

Morphologic and immunohistochemical features of breast nevi

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Abstract

A large number of publications recognize that there are melanocytic lesions with microscopic features similar to melanoma, related to their location. Those locations are represented by the ear, the milk lines (axillary, breast, periumbilical and inguinal regions), palms, soles, and flexural regions. Starting from Rongioletti F *et al.* study in 2004, we measured 10 histological parameters on 96 nevi (39 from the breast) and notes with 0 if absent or impossible to evaluate and with 1 if present. The score for each lesion ranged from 0 to 7 and we compared the features of the breast nevi with the nevi from the control sites and found that breast nevi present more atypical features than the nevi from the other sites (absence of demarcation of melanocytes at lateral margins, nests and dyscohesive pattern and melanocytic atypia). We also performed immunohistochemical examination on lesions that presented three or more of the examined histological parameters, but the results were not suggestive. The conclusion of this study is that the atypical features of the breast nevi are only site related atypias and have no hormonal influences.

Keywords: breast, nevus, melanocytic, atypias, melanoma.

Introduction

It is recognized in a large number of publications that there are some sites of melanocytic nevi that can present aspects similar to melanoma. Those sites include the head and neck (specially the ear), the "milk line" (axillary, breast, periumbilical, and inguinal), acral regions (palms and soles) and flexural regions. The microscopic aspect of these nevi necessitates sometimes differentiation from melanoma [1–3].

The milk lines are the progenitors of mammary glands and nipples. All the mammals (both male and females) present a pair of symmetrical mammary ridges, along which the mammary gland the areolas and the nipples develop. Those ridges appear around six weeks of fetal life and they go from the axillary region, down the torso to the groin and sometimes to the inferior limbs [2–4].

Around the ninth week, most of the milk lines disappear, except those on the torso. Occasionally, the milk lines persist and therefore some individuals may present ectopic mammary tissue or multiple nipples.

In 2004, Franco Rongioletti and a group of dermatopathologists affiliated to *Melanocytic Lesions Group of the Italian Association of Dermatopathology*, performed an inter-institutional study on 198 melanocytic nevi localized on the breast (101) as well as other sites such as thorax, extremities (except palms and soles). For each lesion were measured 10 parameters: asymmetry, absence of marginal melanocytic demarcation, lentiginous proliferation, presence of melanocytic nest with dyscohesive pattern, intra-epidermal melanocytes,

involvement of hair follicle, absence of dermal maturation, melanocytic atypias, papillary dermis fibroplasia and the presence of the dermal lymphocytic infiltrate. This study demonstrated that the nevi in the flexural sites (exclusively axillary and inguinal) present a more dyscohesive pattern, similar to the genital nevi [5–9].

Breast nevi instead, do not have many histological differences from the flexural ones, but they have more atypical features such as intra-epidermal melanocytes, melanocytic atypias and dermal fibroplasia. The role of the estrogen in the biologic behavior and the histological aspect is still unsure, because there were no major differences between males and females. Starting from this study, we have analyzed the breast nevi (as part of the milk line) in order to obtain a histopathological and immunohistochemical characterization [1, 3–5, 10, 11].

Materials and Methods

This study includes 96 melanocytic nevi localized on the torso, 39 of them from the breast. Those lesions have been diagnosed in the Department of Pathology, Emergency County Hospital of Constanța, Romania, during 2007–2011. They have been categorized by conventional criteria in junctional, dermal, combined and dysplastic nevi.

For every one of these nevi we measured the 10 parameters proposed by Rongioletti F *et al.* [5], such as: (1) asymmetry, (2) absence of demarcation of melanocytes at lateral margins of the lesion, (3) lentiginous proliferation of melanocytes for three

consecutive papillary ridges, (4) nested and dyscohesive pattern with bridging of nests between rete ridges, (5) intra-epidermal melanocytes singly or in groups, above the basal layer over the length of three rete ridges, (6) involvement of the hair follicle by melanocyte proliferation, (7) absence of maturation of deep dermal melanocytes, (8) melanocyte atypia including one of the following: prominent nucleoli, irregular chromatin pattern, increase of nuclear/cytoplasmic ratio, finely distributes melanin pigment within the cytoplasm (smoky cytoplasm), (9) fibroplasia of the papillary dermis, and (10) lymphocytic infiltrate in the dermis.

Each parameter was scored 1 if present and 0 if absent or impossible to evaluate. For every lesion, we established a score with a minimum value of 0 and maximum of 10. The score of the nevi from the breast was compared with the score from the nevi in the other sites of the torso and also the scores were compared between man and women as well as different age groups.

For lesions with scores above 3, we used specific melanocytary immunohistochemical stains such as HMB45, Melan A, and S100, proliferation markers (Ki67) and estrogen receptors markers.

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissues cross sections using a Dako EnVision™ + Dual Link System HRP (Dako, Carpinteria, CA, USA) according to the manufacturer's instruction and counterstained with Hematoxylin.

The results were statistically analyzed by Student *t*-test and *chi*-square test using Microsoft Excel and MedCalc packages.

Results

We collected 96 nevi, 39 of them from the breast and 57 from the anterior thorax. Out of these nevi, 86 are from women (37 from the breast and 49 from other sites of the thorax) and 10 from men (two from the breast and 8 from the thorax).

The mean age of the patients was 32.68 ± 11.7 years, with no major differences in the two locations (mean age of 33.67 ± 13.53 years in breast lesions and 32.01 ± 10.35 years on thorax). Also, there are no significant differences in males and females: 30.15 ± 15.71 years in breast lesions of men and 32.01 ± 0.35 years on lesions of thorax in men, and 33.66 ± 13.53 years in women with breast nevi, and 31.81 ± 10.45 years in women with nevi on thorax except the breast area.

From the 96 nevi we studied, 68 are intradermal (31 on the breast area and 37 in the control sites), 12 compound (three on the breast and nine in other areas of the thorax), 10 junctional (four on the breast and six in the control sites).

From all the nevi, six are dysplastic, one on the breast and five in the control sites, all of them being compound histological type.

The total score obtained for the every lesion varies between 0 and 7, with a mean value of the score of 1.48 ± 1.6 . The mean value for the breast nevi is $1.84 \pm$

1.82 and the mean value of the nevi on the control sites is 1.22 ± 1.54 ; thus, the score of the breast nevi is significantly higher than the score of the nevi located on the thorax ($p=0.383$). For women with breast lesions, the mean score of 1.84 ± 1.82 and men with the same kind of lesion have a mean score of 1.38 ± 1.50 ($p=0.7854$). The control sites nevi also present no differences between sexes (mean value for women of 1.25 ± 1.56 and for men of 1.22 ± 1.54) (Figure 1).

Considering the score of the breast nevi compared to the nevi on the control sites, we encountered the following differences: asymmetry was present in 12.83% of breast lesions and 14.04% of control sites lesions ($p=0.6441$); the absence of demarcation of melanocytes at lateral margins of the lesion was present in 7.7% of the breast lesions and was not present in any nevus located in the control sites ($p=0.0831$); the lentiginous proliferation of melanocytes for three consecutive papillary ridges is encountered in 17.95% of the breast lesions and 14.04% of the control sites nevi ($p=0.6157$); the nested and dyscohesive pattern with bridging of nests between rete ridges was encountered in 33.34% of breast nevi and 15.79% of nevi in the control sites ($p=0.0572$) (Figure 2); intra-epidermal melanocytes singly or in groups, above the basal layer over the length of three rete ridges were found in 7.7% of the breast nevi and 5.27% of the control sites nevi ($p=0.6541$); the involvement of the hair follicle by melanocyte proliferation was present in 5.13% of the breast lesions and 5.27% of the nevi in other sites of the thorax ($p=0.9769$); the absence of maturation of deep dermal melanocytes was present in 5.13% of the breast nevi and was not encountered in nevi of the control sites ($p=0.1599$); melanocyte atypia including one of the above-mentioned criteria was found in 25.65% of the breast nevi and 12.29% of the control sites nevi ($p=0.0660$) (Figure 3); the fibroplasia of the papillary dermis was encountered in 28.21% of the breast nevi and 29.83% of the control nevi ($p=0.8653$); lymphocytic infiltrate in the dermis was present with different intensities in 38.75% of the breast nevi and 28.08% of the control sites nevi ($p=0.2979$).

The immunohistochemical examination revealed positive reaction for the melanocytary markers in all the nevi regardless their location. The proliferation marker Ki67 had a mild positive reaction only in two cases of breast nevi (nevi with a total score of 6 and 7), a weak positive reaction in three breast nevi (all with a total score of 4).

The rest of the breast nevi that we used immunohistochemistry on were negative for Ki67. Immunohistochemistry was practiced on nine cases on nevi from the thorax except the breast and as mentioned above they all presented with positive reaction for all the melanocytary markers.

The expression of Ki67 for the control sites nevi was variable depending on the score obtained on the morphologic examination. Therefore, three (33.33%) cases presented a weak positive reaction, and the rest of the cases were negative for Ki67. On the selected cases

for immunohistochemical examination, we practiced hormonal stains, particularly ER. The reaction to ER was

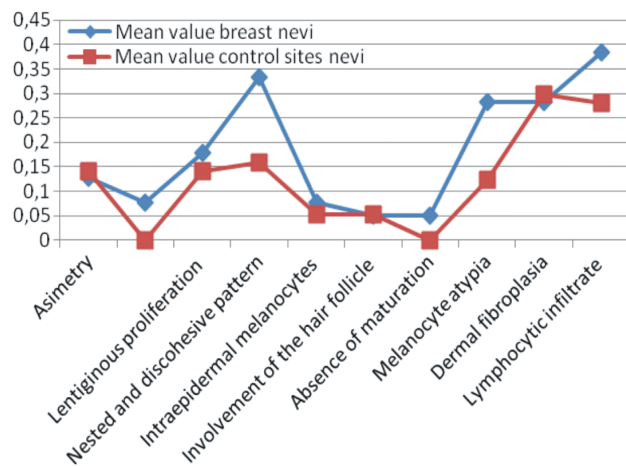


Figure 1 – Mean value of scores for the histological parameters.

not suggestive; all the cases had a negative expression regardless the location or patient' gender (Figure 4).

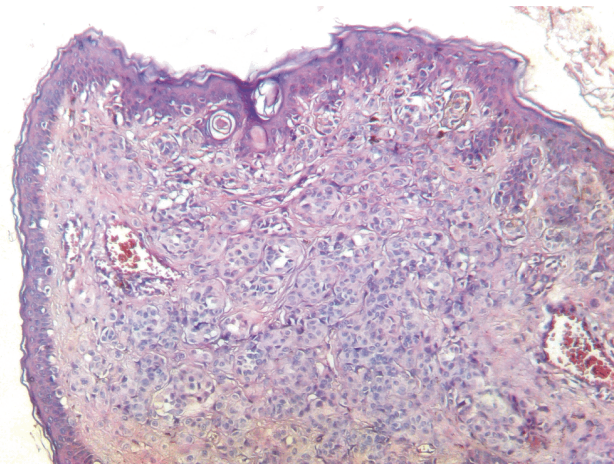


Figure 2 – Breast nevus with nested pattern and bridges between ridges (HE stain, ob. ×10).

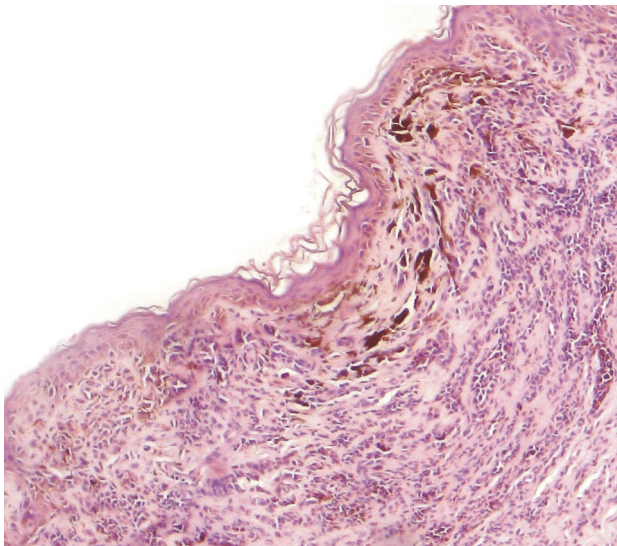


Figure 3 – Breast nevus with atypical cytology (HE stain, ob. ×10).

Discussion

It is known for many years that a group of nevi in different anatomic sites that may simulate both dysplastic nevi and melanoma [1, 3, 6, 7, 12, 13]. These nevi have been termed generically “nevi of special sites”. As said above, the anatomical sites that have been included are acral locations, genitalia, breast, scalp, ear, flexural regions and the conjunctiva. Some of these locations offer a physical explanation for the atypical features of the nevi (thick corneum and dermatoglyphs of the soles and palms) but it is speculative [11].

Most of the nevi from these anatomic sites are identical clinically and histologically from the “banal” nevi (torso, arms, except acral regions, and other locations), and only a small part of them show atypical features that overlap dysplastic nevi or melanoma. Therefore, the anatomical arguments, hormonal influences or effects of repeated friction are not sufficient to explain their atypical aspect. Even though they may show severe modifications, their behavior is completely benign.

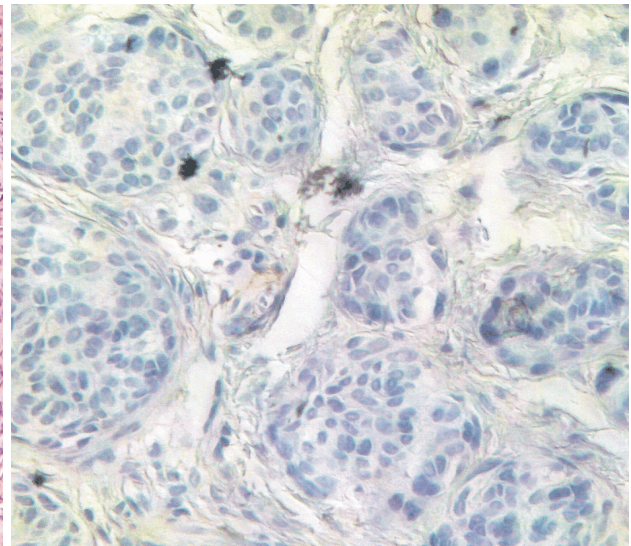


Figure 4 – Breast nevus with negative estrogen receptors expression (ob. ×40).

The acral nevi tend to be more cellular than most of the nevi, with a predominant lentiginous than nested pattern and occasionally with pagetoid cells [14–18].

The nevi of the there genitalia present with confluent melanocytic nests, with various sizes and shapes, and more dyscohesive melanocytes. There may be present a lentiginous growth as well as adnexal involvement. The presence of pagetoid cell is uncommon in genital nevi [11, 19].

The flexural sites include several locations such as axillary, umbilical, inguinal, antecubital, popliteal fossa, pubic, scrotal, perineal and perianal and depending on each constitution the folds of the neck and abdomen [3, 11].

In one study, Rongioletti F *et al.* found 55.5% of the flexural nevi as atypical with features similar to genital nevi (pleomorphic nesting pattern and lack of cellular cohesion). Cytologic atypia is not often found and when present it is mild and restricted to the junctional and papillary dermal component of the nevus.

David E. Elder mentioned in an article published in 2006 in *Modern Pathology* that “it is just as important to recognize truly dysplastic nevi as such, as it is to avoid overdiagnosis of the nevi of special sites as dysplastic nevi, or as melanoma”.

The breast nevi morphology do not differ very much from the nevi in other locations, however they presented with more atypical features than nevi from other sites.

Rongioletti F *et al.*, in 2004, proved that breast nevi showed more frequent intra-epidermal melanocytes above the basal layer, atypical cytology and papillary fibroplasia. Their data could not confirm that these features are under estrogenic influences because there were no differences between males and females [5, 20]. The atypical features observed by Rongioletti F *et al.* did not correspond to any unusual biological behavior, meaning that they do not represent a risk for melanoma [5, 21]. Rongioletti F *et al.* concluded that “to avoid undue concerns, dermatopathologists should be aware that breast nevi may show a higher degree of atypical features than nevi from elsewhere”.

We studied the breast lesions as part of the milk line in order to observe if any connections are present due to their common embryologic origin. The results we obtained were not very different from the ones of Rongioletti F *et al.*, therefore the morphologic features the most present in our cases of breast nevi were the nested or dyscohesive pattern (33.34%), melanocytic atypias (25.65%), dermal fibroplasias (28.21%) and the dermal lymphocytic infiltrate as the most present of all (38.75%).

Though the frequent presence of these parameters, the difference between breast nevi and control sites nevi was not statistically significant, with a *p* close to the significant value of ≤ 0.05 only for nested and dyscohesive pattern (0.0572) and melanocytic atypias (0.066).

There are no important differences between these values in men and women. Corroborating this aspect with the inconclusive immunohistochemical examination with estrogen receptors [22], we therefore considered that the modified aspect of the breast nevi is not hormonally influenced.

The melanocytic specific markers were positive in all the examined nevi. The Ki67 expression in breast nevi has a tendency to be stronger in nevi with a higher score (two cases with moderate reaction with scores of 6 and 7, with marked atypia, which were considered to be dysplastic nevi). The control sites nevi presented three cases out of nine with weak Ki67 expression, also in dysplastic nevi. Therefore, this examination was not very conclusive to our study.

✉ Conclusions

We can conclude that these atypical features that can be encountered in breast nevi are only site related atypia so far not related to any hormonal influence. It is important for any pathologist to be aware of the atypias on the breast nevi as well on other specific sites, to

avoid overdiagnosis of malign melanoma and unnecessary concern from the patient.

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