Case Report

Clinical and Pathological Analysis of Two Cases of Cutaneous Malignant Melanoma

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ABSTRACT: The purpose of this study was the clinical and histo-immunohistochemical analysis of two cases: a cutaneous pigmented facial malignant melanoma and a lumbar congenital nevus with malignant transformation. A series of clinical elements raised the suspicion of some malignant melanocytic lesions and the histopathological analysis through the paraffin embedding technique confirmed the clinical suspicion. The immunohistochemical analysis using the streptavidin-biotin-peroxydase method of the facial malignant melanoma showed: S100 protein intense and diffuse positive, Tyrosinase diffuse positive, HMB45 strong and focal positive, Cyclin D1 positive in approximately 40% and Ki-67 positive in almost 70% of the tumor cells. The malignant melanoma developed on the nevocellular nevus displayed: S100 protein intense and diffuse positive, both in the nevus cells and in the malignant melanocytes as well, Tyrosinase intense and diffuse positive in the malignant melanocytes, poor and focal positive in the nevus cells and HMB45 intense and focal positive in the malignant cells and positive in the isolated nevus cells. Cyclin D1 was positive in about 70% of the malignant cells, but negative in the nevus area and Ki-67 was found positive in relatively 30% of the malignant melanocytes, also in less than 1% of the nevus cells. The pattern and the intensity of the Tyrosinase and HMB45 immunoexpression are important in the differentiation of the nevus cells from the malignant melanocytic cells. The immunoexpression of Cyclin D1 does not correlate directly with the proliferating activity of the malignant melanocytic cells in all types of malignant melanomas.

KEYWORDS: *malignant melanoma, malignant transformation of congenital nevus, immunohistochemistry, tumor markers*

Introduction

The cutaneous malignant melanoma (MM) continues to persist nowadays, due to its serious issues in evolution and prognosis, which always remain unpredictable, especially when the patient fails to attend the physician on time. The diagnosis methods for the disease detection in its precocious state are based, essentially, on the morphoclinical, dermoscopic, microscopic and immunohistochemical aspects that facilitate the assessment of the tumor's depth in millimetres, this matter being a decisive factor in stating a prognosis even more accurate. We also cannot yet disregard that there are authors who consider these data insufficient for an exact evaluation of the risk regarding the tumor progression [1, 2].

We focus our attention on two cases submitted at the Dermatology Clinic within the County Clinical Emergency Hospital from Craiova in September 2014, as well as April 2015.

The first case was patient P.E., woman, aged 67, who was hospitalized due to the apparition on the left cheek of a roundly-oval tumor lesion, with the diameter of 1/1.5 cm, of dark-brownish colour with pigmented halo around and a small central ulceration.

The family medical history presented nothing conclusive. From the personal medical history of the patient, however, we noted: a total hysterectomy at age 48 as a result of a uterine fibroma, thyroidectomy by cause of a papillary carcinoma in 2013, the patient also being a 1st degree hypertensive.

The general clinical examination was within the normal limits, and the ultrasonography did not detect suspicious lymph nodes. The dermoscopic examination was carried out with a FotoFinder dermoscope which revealed an asymmetrical lesion with polymorphism and polychromatism with atypical pigmentary network and the presence of the "veil" characterizing the malignant melanoma (Fig.1a). The surgical intervention was decided, excising the tumor "in toto", with oncological safe margins and the sample was sent for histopathological analysis (Fig.1b).

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Fusing the evidence of the clinical examination with the dermoscopic, histopathological and immunohistochemical analysis, the diagnosis of malignant melanoma was obvious.

The second case was the patient P.G., a male aged 57, who, in April 2015, presented himself for hospitalization with a round tumor lesion of 1.2 cm in diameter, of brown colour, situated in the lumbar region (Fig.2 a,b).

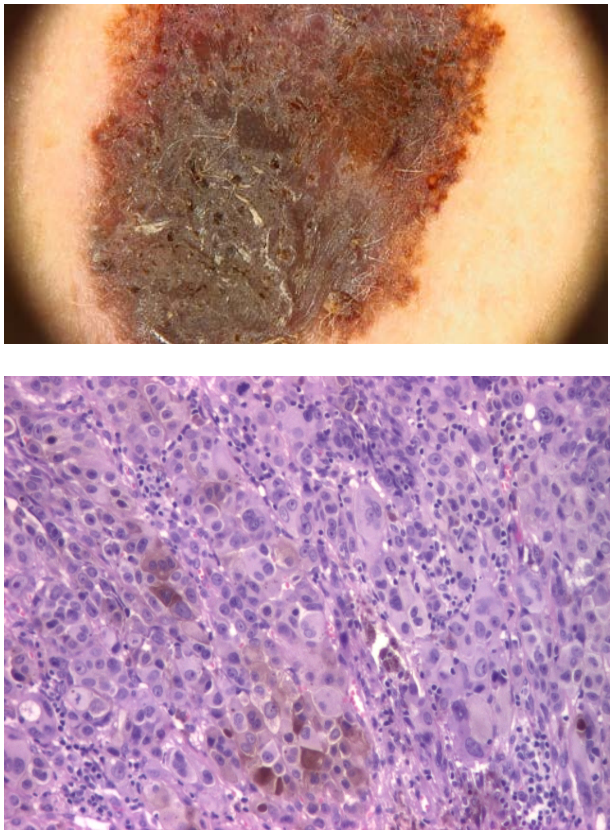

Fig.1. Patient with facial malignant melanoma: a. FotoFinder dermoscopic image; b. HE staining, x100.

From the family medical history nothing significant was recorded. Regarding the personal history, it is worth mentioning that the patient displayed numerous facial actinic keratosis, being a 2nd degree phototype, with a hypersensitivity to the sun. We also disclose the obesity (90 kg at 1.68m height) with Corporal Mass Index 32, non-smoker and occasional alcohol consumer.

From the patient's statements resulted that the tumor lesion was present since childhood, growing very slowly and gradually over the years, with no subjective symptomatology. It is to be emphasized that the tumor was traumatized repeatedly, without being visibly altered. Yet, a year ago, after a new trauma, the tumor started to expand, but without being painful. Local examination revealed that the tumor was cherry-sized, of dark-brownish colour towards black, with small prominences (blackberry aspect), inconsistent pigmentation and some pilosity.

This clinical feature determined us to consider a malignant melanoma, the rapid and recent development, proeminences on the surface and marbled chromatism supporting this idea. Simultaneously, the tumor also offered some contradictory malignant transformation arguments, such as: it was a pilose nevus tumor, which is commonly benign and does not present a red inflammatory halo. Nevertheless, the surgical excision was extensive, with oncological safe borders.

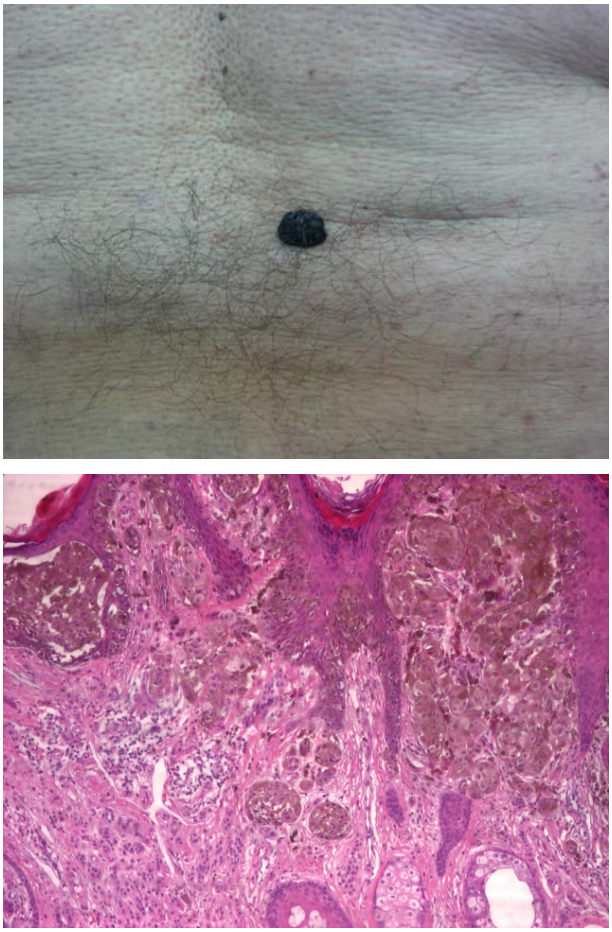

Fig.2. Patient with lumbar malignant melanoma developed on congenital nevus: a. Clinical aspect; b. HE staining, x40.

Material and Methods

The tumor lesions in both cases were surgically removed "in toto" with safety borders and "per-primam" suture, then sent to the Pathology Laboratory for a histopathological analysis.

The excised tissue fragments were fixed in buffered formalin 10% and they were processed using the classical paraffin embedding

technique. The paraffin blocks were sectioned at 3-4 microns thick segments which were initially stained with the usual Haematoxylin-Eosin staining. Subsequently, serial sections were performed and displayed on glass slides coated with Poly-L-lysine for the immunohistochemical examination.

The immunohistochemical approach was the two-step technique with streptavidin-biotin-peroxydase as secondary antibody (LSAB plus kit, DakoCytomation, Denmark), and the substrate chromogen used for visualizing the immunoreactions was AEC (3-amino-9-ethylcarbazole). The primary antibodies used were: S100 Protein (polyclonal, DakoCytomation, dilution 1:500), HMB45 (clone HMB45, DakoCytomation dilution 1:50), Cyclin D1 (clone P2D11F11, Novocastra dilution 1:50), Tyrosinase (clone T311, Novocastra, dilution 1:40) and Ki-67 (clone MIB1, DakoCytomation, dilution 1:100).

Results

The histopathological investigation of the first case indicated a microscopic structure of malignant melanoma with epithelyoid cells, intensely pigmented and ulcerated, with significant inflammatory reaction, Clark level IV, with perivascular invasion and Breslow's depth of 3.5 mm (Fig.1b).

The immunohistochemical inquiry of the facial malignant melanoma case established: S100 protein intense and diffuse positive in the tumor cells (both at nuclear and cytoplasmic level), Tyrosinase diffuse positive in the tumor cells (with cytoplasmic pattern) (Fig.3) and HMB45 strong and focal positive in the cytoplasm of the malignant tumor cells (Fig.4). Cyclin D1 was positive in around 40% of the nuclei of the tumor malignant cells (Fig.5a) and Ki-67 was positive in nearly 70% of the nuclei of the tumor malignant cells (Fig.5b), (Table 1).

Table 1. Immunoexpression of analysed markers in the two cases of malignant melanoma

Antibodies	**Expression in facial malignant melanoma**	**Expression in lumbar malignant transformed nevus**
Protein S100	Intense and diffuse positive	Intense and diffuse positive in both components (nevus and MM)
Tyrosine	Diffuse positive	Intense and diffuse positive in the MM area, poorly focal positive in the nevus
HMB45	Intense and focal positive	Intense and focal positive in MM, positive just in the isolated nevus cells
Cyclin D1	Positive in approx. 40% of the tumor cells nuclei	Positive in approx. 70% of the MM cells nuclei and negative in the nevus area
Ki 67	Positive in approx. 70% of the nuclei of tumor malignant cells	Positive in approx. 30% from the nuclei of MM cells and in under 1% from the nevus cells

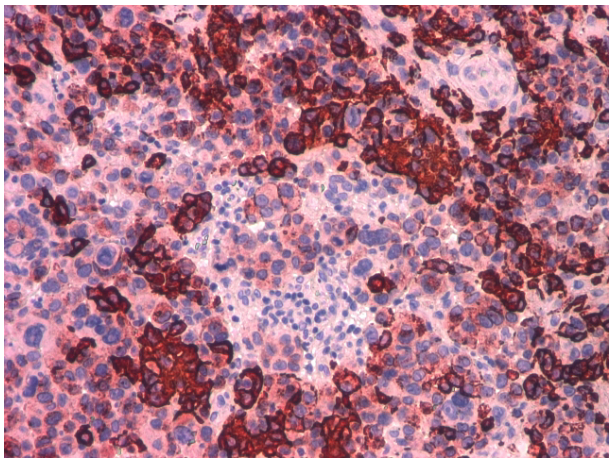

Fig.3. Patient with facial malignant melanoma: Tyrosinase immunostaining, x100.

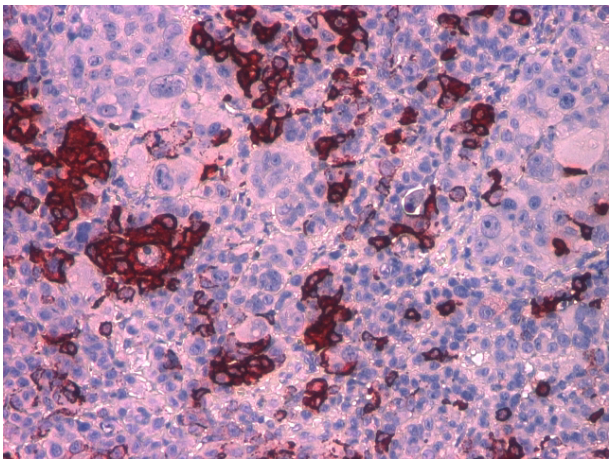

Fig.4. Patient with facial malignant melanoma: HMB45 immunostaining, x100.

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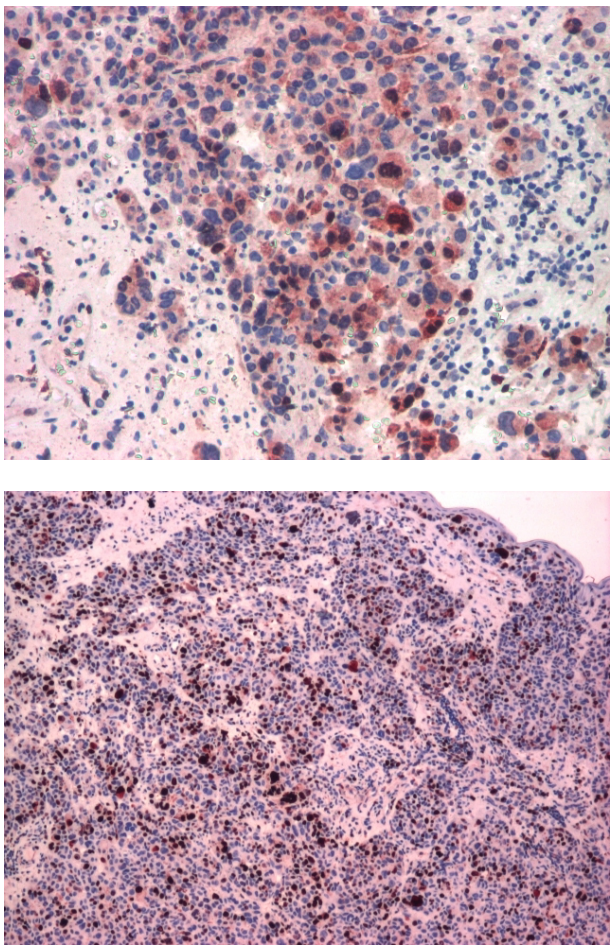

Fig.5. Patient with facial malignant melanoma: a. Cyclin D1immunostaining, x100; b. Ki-67 immunostaining, x40.

In the second case, the histopathological research confirmed the clinical suspicion, showing a microscopic structure of nevocellular nevus with malignant component, with areas of malignant melanoma extremely pigmented, with a Clark level III, ulcerated, with a moderate inflammatory infiltrate and Breslow's depth of 1.3 mm.

The immunohistochemical examination of the malignant melanoma case developed on the nevocellular nevus highlighted: the immunostaining of the S100 protein was intense and diffuse positive (a nuclear and cytoplasmic level) in both cellular components (the benign and the malignant cells), Tyrosinase was intense and diffuse positive in malignant melanocytes, poor and focal positive in the benign nevus cells (at cytoplasmic level) (Fig.6) and HMB45 was strong and focal positive in the malignant cells, positive only in the isolated nevus cells (at cytoplasmic level) (Fig.7). Cyclin D1 determined a nuclear positivity in nearly 70% of the malignant cells and negative in the nevus area (Fig.8a) and Ki-67 was positive in around 30% of the nuclei of the malignant melanocytes, also in under 1% from the nevus cells (Fig.8b), (Table 1).

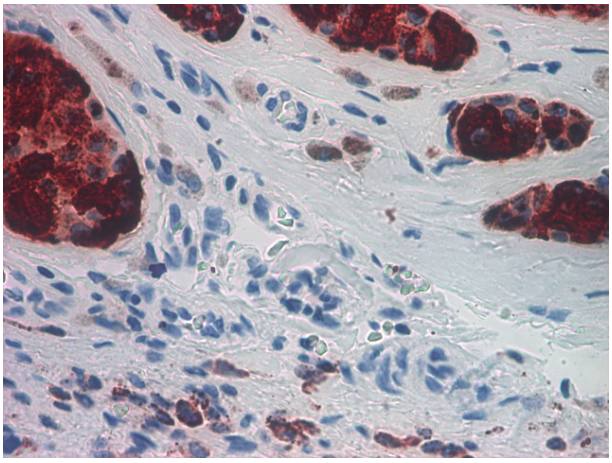

Fig.6. Patient with lumbar malignant melanoma developed on congenital nevus: Tyrosinase immunostaining, x200.

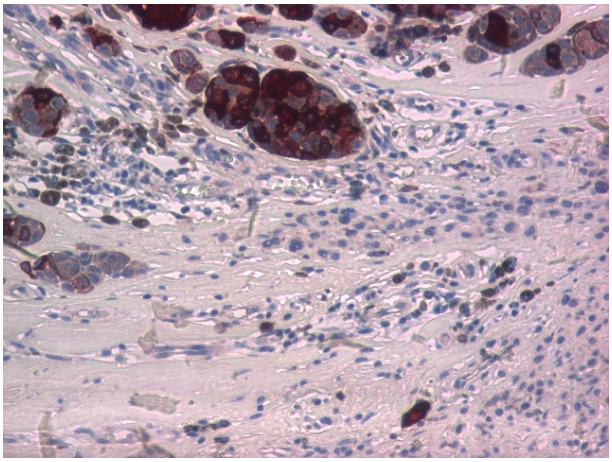

Fig.7. Patient with lumbar malignant melanoma developed on congenital nevus: HMB45 immunostaining, x100.

Discussions

We emphasize the fact that the malignant melanoma is a tumor which may develop in 30% of the cases from a pre-existing nevus, but may also occur "de novo" on an apparently normal skin. Topographically, the most frequent locations are on the torso of men and on the inferior limbs of women. The risk factors for developing such a tumor are multiple, among which it is worth mentioning the genetic predisposition and the individual phototype, the majority being phototype I: blond or red haired persons with prolonged exposure to the sun [3, 4]. Our cases are enclosed in I respectively II phototype.

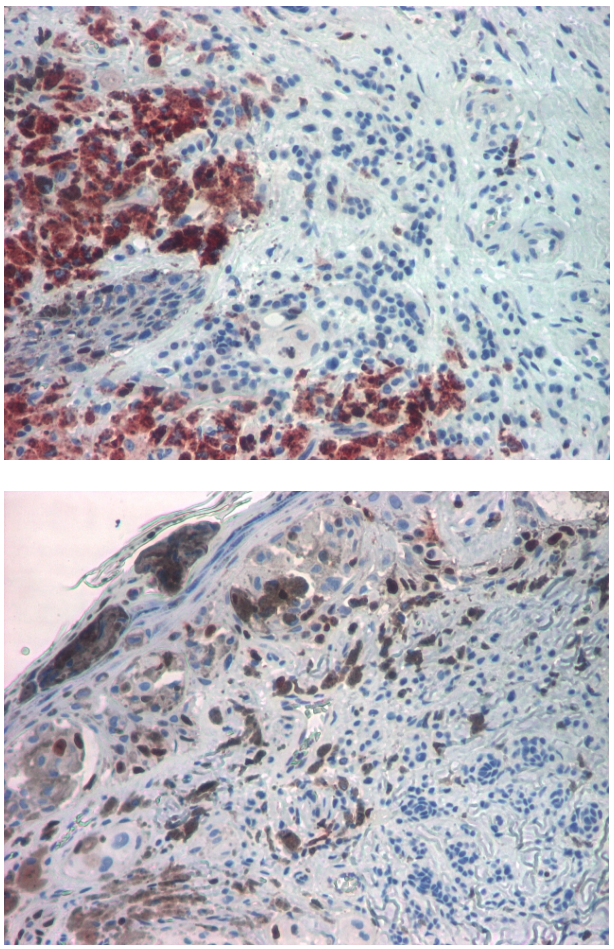

Fig.8. Patient with lumbar malignant melanoma developed on congenital nevus: a. Cyclin D1immunostaining, x100; b. Ki-67 immunostaining, x100

Considering the studies of Mishima and collaborators from 1989, it is notoriously understandable that the malignant melanoma can develop from three types of cells: nevocytes (derived from nevoblast, component of junctional nevus), epidermal melanocytes from the normal skin or from the Dubreulih melanosis and from dermic melanocytes (consisted in the blue nevus), and rarely [5]. It is important to assert the fact that the cutaneous malignant melanoma has a very large potential of metastasis, constituting the most frequent cutaneous tumor which metastases in the gastro-intestinal tract. Approximately 50% of the patients with digestive metastasis of MM have concomitantly other metastasic sites [6, 7].

Immediately upon a melanoma is clinically suspected, besides the paraclinical indispensable examinations (dermoscopy, biopsy, histopathological and immunohistochemical examination, confocal microscopy etc.), the determination of prognosis indexes such as Clark and Breslow have an apart priority. The clinical observations attest that also the topographic position on the cutaneous area has, likewise, a prognosis implication, the tumors located on the extremities having a more favorable forecast than the axial ones: head, neck, torso [8, 9]

We considered purposive to approach the case of P.G., on the grounds that, undoubtedly, the pilose blackberry-shaped nevocellular nevus does not have the malignant transformation tendency. In the mentioned case, additional investigations were imposed, for a more complete diagnosis to formulate a realistic prognosis. Accordingly, the following examinations were necessary: the ultrasound evaluation, CAT scan, MRI, current laboratory analysis and last but not least, the immunohistochemical examination of the biopsied sample, taking into account that we dealt with a relatively young patient with Clark level III, which associates to a high percentage of remote metastasis.

The immunohistochemical analysis of our cases displayed that the Tyrosinase and HMB45 had the same expression in the melanoma area of the malignant nevus, along with the facial MM (Table 1). To this extent, HMB45 and Tyrosinase presented a high intensity in the melanocytic malignant cells, with focal or diffuse pattern, whilst the nevus component expressed the same markers poorly, with focal pattern or just in isolated cells. Oppositely, the S100 protein presented an intense and diffuse character, both in the nevus and malignant melanocytic cells as well. These results advocate the significant role of the expression of Tyrosinase and HMB45 in the distinction of the melanocytic nevi from malignant melanomas. A series of studies have demonstrated the function of melanocytic differentiation antigens (S100 protein, HMB45, Melan A and Tyrosinase) in the distinction between malignant melanomas and common or dysplastic melanocytic nevi [10, 11]. HMB45 is particularly helpful in detecting the pattern of “maturation” of nevi, the descreasing expression of this marker with increasing depth in the dermis or diffuse expression throughout the lesion is suggestive of benign diagnosis, i.e. nevus. In contrast to nevi, primary cutaneous melanomas usually express HMB45 in a patchy pattern, with isolated or clustered cells throughout the dermis. Tyrosinase expression is very similar to HMB45 labeling [10].

Surprisingly, we remarked that an increased level of Cyclin D1 (70%) from the melanoma

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developed on congenital nevus was associated to relatively reduced value (30%) of the proliferation marker Ki 67, implying that the Cyclin D1 was in an inactive configuration. Consequently, it appears that the overexpression of Cyclin D1 did not have the role of accelerating the cellular cycle progression in the studied case. This prospect was according to the studies of Florenes V.A. and collaborators in 2000 [12], and also of Kranenburg O. and collaborators, who proved that in neuronal cells (related as origin with the nevi and melanocytic cells), the moderate expression of cyclin D1 stimulated the cellular proliferation, while the overexpression of cyclin D1 lead to apoptosis [13].

Similarly, the increased proliferated activity (Ki 67 positive in about 70% of the malignant cells) of the facial MM case, did not associate to very high levels of Cyclin D1 (40% of the tumor cells), suggesting that even other mechanisms might be involved in the aggression and unfavorable evolution of the malignant melanomas.

Recent studies have shown that the immunoexpression of Cyclin D1 does not have an impact over tumor progression and clinical evolution of all types of melanomas. Thus, the results of the hereby study were in concordance with these studies which demonstrated that whilst in the superficial melanoma exists a relationship between the expression of Cyclin D1 and that of Ki 67 ($p=0.0001$), for the nodular subtype of MM there are no such statistically significant correlations [14].

Likewise, numerous speciality studies have confirmed the importance of Cyclin D1 in regulating progression through the G1 restriction point by activating CDK4/6 [12]. Although, an association to classical markers of proliferation, such as Ki 67, has not always been achieved [15, 16].

Conclusions

The only treatment with a considerably efficiency in the primary cutaneous melanoma is the precocity of the diagnosis and the surgical excision.

Furthermore, it seems that an efficient management needs to concern, primarily, the prophylaxis, especially at phototype I persons, of whom professions require an elongated exposure to the sun.

Trying to rank the factors which can extend the survival of patients, the main place is occupied by the precocious detection of the cases and their correct coordination foreseeing the surgical treatment in useful time, a proper systemic treatment of the melanoma stage and also a post-therapeutic follow-up on long terms.

The immunohistochemical techniques, along with the interpretation of the pattern and the intensity of the immunoexpression of Tyrosinase and HMB45, play an important role in the differentiation of melanocytic nevi from malignant melanomas.

The immunoexpression of Cyclin D1 does not correlate directly with the proliferating activity (determined with Ki-67) of the tumor cells in all types of malignant melanomas, failing to have such an impact over the tumor progression and the clinical evolution.

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