a dermoscopic pattern seen in enlarging nevi revealing a peripheral rim of small, homogeneously sized brown globules, corresponding to melanocytic nests at the junctional shoulder of the lesion (7). The globules are considered to be a sign of growth of flat nevi. After some years these nevi enter a state of senescence resulting in a flat reticular or homogeneous aspect and disappearance of the peripheral globules. These nevi with female predominance are preferentially localized on the trunk.

Figure 2:

Actively growing nevus with circular rim of brown globules and dots corresponding to large junctional nests at the shoulders of the lesion (→).



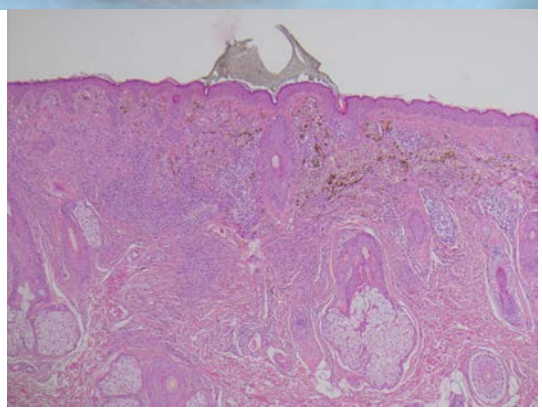
Another group of nevi are those removed due the appearance of a new suspicious black, blue, brown, yellow or white dot. This new dot can easily be marked and diagnosed readily without the need for deeper

cuts (**Figures 3-5, 9**). In this type of nevus, the area of concern can be missed without EVD with DD.

Flat nevi with a blue dot correspond mostly to nevi with a focal area of pigmented epitheloidal differentiation. They are probably a minor variant or related to deep penetrating nevi, since in our experience these lesions also show conservation of nuclear β-catenin. In deep penetrating nevi an activation of the β-catenin signalling has recently been demonstrated. This activation probably prevents these cells from maturing and entering senescence (8).

Figure 3:

Nevus with blue dot corresponding after dotting and guided cutting to a benign focus of pigmented epitheloidal differentiation in a flat compound nevus.

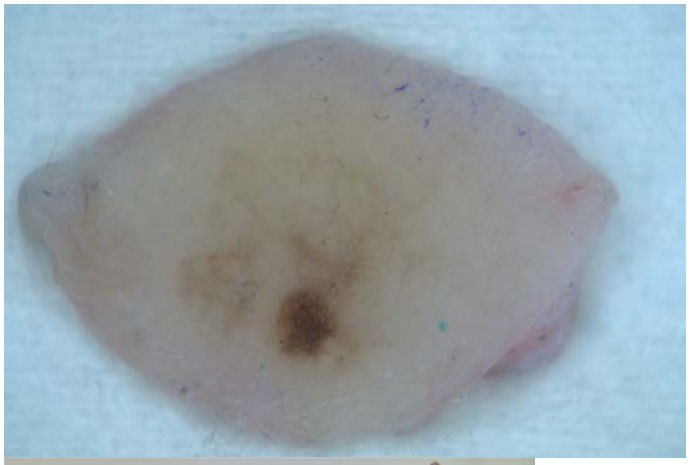


A black dot can correspond to melanin or blood in the horny layer. Brown-black dots are often caused by benign lentiginous hyperpigmentation of the basal layer, probably an irritative phenomenon.

We have observed grouped yellow dots in some nevi, which, after dotting, correspond to nests of balloon cell differentiation in the background of a classic compound nevus. This observation has resulted in the addition of 'balloon cell nests' to the list of differential diagnosis of yellow dots in dermoscopy.

Figure 4:

Nevus with brown-black dot corresponding after dotting and guided cutting to focal area of benign lentiginous hyperpigmentation.



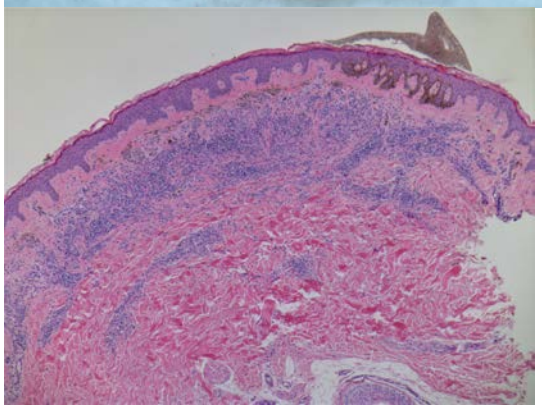
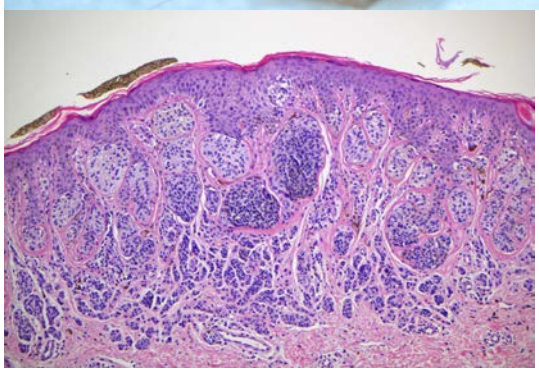


Figure 5:

Yellow globules in a Miescher nevus after dotting traced to be benign balloon cell nests.



EVD with DD also permits marking and guided cutting of focal areas, like crusts and erosions. This can be important for the diagnosis of irritated, traumatized (**Figure 6**), eczematous and recurrent nevi (**Figure 7**). The EVD of recurrent nevi is often diagnostic, namely centrifugal radial brown to black pigmentation in a structureless white background (scar).

Figure 6:
Nevus with focal posttraumatic crust formation.

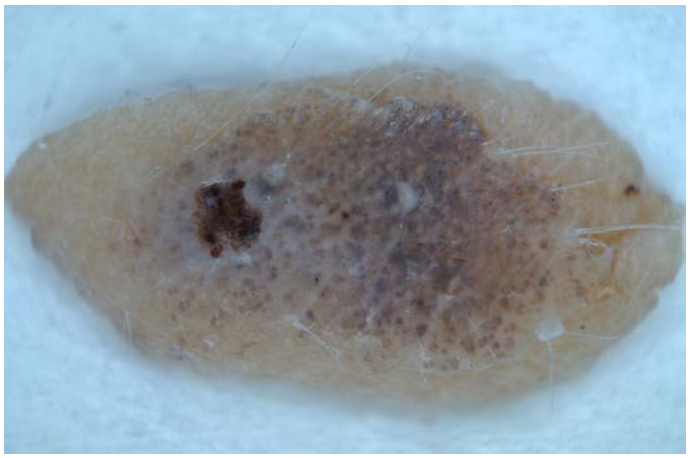
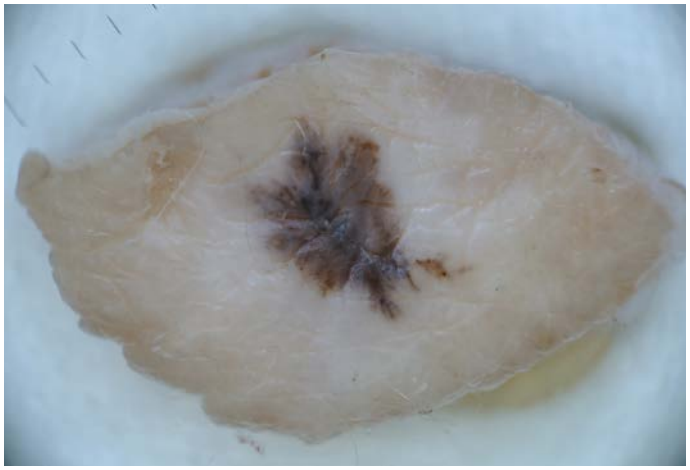


Figure 7:
Recurrent nevus after laser therapy with centrifugal, brown-black pigmentation radiating from the center of the scar.



Another subtype of nevi that we frequently detect with EVD and DD are fibrosing/sclerosing nevi. Moreover, we can subtype them as superficial (**Figure 8**), focal, perifollicular (**Figure 9**) and deep sclerosing forms (**Figure 10**). In these lesions pseudomelanomatous features may be seen. Without correct interpretation and dermoscopic correlation these lesions might be wrongly diagnosed as melanomas.

Figure 8:
Flat lesion with confluent areas of depigmentation, corresponding to superficial fibrosis and loss of pigmentation.

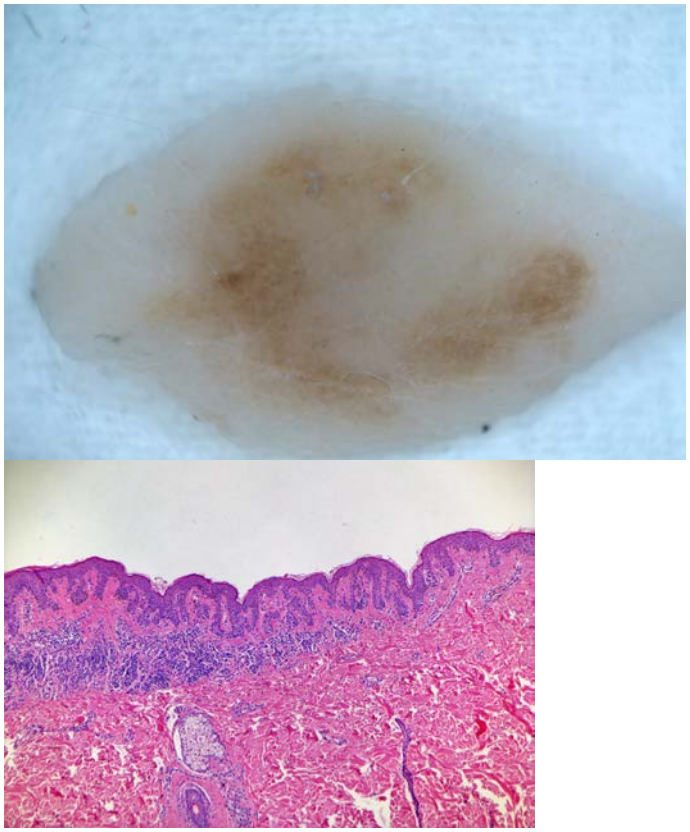


Figure 9:
White dot (→) in nevus corresponding to perifollicular fibrosis with depigmentation.

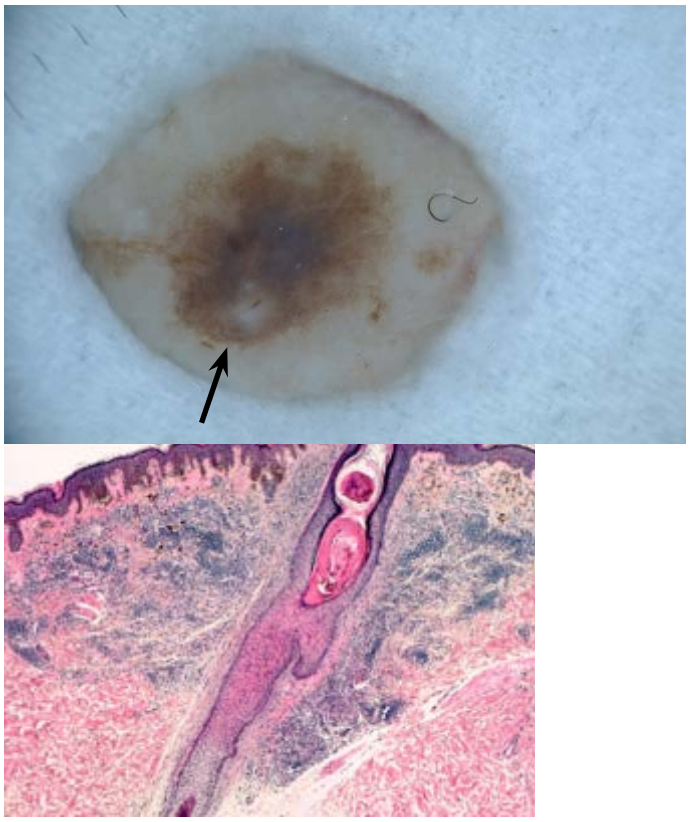
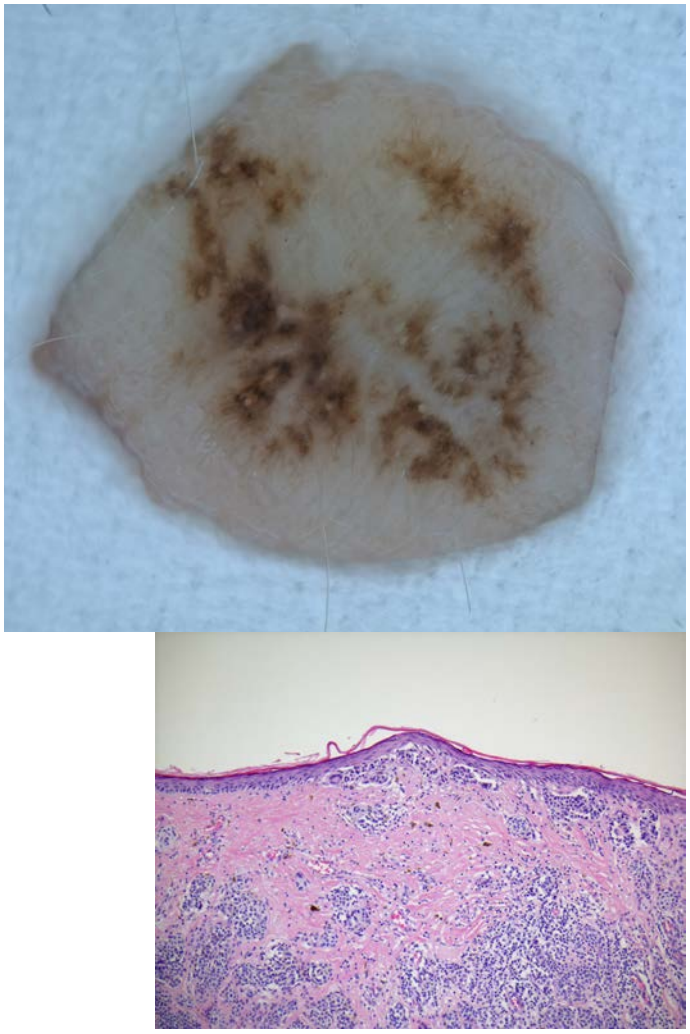


Figure 10:

Flat lesion with irregular but sharp delineated areas of depigmentation corresponding to sclerosis with pseudomelanomatous histologic changes of the recurrent type at the junction.



A further retrospective statistical analysis of the clinical features (such as age, gender and localization) of these subtypes, resulting from the use of EVD with DD, could tell us more about their exact nature. This could help to elucidate whether they form separate clinicopathological entities with possible prognostic and therapeutic consequences. It might also teach us more about the pathways of senescence, natural evolution or growth arrest of nevi.

Furthermore, a more thorough look at the group of nevi with inflammation (e.g. halo nevus and lichenoid or eczematous nevus) might inform us about possible inflammatory pathways involved in the natural evolution, maturation and growth arrest of these lesions.

Since 1997, when Wallace Clark described the so-called 'dysplastic nevus' (9), there has been a lot of controversy about the exact nature of this type of mole, and its clinical and histological definition has been the subject to different views (10). Clinically, these are flat nevi with some features that may resemble melanoma. On dermoscopy, these lesions are well-delineated with irregular borders and variation of colors. Since the diagnosis of a 'dysplastic nevus' requires histologic evaluation, the term 'atypical nevus' is considered more appropriate for the clinical classification. Although a universally accepted definition for dysplastic nevi does not exist, they are retained to be clinically relevant and considered to represent a marker for melanoma development. It is, however, not possible to predict which lesions might progress to melanoma and which ones may serve as predictors of melanoma risk.

Most pathologists still use the term 'dysplastic nevus', others prefer 'atypical nevus' or 'Clark nevus'. Regardless of what they are named, they are usually graded into mildly, moderately or severely dysplastic. Only in the case of severely dysplastic lesions, which are difficult to distinguish from melanoma *in situ*, a 0.5cm broader resection is advised. The low-grade lesions are considered benign.

Moles removed as 'dysplastic nevus' represent an important fraction of the melanocytic lesions examined by pathologists. Since a universally accepted pathological definition of dysplastic nevi is lacking, the diagnostic term 'dysplastic nevus' is used for a broad spectrum of pigmented lesions. First of all, there is a lack of concordance between clinically atypical nevi and histological dysplasia. Moreover, some clinically dysplastic nevi have no histologically dysplastic features, while clinically non-atypical lesions might have. Nevi with some atypia that do not correspond to classic subtypes, such as flat Clark, Miescher or Unna nevus, are often diagnosed histologically 'dysplastic'. This because of a prudent reaction not to underdiagnose a potentially malignant lesion. Even though overdiagnosis will usually be without consequences, some of these dysplastic nevi may be incorrectly diagnosed as thin melanomas. However, with good clinicopathological correlation, many of these lesions can be diagnosed as benign reactive, traumatized, inflamed, fibrotic/sclerotic, actively growing or phenotypically heterogeneous nevi.

In one third of melanomas, a pre-existing nevus is found, mostly of a non-specific congenital type (11). In a smaller part of melanomas however, the melanoma is arising in a pre-existing flat dysplastic nevus (12), strongly supporting the existence of an authentic dysplastic nevus that may evolve into melanoma. The existence of a genetically intermediate type of nevus has recently been proposed by Shain et al. (13). The criteria to recognize this 'authentic' dysplastic nevi and their exact morphological counterpart is unknown.

Apart from molecular analysis, a possible way to identify the exact nature of different types of flat nevi

is a clinicopathologic approach. As already mentioned, with EVD with DD-guided clinicopathologic approach, we can make a specific benign clinicopathologic diagnosis in many flat nevi excised as an 'atypical nevus'. In these lesions, none or only mildly dysplastic features are seen. A small remaining group of flat nevi however is diagnosed as moderately or severely dysplastic. The revision of these lesions allowed us to identify a subtype of nevus with distinctive *ex vivo* dermoscopic and histopathological features. On EVD, they appeared as flat lesions with an orange-brown peripheral color and with areas of an irregular broken, or even linear, pigment network and a variable blue-white center (**Figure 11**). In most cases, the diameter of these lesions varies from 5 to 10mm. The orange color is probably related to the skin type of the patients (type I or II) and the superficial junctional localisation of the melanin pigment. On histology, these lesions contain a variable number of small to medium-sized melanocytes with a finely granular pigmented cytoplasm, sometimes conferring a gray color. This type of melanocyte corresponds to what has previously been described as 'pulverocytes' (14).

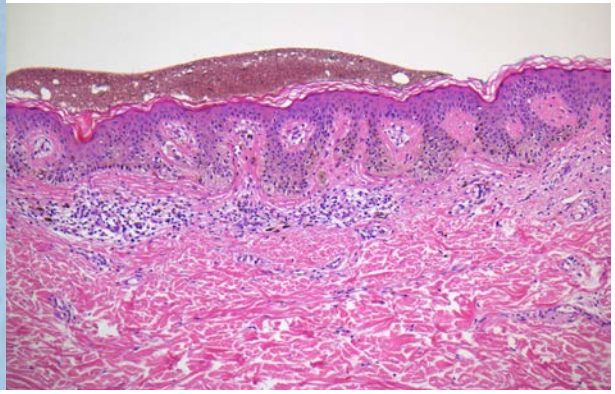
These pulverocytes are mainly localized along the asymmetric, junctional shoulders of these lesions. The cases with a blue center contain numerous melanophages. The shoulders are composed of irregularly growing melanocytes, linearly arranged along the basal layer of the epidermis or forming small irregularly placed nests. In most of these lesions, the nests show variable horizontal fusion.

The irregular broken up network seen on EVD, can partially be explained by the peculiar linear gray pigmentation of basal epithelial cells. In contrast to the normal basal keratinocytes, where the pigment is in an apical perinuclear position, the dusty pigment is present at the bottom of these basal cells. However, due to hyperpigmentation, the distinction between lentiginous pulverocytes and pigmented epidermal basal cells can be difficult to make. A melan A stain is necessary to identify the exact number of melanocytes, their growth pattern and the grade of atypia in the shoulders of these lesions.

Interestingly, immunostain for p16 has shown a variable loss of the protein in these lesions, from scattered cells to the entire pulverocytic component. There might be a correlation between p16 loss and the degree of atypia. Some lesions with severe atypia (lesions with some epidermotropism, irregular fusion of junctional nests and clear random atypia) show complete loss of p16. These data correspond well to the existence of a so-called 'intermediate lesion', gradually evolving from nevus into a melanoma (10). In their study, Shain et al. have defined the succession of genetic alterations during melanoma progression and identified an intermediate category of melanocytic neoplasia, characterized by the presence of more than one pathogenic genetic alteration and distinctive histopathological features. Homozygous loss of p16

Figure 11:

Example of orange pulverocytic nevus showing a flat atypical lesion with orange-brown broken up network at the periphery and more homogeneous blue-gray center corresponding to irregularly growing gray pulverocytic melanocytes along the rete ridges.



seems to be a late event in this genetic cascade, since it was observed only in fully developed melanomatous lesions.

As such, the 'pulverocytic orange nevi' could represent a genuine 'dysplastic nevus'. So far, we have collected over 280 of these lesions. Further molecular analysis of these lesions is required to better define their nature and their potential capacity to evolve into melanoma. Moreover, some patients have had multiple atypical pulverocytic nevi and were diagnosed as patients with a dysplastic nevus syndrome or with the so-called 'signature nevi'. We are actually performing a retrospective analysis of clinical features like gender, age, localization and personal and/or family history of these nevi. In this way, we want to examine whether morphological features can be identified to separate them from other flat nevi and to determine their risk of progression towards melanoma.

CONCLUSION

EVD with DD allows adapted sectioning of skin lesions and permits a more accurate histological diagnosis in less time, compared to the standard method of random transverse cutting. It allows better section margin evaluation, better visualization of skin lesions, and specific marking and evaluation of focal or suspicious alterations. The method is easy to implement in a dermatopathology setting.

Especially for classification and interpretation of flat nevi, the method adds a new dimension to the clinicopathologic approach of these lesions. Besides a more confident and specific diagnosis, it might lead to more insight in the pathologic correlate of specific features observed in dermoscopy, as well as to a more accurate delineation of specific subtypes of melanocytic nevi. ■

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