Diagnostic spectrum of nodular melanoma

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Summary

Nodular melanoma represents 10 to 30% of all melanomas and circa 50% of those with a Breslow thickness greater than 2 mm. The main cause of death among malignant cutaneous tumors, it presents a high metastatic potential even in the early growth stages, making an early diagnosis fundamental. It is more common in men than in women, its onset is in later age, in any part of the body, more often on the neck and head. Due to its clinical characteristics (symmetrical, nodular, amelanotic/hypomelanotic or highly pigmented lesion) nodular melanoma is difficult to diagnose with the ABCD rule (Asymmetry, irregular Borders, Color variation and Diameter). For this reason it is preferred to use the EFG rule which takes into consideration Elevation, Firmness and rapid Growth pattern.

Histologically, nodular melanoma is characterized by the lacking of atypical melanocytes in the epidermis (up to 3 crests) beyond the lateral margins of the malignant dermal cellular component, with theca cells and/or confluent aggregates without maturation phenomena. The dermoscopic structures mainly related to nodular melanoma are asymmetry of the pigmentation pattern, blue-white veil, blue-black areas, milky red areas, irregular dots and globules, atypical vascular pattern.

Recently reflectance confocal microscopy has permitted a close dermoscopic-confocal correlation of melanocytic and non-melanocytic skin lesions. RCM features of nodular melanoma show atrophic or thin epidermis, sheet-like structures at the dermo-epidermal junction and the presence of single or variable-in-size clusters of atypical melanocytes in the upper dermis in proximity or overlapping enlarged and convoluted vessels.

In the diagnostic challenge of nodular melanoma, integration between clinical parameters and non-invasive skin techniques are needed to warranty a correct evaluation of pigmented, hypopigmented or achromic nodular lesion.

KEY WORDS: dermoscopy; diagnostic imaging; nodular melanoma.

General features

Nodular melanoma is defined as an invasive melanoma lacking a significant component of epidermal tumor cells beyond the dermal invasive component (1). It represents 10 to 30% of all melanomas (second for frequency after superficial spreading melanoma SSM) and about 50% of melanomas with a depth greater than 2 mm (2). It is the main cause of death among cutaneous malignant tumors despite the fact that it makes up only from 9 to 15% of invasive melanomas (1). Due to its rapid vertical growth and lack of the radial growth phase, often it is not diagnosed until it has advanced locally. Hence it is associated with a relatively poor prognosis (3). It presents a high metastatic potential with the tendency to metastasize in the early stages of growth; thus an early diagnosis is fundamental (3). Males are more inclined towards nodular melanoma whereas the other three subtypes (SSM, lentigo maligna melanoma and acral
basis of histologic criteria nodular melanoma presents pleomorphism and prominent nucleoli (4). On the contrary, in a variable combination, with hyperchromasia, fusiform, epitheliod nevoid or pleomorphic morphology, cells and or confluent aggregates without maturation signs of the malignant dermal component, with thecal arrangement of the basal cell layer (up to three crests) besides the lateral margins of the indifferent melanocytic component. By definition the nodular melanoma is characterized by the limited presence of atypical melanocytic cells, withcha cells and or confluent aggregates without maturation phenomena, with atypical melanocytes having fusiform, epitheloid nevoid or pleomorphic morphology, in a variable combination, with hyperchromasia, pleomorphism and prominent nucleoli (4). On the basis of histologic criteria nodular melanoma presents the following characteristics: as regards the Breslow thickness it is seen that circa 50% of melanomas with a depth greater than 2 mm belongs to the nodular subtype (3). Hersey et al. compared melanomas having a Breslow thickness > 3 mm with melanomas having a Breslow thickness < 0.75 mm. It was demonstrated that circa 62% of the melanomas > 3 mm were of the nodular type with a limited prevalence (2%) in the forms > 0.75 mm. As a result of its nodular radial growth phase and the presence of rapid vertical growth, nodular melanoma nearly always presents Clark Level III and an innate propensity to metastasize (8). Liu et al. showed that the fast median growth rate corresponds to 0.49 mm each month for nodular melanomas compared with the slow growth rate of 0.12 mm every month in the progression of superficial spreading melanomas (9). Furthermore, nodular melanoma also has a greater tendency towards ulceration with respect to the other subtypes (1). In particular, in all nodular melanomas ulceration is correlated with poorer disease free survival (DFS) and worse overall survival (10). Nodular melanoma presents a higher mitotic rate with respect to the other subtypes (11). A study of 2397 cases of melanoma carried out in Australia demonstrated how melanomas with higher mitotic rates develop in men, in patients over age 70, in patients with a history of solar keratosis. Moreover, these melanomas are found more frequently on the head and neck and are more often amelanotic (11). The association of a high mitotic rate has also been seen with the nodular subtype, greater depth of the tumor and ulceration (11). With regard to the phenomena of regression and inflammation, they may be present in nodular melanoma but always to a minor degree respect to the form of superficial diffusion.

Molecular and genetic classification of nodular melanoma

In recent years molecular alterations associated with melanoma have been identified, in particular mutations of genes that encode by kinases involved in the transduction pathway of the Ras/RAAF/MEK/ERK Mitogen-Activated Protein Kinase (MAPK) signal, responsible for the regulation of the cellular proliferation and differentiation processes (12). Approximately 2/3 of melanomas presents mutation of the BRAF gene and the remaining percentage of cases presents mutation of the NRAS gene (12). They both have in common activation of the MAPK pathway whereas mutation of NRAS also activates the AKT pathway (12). As concerns BRAF, the most frequent mutation (V600E) involves expression of exon 15. BRAF mutation is associated with a young age, intermittent solar exposure, localized on the trunk and limbs, a large number of melanocytic nevi, few freckles, easily tanned, outbreak on skin lacking solar elastosis and correlation with the superficial diffusion histotype (13). The mutations expressed by c-KIT receptor are observed in 1 to 3% of melanomas, with greater frequency in acral-lentiginous melanomas, mucosal melanomas and melanomas on sites affected by
Diagnostic spectrum of nodular melanoma

chronic exposure to the sun (13). In nodular melanoma a minor frequency of BRAF mutation was seen respect to the superficial spreading subtype (12). In a study published in 2006 it was found that BRAF mutated in 64.3% of superficial melanomas and in only 36.4% of nodular melanoma cases (12). Instead, NRAS appears mutated more frequently in the nodular subtype than in the superficial (14). Still more recently it has been demonstrated how in nodular melanoma there is not an exclusive mutation of BRAF or NRAS: these alterations may be present simultaneously in the same tumor sample (15). When the two mutations were present together, one presented with a high frequency and the other with a low frequency (15). It has been seen how particular chromosomal alterations can be correlated to a greater extent with a particular melanoma subtype: in the nodular subtype we more frequently find a deletion on 1p36 as this region is strongly connected to the vertical growth or aberrations in the long arm of chromosome 10 or the complete loss of this chromosome (16). Recently the role of CIP2A (alkaline phosphatase inhibitor 2A) has also been studied. CIP2A is an important oncogene that contributes to the progression of the tumor by regulating cMKI and AKT (17). By means of immunohistochemical analysis it has been seen that the CIP2A levels increased in the tumor cells and its nuclear or cytoplasmatic localization (17). In nodular melanoma there is a high cytoplasmatic expression of CIP2A correlated with an increase in survival, whereas in SSM a high nuclear expression of the protein is correlated with poor survival. This suggests that CIP2A has different functions depending on its molecular context and histologic subtype (17).

Dermoscopic features

Dermoscopy (dermatoscopy; epiluminescence microscopy, microscopy of the cutaneous surface) is an in vivo technique that certainly aids in the evaluation of cutaneous lesions and in the early diagnosis of melanoma in particular. In fact, dermoscopy is able to increase the diagnostic sensitivity of melanoma by 10 to 35% compared with clinical observation alone (18-20). Dermoscopic characterization of nodular melanoma is limited owing to the diagnostic difficulties posed by the morphologic features of the lesion and the dermal component of nodular malignant proliferation. Often nodular melanoma is misclassified as non-melanoma and complex instrumental methods may be useful to evaluate the lesion and to improve diagnostic assessment. Recently three important studies have been carried out concerning the dermoscopic evaluation of NM, the objective being to obtain a better classification of the vascular, chromatic and structural features and compare them with the other cutaneous lesion subtypes. In general, the histologic-dermatologic correlation is conditioned by the overall picture of the pheomelanotic and/or eumelanotic pigmentation, localization of the pigment, speed of the vertical growth pattern, neoangiogenesis and the relative presence of the peripheral component of the epidermis, dermal-epidermal junction and papillary dermis. Menzies et al. analyzed 467 cutaneous lesions including NM, invasive non-NM, 115 nodular benign melanocytic tumors and 135 nodular non-melanocytic tumors (1). It emerged that NM has a greater mean depth compared to non-NM, but is much more pigmented compared to nodular non-melanocytic tumors (1). At dermoscopic analysis pigmented NM in comparison to invasive non-NM more frequently presents the following features in order of prevalence: symmetrical pigmentation pattern, large diameter vessels, homogeneous blue pigmentation areas, symmetrical form, predominant peripheral vessels, blue-white veil, black, pink color, milky pink-red areas (1). Respect to nodular lesions non-melanoma presents more frequently: peripheral dots-globules, multiple brown dots, irregular black dots-globules, blue-white veil, pseudopods, homogeneous blue pigmentation, 5 to 6 colors, black color (1). Comparing the amelanotic/hypomelanotic lesions (NM vs non-melanoma nodular lesions) the patterns positively correlated with NM are (in order of ORs): blue-white veil, atypical vascular pattern, homogeneous blue pigmentation, 5 to 6 colors, black color, white central macula, blue color, more than one shade of pink, predominant irregular linear vessels, irregular dots and globules, irregular pink-red areas, irregular depigmentation, black or brown globules, irregular spots, red globules, irregular dots-globules, hairpin vessels (1).

Pizzichetta et al. compared 457 lesions that had been diagnosed histologically as nodular melanoma, SSM, benign pigmented nodular melanocytic lesions (dermal nevi, blue nevi) and pigmented non-melanocytic nodular lesions (seborrheic keratosis, basal cell carcinoma). Through the use of univariate and multivariate analysis it was sought to make evident which characteristics are the most frequent at dermoscopy of nodular melanoma (2). By comparing NM with SSM in the univariate analysis it was found that ulceration, homogeneous disorganized pattern, homogeneous blue pigmented areas, multiple colors (>3), combination of polymorphic vessels, milky red areas, and symmetrical form were more frequent in NM compared with SSM (2). Comparing these results with those of Menzies both appear to be in agreement on the homogeneous blue pigmentation areas and the symmetrical form, while in Menzies’ study a positive correlation was also found with the symmetrical pigmentation pattern, pink color, blue-white veil and black color (2). The inconsistency between the two studies is probably due to the different size of the samples and the use of different terminology (2). At the multivariate analysis (used to estimate the independent effect for each factor that presented a significant result in the univariate) the only characteristics able to distinguish NM from SSM are ulceration, homogeneous disorganized pattern and homogeneous blue areas (2). Comparing NM with melanocytic nevi, seborrheic keratoses and basal cell carcinomas at the univariate, many of the characteristics are in agreement with Menzies’ study: peripheral black dots and globules,
irregular black dots and globules, blue-white veil, homogeneous blue pigmentation, multiple colors, black color, irregular spots, irregular dots and globules, blue-black structures and asymmetrical form (2). In this study, however, other patterns were found such as irregular linear vessels with red globules and areas, more than one shade of pink, homogeneous red areas, homogeneous pigmentation pattern, atypical pigment reticulum, shiny white structures, predominant blue clusters, patterns with no distinctive characteristics, light brown areas with no structures and irregular depigmentation (2).

At the multivariate analysis, instead, the following are more frequent: asymmetrical pigmentation, blue-black pigmented areas, homogeneous disorganized pattern, combination of polymorphic vessels and milky red globules-areas and polymorphic vessels associated with homogeneous red areas (2). The two studies have contributed to drawing attention to the importance of the vascular structures. Polymorphic vessels, milky red globules and areas and homogeneous red areas are particularly correlated with nodular melanoma (2). The polymorphic vessels can be irregular linear, dotted, hairpin, helicoidal, branched. The milky red globules are ovoid or polygonal structures of a pink-white color that often present an irregular linear or helicoidal vessel at the center (2). The milky red areas are areas with more than one pink shade, probably corresponding to areas with increased vascular volume and represent an increased vascular volume that reflects a neoangiogenesis (2).

The combination of blue and black areas involving at least 10% of the surface of the lesion was studied by Argenziano et al. and seems to have an important role in the dermoscopic diagnosis of nodular melanoma (21). Starting with 283 dermoscopic images of benign and malignant lesions, the standard criterion for melanoma was compared with the presence of blue-black areas (BB). It was noted that combined use of the two criteria is correlated with a higher positive predictive value for malignity (90.6%) and with 93.2% of negative predictive value for nodular melanoma (21).

Many characteristics included in the standard criteria for melanoma cannot be expressed in nodular melanoma because they refer to epidermal and upper dermal component such as atypical pigment network, irregular pigmentation, atypical brown globules, brown areas lacking structures, regression (21). In previous studies a blue-white veil and atypical vessels were often predictive of melanoma. When the standard criteria are absent the diagnostic relevance of the blue-white veil and atypical vessels is limited because these two characteristics can be present in both benign and malignant lesions (21). The addition of the BB criterion seems to have overcome this limit: the combination of blue and black indicates that the pigment is not only present in the medio-deep dermis (blue color) as seen in hemangiomas and blue nevi, but is also present in atypical melanocytes of the epidermis (black color). Hence the BB criterion seems to be useful in improving the dermoscopic diagnosis of nodular melanoma (21).

**Dermoscopic synopsis**

On the basis of the data in the literature we can distinguish a pigmented form, an achromic form and a hypopigmented form of nodular melanoma.

**Pigmented Form:** irregular pigmentation with homogeneous blackish color or variable polychromy (>3 colors), blue-white veil, pink areas, blue-black areas (blue-black rule) (21), milky red areas. Compared with other types of cutaneous lesions, we more often found a homogeneous disorganized pattern, ulceration, irregular dots and globules, irregular streaks, atypical vascularization (Figures 1, 4).**

**Achromic Form:** lesion completely devoid of pigment where the reddish colour represents the substrate of a nodular melanoma where the different expressions of vascular pattern may help the clinician in the diagnosis (Figure 2). However nodular achromic melanoma is a true diagnostic challenge and it is often misclassified as non melanoma skin cancer.

**Hypopigmented Form:** hypomelanotic spectrum subdivided in partially pigmented melanomas, in which the pigmented area can cover an area ≤ 25% of the total surface and lightly pigmented melanomas, in which a light brown, light blue or light gray pigmentation is generally present on all or part of the lesion (at least 25% of the surface). In this form of melanoma it is important to carefully evaluate the presence or absence of pigmentation and/or dermoscopic melanocytic structures on the edge (1) (Figure 3).
Reflectance confocal microscopy features

In the last few years reflectance confocal microscopy (RCM) has opened a new dimension in non-invasive optical digital biopsy of skin tissue (22-24). RCM analyzes different horizontal layers of the epidermis, dermo-epidermal junction and papillary dermis and permits a close derooscopy/confocal/histological correlation. Furthermore skin tissue is evaluated at the cellular level in relation to its histological counterparts especially in melanocytic nevi and melanoma. The melanoma spectrum using RCM is characterized by different cytologic and architectural features mainly related to the presence of atypical cells at the dermo-epidermal junction, rounded pagetoid cells in the epidermis, disarrangement of the rete ridge in superficial spreading melanoma and cerebriform nests in nodular melanoma (22, 23). Based on the dermal vertical growth and the limited extension of histologically atypical cell infiltrate in the epidermal layers of nodular melanoma, RCM features show: 1) atrophic or thin epidermis commonly spared by RCM pagetoid cells or limited to few cells focally distributed; 2) dermo-epidermal junction destroyed with pleomorphic cells in sheet-like structures; 3) presence of single or variable-in-size clusters of atypical melanocytes in the upper dermis in proximity to or overlapping enlarged and convoluted vessels (22, 23). The presence of cerebriform nests is a predictive marker of nodular melanoma that is characterized by low reflective atypical clusters of melanocytes with granular cytoplasm without distinct nuclei and bordered by brighter collagen structures (Figures 4, 5). RCM evaluation may be limited by reduced-depth laser penetration, but the presence of the thin epidermal layer of nodular melanoma permits the analysis of the dermo-epidermal junction and upper dermis, thus adding information to the cytological and architectural morphology of the malignant lesion (22, 23). In the dermo-epidermal junction and the dermal component, nodular melanoma and the nodular phase of superficial spreading melanoma share similar confocal and histologic parameters (22). However epidermal disarrangement...
and moderate or widespread pagetoid infiltration are specific RCM markers of superficial spreading melanoma, whereas thin or preserved epidermis with or without few pagetoid cells is characteristic of nodular melanoma (22).

Final remarks

Nodular melanoma is a subtype of melanoma distinct from superficial spreading melanoma from the clinical point of view and for its dermoscopic/confocal and histologic features. It is defined as an invasive melanoma that lacks a significant component of epidermal tumor cells besides the invasive dermal component. It represents from 10 to 30% of all melanomas. Due to its rapid vertical growth and the absence of a radial growth phase it is diagnosed already in an advanced phase and represents the main cause of death among cutaneous tumors. Clinically it generally presents as a symmetrical, regular nodule or papule with clean edges and a hard consistency. For these reasons the clinical diagnosis of nodular melanoma is more difficult as clinically it does not reflect the ABCD criteria which, on the other hand, are useful in the superficial spreading subtype. Therefore, it is preferred to use the EFG rule (Elevation, Firmness, rapid Growth rate). Histologically nodular melanoma is characterized by the absence of atypical melanocytes in the epidermis (up to 3 crests) beyond the lateral margins of the malignant dermal component, with theca cells and/or confluent aggregates without maturation phenomena. The chromatic spectrum presents: amelanotic/hypomelanotic with little or no trace of pigment or strongly pigmented with a homogeneous blackish color or with a polychromy variable from blue to black up to pink and gray. The utilization of dermoscopy in the last twenty years has made it possible to improve the accuracy of the diagnosis of melanomatous lesions, even if this method is closely correlated with the experience of the dermatologist. The study of lesions with histopathologic diagnosis of nodular melanoma has made it possible to identify criteria for dermoscopic diagnosis. Dermoscopic structures closely correlated with nodular melanoma are asymmetry of the pigmentation pattern, blue-white veil, blue-black areas, milky red areas, irregular dots and globules, irregular streaks, atypical vascular pattern with polymorphic vessels. Recently, the use of RCM has been able to add information to dermoscopy and highlight cytological and architectural patterns of melanoma where the presence of cerebriform nests are specific markers of nodular melanoma. In the spectrum of melanoma progression a dermatologist has a crucial role in the identification of early melanoma. However, the combination between clinical parameters and non-invasive skin techniques are needed to warrant a correct evaluation of a pigmented, hypopigmented or achromic nodular lesion. In the diagnostic challenge of nodular melanoma integration of RCM in the clinical and dermoscopic assessment might also provide additional quasi-histological criteria in the decision-taking process.

Financial disclosure

None reported.

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