CONSENSUS DOCUMENT

Protocol Proposal for the Histological Report of the Primary Tumor in Patients with Cutaneous Melanoma From the Task Force for Cutaneous Melanoma of the Valencian Community

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Abstract. This text compiles a histological protocol proposal for cutaneous melanoma agreed by the Task Force for Cutaneous Melanoma of the Valencian Community. It brings together the protocol itself and, in addition, includes a description of each of the proposed variables that has shown to have a greater prognostic impact in previous works.

Key words: melanoma, histology, protocol.

PROPUESTA DE PROTOCOLO PARA EL INFORME HISTOLÓGICO DEL TUMOR PRIMARIO DE LOS PACIENTES CON UN MELANOMA CUTÁNEO DEL GRUPO DE TRABAJO PARA EL MELANOMA CUTÁNEO DE LA COMUNIDAD VALENCIANA

Resumen. En este texto se describe una propuesta de protocolo histológico para el melanoma cutáneo realizado de forma consensuada por el Grupo de Trabajo para el Melanoma Cutáneo de la Comunidad Valenciana. En él se recoge el propio protocolo y, además, incluye la descripción de cada una de las variables propuestas que ha demostrado tener un mayor impacto en el pronóstico en los trabajos previos.

Palabras clave: melanoma, histología, protocolo.

Introduction

The prognosis of cutaneous melanoma varies according to the patient and depends on a wide range of factors. Knowledge of some of these factors has allowed different risk groups (stages) to be defined. Staging can help indicate the most appropriate clinical approach (in both diagnostic and therapeutic terms). The histologic features of the primary tumor constitute one of the most crucial known factors for establishing risk groups.

Clark et al[1] were the first authors to recognize that the anatomic region invaded by the tumor was important for determining the prognosis of melanoma. They therefore established what are nowadays known as levels of invasion or Clark levels. Then, in 1970, Breslow[2] described the use of an ocular micrometer to accurately measure the primary tumor thickness. For a long time, these 2 features were the most widely used prognostic variables by pathologists for predicting the biological behavior of the melanoma.

During the last 2 or 3 decades, many studies have confirmed beyond doubt that the tumor thickness, or Breslow thickness,[2] is the most important histologic prognostic factor for patients with localized cutaneous melanoma,[3-8] that is, for patients with no evidence of lymph node involvement or blood-borne metastases

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(stages I and II according to the most recent staging system recommended in 2001 by the American Joint Committee on Cancer[9]). However, it is known that the predicted outcome does not occur in a variable percentage of patients. Thus, all disease experts agree on the need to seek new prognostic models that provide a better understanding of the biological behavior of cutaneous melanoma. To this end, a number of research groups have conducted numerous studies that define several histologic characteristics apparently with additional prognostic value.[4-8,10-35] In fact, when the aforementioned current staging system was published, the authors encouraged further investigation of other histologic variables that were not included in the final system.[9] Such variables included tumor growth phase, histologic subtype, ulceration, presence of microscopic satellites and vascular invasion, characterization of inflammatory infiltrate, mitotic activity, predominant cell type, and presence of regression.

It is important to remember that most of the studies conducted to date have attempted to use models based on readily reproducible clinical and histologic variables. Thus, the most widely studied histologic variables are those which can be analyzed using paraffin-embedded tissue with hematoxylin–eosin staining. These variables include a certain degree of human judgment and so are subject to measurement errors. This error can be quantified by determining the reliability index, which measures the level of interobserver agreement. Studies have measured this interobserver variability, and the findings go some way to explaining some of the contradictory results in the literature.[36,37] For example, low interobserver variability has been reported for measurement of tumor thickness, ulceration,[36,37] and, more recently, assessment of mitotic index.[36]

Another problem associated with the high variability reported in some studies is that the interpretation of variables depends on a strong subjective component. This is the case, for example, in the assessment of inflammatory infiltrate or histologic type and leads to a large interobserver variability.[36,37] In addition, some studies cannot be readily compared because a precise definition of the variables used is lacking.[15,24]

It may be that in the medium or long term, the use of molecular biology techniques will give us a more accurate understanding of the biological behavior of certain tumors and allow greater tailoring of treatment for each melanoma patient. However, such objectives are still a long way off in the current health care setting. It therefore appears necessary to continue to record histologic data after standard hematoxylin–eosin staining of paraffin-embedded tumor tissue. Although there are many histologic protocols that generally include similar variables, there is a lack of unanimity regarding how all these variables are recorded. The aim of this article is to propose a protocol for recording the histologic features of the primary tumor and define each of them in accordance with the most precise and reproducible criteria available at present.

Protocol for Recording Histologic Data

The table shows the proposed form for recording histologic data. In addition to the patient's details, it is also important to record the site from where the sample was taken and the type of sample in the pathology report (excisional or incisional biopsy, punch biopsy or shave biopsy). A common practice in most referral centers is to request the original block (the block and sections) or at least a representative sample (one or several sections) when patients are referred to the center to complete treatment or perform follow-up. It is therefore important to state in the report what material was available for diagnosis as this might limit the value of the report in some cases.

The following points present the definitions and some comments on the variables recorded in the protocol.

1. *In situ or invasive melanoma.* An in situ melanoma is defined as one that is confined to the epidermis and so has not penetrated the epidermal basement membrane.
2. *Histologic type.* Four main histologic subtypes are defined: superficial spreading melanoma, lentigo maligna/lentigo maligna melanoma, acral lentiginous melanoma, and nodular melanoma.[1,38,39] These 4 variants are differentiated by the presence or absence of a radial growth phase (nodular melanoma lacks a radial growth phase) and, should one be present, the type of intraepidermal component.[39,40] The intraepidermal component can be pagetoid (superficial spreading melanoma) or lentiginous (lentigo maligna and lentigo maligna melanoma when photoaged skin is involved, acral lentiginous melanoma if palms and soles are involved, and mucosal melanoma).
Other less common variants have been reported but they have been the subject of fewer studies given their low incidence and so their prognostic value is unclear. One variant worthy of mention however is desmoplastic melanoma,[39,41] which may present with or without an epithelial component. In histologic terms, this variant comprises isolated spindle cells or fascicles or nests of cells within the desmoplastic stroma. One of the keys to its diagnosis is the presence of cell bundles in which the nuclei, with spindle-shaped morphology, are tightly arranged in parallel to one another such that they form a herringbone pattern. This variant is neurotropic in 50% of the cases.

Other forms of melanoma have also been described such as spitzoid melanoma, animal-type melanoma, and blue-nevus-like melanoma.[39]

3. *Growth phase*. Two growth phases can be distinguished: radial and vertical growth.[21] The essential difference between the two lies in their capacity for proliferation of tumor cells (that is, tumorigenicity) in the dermis. Melanomas are considered to be in the radial growth phase when they only have an epidermal component (in situ melanoma). Also classified as being in the radial growth phase are those melanomas that have tumor cells in the dermis either in isolation or forming nests smaller than those in the epidermis and that are not undergoing mitosis (microinvasive melanoma in radial growth phase). In contrast, the vertical growth phase includes melanomas that have tumor cells in the dermis, some of which are undergoing mitosis, or cells that form larger nests than those found in the epidermis.

Patients with melanoma in the radial growth phase (regardless of whether tumor cells are present in the dermis) have a survival close to 100 % at 8 years.[7,23,42] In addition, patients with melanomas in this phase do not display sentinel lymph node metastasis,[27] an observation which could be used to select patients for lymph node dissection.

It is important to point out that the interobserver variability is moderate and so a certain degree of previous experience and learning is required. Nevertheless, levels of agreement similar to those obtained with the Breslow thickness can be achieved.[43]

4. *Breslow thickness*. The definition of this variable has remained largely unchanged ever since it was first introduced by Breslow[2] in 1970. It is a quantitative measure of the extent of tumor invasion of the dermis. The measurement is made with a calibrated ocular micrometer. The depth is measured, in millimeters, from the most superficial level of the granular layer perpendicularly downwards to the deepest point of dermal invasion by the tumor mass. Invasion of the adventitial dermis is excluded unless this is the only site of dermal invasion. In this case, the depth is measured from the inner luminal surface of the eccrine gland or the lumen or inner aspect of the outer root sheath epithelium of the hair follicle. If the tumor is ulcerated, the measurement is taken from the base of the ulcer.

Future studies should clarify how to perform the measurement in those cases in which the primary tumor is confined exclusively to the dermis[44] (if this so-called dermal melanoma comes definitively to be considered a well-differentiated form of melanoma) in terms of whether the measurement should be taken

Table. Protocol for Histological Reports

Histological Protocol for Cutaneous Melanoma

Hospital No.   Biopsy No.
Name:
Surnames:
Sex: Age: Site: Type of sample:

1. Melanoma: ❏ In situ   ❏ Invasive

2. Histologic type: ❏ Superficial spreading
                      ❏ Type/associated with
                         lentigo maligna
                      ❏ Nodular
                      ❏ Acral lentiginous
                      ❏ Mucosal
                      ❏ Desmoplastic
                      ❏ Others: ....

3. Growth phase:
   ❏ Radial   ❏ No   ❏ Yes: ❏ Intraepithelial
                                  ❏ Invasive
   ❏ Vertical   ❏ No   ❏ Yes:

4. Maximum tumor thickness (Breslow thickness): .... mm

5. Clark level

6. Ulceration: ❏ No
                 ❏ Yes .... mm

7. No. mitoses/mm^2 [a]:

8. Regression: ❏ No
                 ❏ Yes: ❏ < 50 %
                          ❏ > 50 %

9. Lymphocytic infiltrate: ❏ Peritumoral: ❏ absent
                                           ❏ discontinuous
                                           ❏ brisk

                           ❏ Intratumoral: ❏ absent
                                           ❏ weak/moderate
                                           ❏ brisk

10. Vascular invasion: ❏ No   ❏ Yes

11. Perineural invasion: ❏ No   ❏ Yes

12. Microscopic satellites: ❏ No   ❏ Yes

13. Associated   ❏ None
    melanocytic   ❏ Common melanocytic nevus
    lesion: ❏ Dysplastic nevus
                     ❏ Congenital melanocytic nevus
                     ❏ Other: ....

14. Predominant cell type: ❏ Epithelioid
                           ❏ Spindle cell
                           ❏ Spitzoid/nevoid
                           ❏ Balloon cell
                           ❏ Others: ....

15. Actinic elastosis of the dermis
    of healthy skin around the tumor: ❏ No   ❏ Yes

16. Was resection of the melanoma complete?
                     ❏ Yes
                     ❏ No: ❏ laterally
                            ❏ vertically
                            ❏ both

[a]Dependent on the microscope, but a count over 5 fields at 400× magnification is accepted.

from the granular layer or whether the tumor diameter in the vertical plane is what should be taken into account.

5. *Clark level of invasion*. This variable seems to be relevant only for tumors less than 1 mm thick but it should nevertheless be recorded for all patients in view of the possibility that its prognostic value be confirmed in certain other special cases in the future. The Clark levels of invasion are defined as follows[1,38]:

– Level I: Malignant melanocytes are confined to the epidermis (in situ melanoma).
– Level II: Partial infiltration of the papillary dermis by isolated melanocytes or melanocytes in small nests is observed.
– Level III: Tumor cells fill and expand the papillary dermis and extend to the papillary–reticular dermal interface. Such processes can be identified by routine use of a polarizer and condenser lens to take advantage of the birefringence patterns of dermal collagen (collagen fibers of the papillary dermis are vertically oriented whereas those of the reticular dermis are arranged horizontally). At this level of invasion, isolated cells can be observed in the superficial reticular dermis, but they do not spread invasively.
It is worth highlighting that polypoid melanomas have at least level III invasion.
– Level IV: There is significant invasion of the reticular dermis by melanoma cells.
– Level V: Melanoma cells infiltrate the subcutaneous cellular tissue.

6. *Histologic ulceration*. Histologic ulceration is only considered when loss of total epidermal thickness has occurred.[7] The first important point is to ensure that loss of epidermal thickness resulting from an artifact in the processing of the histologic sample is not considered as ulceration. Such loss is usually readily distinguishable given that there is a total lack of fibrin or granulation tissue.[36] The second key distinction is whether or not the ulcer can be attributed to trauma. The clinical characteristics (a prior biopsy, presence of scarring, or a traumatic event in the patient's history) must be available to make this distinction. Without such information, it may be impossible to distinguish between the two types of ulcer, although the presence of epidermal margins with an abrupt squared profile or the presence of underlying V-shaped granulation is also suggestive of traumatic origin.[36] Some authors have defined 2 possible types of tumor ulceration: infiltrative (erosive) and attentuative.[36] Infiltrative ulceration is due to invasion of the epidermis by tumor cells, which disrupt cell junctions thereby ulcerating the epidermis. Attentuative ulceration on the other

hand tends to occur in nodular lesions that reduce the thickness of the epidermis by compression until ulceration finally occurs. Although the prognostic significance of this distinction is unclear, it seems reasonable to record the difference in a prospective pathology report given that the pathogenic mechanism seems to be different. Finally, it is recommended to measure the thickness of the ulcer, given that some authors have observed that this variable might have prognostic value (especially if that thickness is > 3 mm).[45] Other authors have pointed out that if a minimum value for the thickness of ulceration were to be established at a cutoff of, for example, 3 mm as above, the problem associated with evaluating small ulcer foci would be minimized.[36]

7. *Mitotic index (number of mitoses per mm²)*. Given that this variable seems to have returned once again to favor in recent years, it seems appropriate to define it as accurately as possible. The recommended method for measuring the mitotic index is as follows: the complete section is assessed to determine the dermal region of the tumor where there appear to be most mitoses. The number of mitoses is counted in an area of 1 mm² (approximately 5 high-power microscopic fields at a magnification of 400×). The area where the largest number of mitoses has been observed is identified and mitotic cells are counted in successive fields (according to the recommendations of the 1982 International Pathology Workshop[36,40]). With this method, a whole number of mitoses per square millimeter is obtained. The fields can include a mixture of tumor and stromal cells. If there are not enough fields, successive levels should be examined. Here, it is worth repeating that this system was proposed as a revised version of the one presented in the 1972 Sydney Classification of Malignant Melanoma, in which at least 10 high-power fields of the entire lesion were assessed, and the count was expressed as the number of mitoses per 5 high-power fields.[40] The original idea behind the proposal was to avoid the potential error in measurement associated with the optical characteristics of different microscopes. Comparison of the two methods has shown that the new approach improves prognostic value[5] and the reliability index, thereby reducing the interobserver variability.[36,37]

8. *Histologic regression of the tumor*. Regression is characterized by absence of melanoma cells in a focal region of the radial growth phase adjacent to the vertical growth phase, often bordered on one or both sides by the tumor. The epidermis is often attenuated by loss of the epidermal ridge pattern. In the underlying dermis, a thickening of the papillary dermis is observed with an increased number of

collagen fibers parallel to the epidermal surface (nonlaminated fibroplasia), along with weak diffuse lymphocytic infiltrate, presence of melanophages in the papillary dermis, and a variable degree of edema. It is possible to observe telangiectasia, typically oriented perpendicularly to the epidermis.[18,38] This histologic variable seems to be of particular relevance as it is associated with worse prognosis in thin tumors, especially when telangiectasia is extensive (affecting more than 75 % of tumor).[46-48] Our protocol, which aims to simplify data collection, classifies the presence of regression only according to whether it is greater or less than 50 %.

9. *Inflammatory infiltrates.* Infiltration by tumor infiltrating lymphocytes is classified as brisk if there is either diffuse lymphocytic infiltrate affecting the whole of the vertical growth phase of the tumor or lymphocytic infiltrate present in at least 90 % of the circumference of the tumor base. The infiltrate is classed as nonbrisk if it is focal and absent if there are no lymphocytes mixed with tumor cells, even if lymphocytes are present in the perivascular area within the tumor or beyond the tumor border.[18,38] It might also be of interest to indicate whether plasma cells are present because, although an uncommon finding, they have been shown to have certain negative predictive value in a previous study.[49]

10. *Vascular invasion.* This is defined as the unequivocal presence of tumor cells within the vascular lumen (of both lymphatic and blood vessels) adhered to the endothelium.[10,26] Although some authors use immunohistochemical markers (*Ulex europaeus*, CD31, or CD34) for staining, invasion does not appear to be more frequent than when hematoxylin and eosin are used.[26] Immunohistochemical markers should probably therefore only be used in very unclear situations. Recently, it has been proposed to extend the definition of vascular invasion to include the presence of tumor cells adhered to the exterior of the vessel without separating stroma.[26] The rationale behind this proposal is that the worsening in prognosis is similar to that observed when what we might call "true vascular invasion" is present. Although our proposed protocol does not include this point, it would be interesting to confirm its prognostic value in future studies.

11. *Perineural invasion* (neurotropism). This term refers to the presence of melanoma cells infiltrated in the perineurium and/or endoneurium. Such invasion might be hard to detect when only a few hyperchromatic nuclei can be found in the perineural region, which is thickened by fibrosis. In such instances, immunostaining (HNB.45, S-100, MART-1) might be needed to detect neoplastic processes.[39]

12. *Microscopic satellites.* Strictly speaking, microscopic satellites are defined as well-defined nests of tumor cells separated from the tumor mass (of the vertical growth phase) by a collagen layer or by subcutaneous cellular tissue at least 0.05 mm thick (measured with an ocular micrometer).[19,50,51] Free tumor cells or tumor cells separated only by tumor stroma do not qualify as microscopic satellites. It is important to ignore microscopic satellites when measuring the Breslow thickness or the Clark level of invasion.[51]

13. *Associated melanocytic lesion.* Some studies have found improved prognosis in the presence of melanocytic nevus associated with melanoma,[52] although this finding has not been confirmed.[10,11,14] In most cases (43%), the nevus will be of the dysplastic melanocytic type, but common melanocytic nevi, congenital melanocytic nevi, nevus spilus, and blue nevi may also be encountered.

14. *Predominant cell type.* It may be important to record the predominant cell type of the vertical growth phase. Distinction is made between epithelioid cells, including their nevoid variant, and spindle cells. Other cell types such as balloon cells are less common. Although many studies have shown a lack of correlation with cell type, this aspect has not been systemically studied and some authors have observed that tumors formed from well-differentiated spindle cells seem to be associated with better prognosis.[39]

15. *Actinic elastosis of the dermis of healthy skin around the tumor.* Actinic elastosis was included in the protocol because, although strictly speaking it has not been confirmed as a prognostic factor, its presence or absence may be a marker for genetic differences that may have some implications for the patient's treatment.[53]

16. *Was resection of the melanoma complete?* Information on disease in the surgical margins is essential in any report.

Conflict of Interest

The authors declare no conflict of interest.

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