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Diagnostic utility of epithelial and melanocitic markers with double sequential immunohistochemical staining in differentiating melanoma in situ from invasive melanoma

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ABSTRACT

Identification of melanoma in situ and its distinction from invasive melanoma is important because of its significant impact on morbidity and mortality. However, this interpretation can cause pitfalls in the diagnosis even with the use of immunohistochemistry. The aim of this study is to evaluate the diagnostic utility of epithelial makers (AE1/AE3, CK5/6, and p63) combined with melanocytic markers (HMB-45, S-100, or Melan-A) using dual-color immunohistochemical staining, performed on a single slide by sequentially applying the antibodies. In this study, we show 4 cases in which examination of routine hematoxylin and eosin slides did not allow for clear-cut distinction between in situ and invasive melanoma and highlight the utility of the double-staining method. Therefore, we recommend this double-staining method with melanocytic and epithelial markers as a helpful adjunct to the diagnosis of cases with a differential diagnosis between in situ and invasive melanoma.

1. Introduction

Identification of melanoma in situ (MIS) and its distinction from invasive melanoma is important because of its significant impact on morbidity and mortality [1]. Melanoma in situ is a proliferation of malignant melanocytes within the epidermis without invasion into the dermis. The criteria for histologic diagnosis include the following: (I) poor circumscription of the intraepidermal growth of melanocytes; (II) asymmetry; (III) a predominance of individual melanocytes over nests with confluent growth along the dermoepidermal junction, effacement of rete ridges, and pagetoid scatter; (IV) nests of atypical melanocytes with confluence, variations in shape and size, and consumption of epidermis; (V) haphazard distribution; and (VI) involvement of adnexal epithelium [2].

Distinction between MIS and thin melanoma (invasion ≤1.0 mm) may sometimes be difficult on routine slides [3,4]. Pseudomelanocytic nests at the dermoepidermal junction resemble melanocytic or keratinocytic nests and may be seen in the setting of lichenoid inflammation [2]. This interpretation can cause pitfalls even with immunohistochemical stains because it has been described that MART1 can be expressed in such nests of keratinocytes. Therefore, to avoid this possible diagnostic pitfall, the aim of this study was to evaluate the diagnostic utility of combining epithelial makers (AE1/AE3, CK5/6, and p63) with melanocytic markers (HMB-45, S-100, or Melan-A) on a single slide. This was achieved using dual-color immunohistochemical staining performed on a single slide by sequentially applying the antibodies.

2. Materials and methods

Dermatopathologists reviewed the most representative cases with difficult differential diagnosis between MIS or invasive melanoma of the archival material from National Institute of Cancer, Bogotá, Colombia. The inclusion criteria were biopsies with MIS with areas suspicious for dermal invasion on hematoxylin and eosin slides.

Two double-staining immunohistochemical techniques were performed for each case on 4-μm tissue sections mounted on Superfrost Plus slides (Thermo Scientific, Saint-Herblain, France) dried overnight at 37°C before processing. Double-stain IHC was performed on Ventana Benchmark XT automated slide preparation system (Roche Diagnostics, Meylan, France) using 2 different revelation kits: ultraView Universal DAB Detection Kit (Roche Diagnostics) and ultraView Universal Alkaline Phosphatase Red Detection Kit (Roche Diagnostics). This technique concerned melanocityc markers such as MART-1/Melan-A (clone A103), gp100 (clone HMB-45), and S-100 (clone S-100). These markers were combined with epithelial markers on the same slide; the epithelial markers used were CK5/6 (clone D5/16B4) and p63 (clone 4 A4). The melanocytic markers were conjugated with Fast red immunodye and the epithelial markers with diaminobenzidine.

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The dermoepidermal junction and the dermis were examined for the presence of immunohistochemically positive cells and correlated with the same areas in the hematoxylin and eosin slides. We evaluated the reactivity for single or for both markers to determine if the suspicious focus was located at the epidermis or if it was located in the dermis (ie, invasive focus) by the presence of a combination of melanocytes in red and keratinocytes in brown in the first scenario (MIS) and the presence of melanocytes only in the second one (invasive melanoma).

3. Results

We present a double sequential melanocytic technique in 4 cases, the first one corresponding to a melanocytic lesion with multiple nests of atypical melanocytes in the dermoepidermal junction with some isolated, atypical cells in the dermis suspicious of invasion. In this case, we used HMB-45 and CK5/6. The immunostudy indicated that there were melanoma cells in the dermis associated with keratinocytes and thus were interpreted as tangential cuts of the epidermal rete or skin adnexa, whereas the isolated cells did not express HMB-45 and thus were interpreted to be macrophages (Fig. 1). The second case was another melanoma predominantly in situ with a dense lichenoid infiltrate in the papillary dermis and scattered, highly atypical cells in the dermis suspicious of invasion. In the double sequential stain, we used HMB-45 and anti-p63. The atypical cells in the dermis were negative for either antibody, corresponding to reactive changes in fibroblast and endothelial cells. Furthermore, the immunohistochemical study delineated the in situ component including the involvement of skin adnexa (Fig. 2). The third case was very similar to the second one; it had a more prominent lymphocytic infiltrate in dermis along with some atypical cells with no apparent connection to the epidermis. However, the combination of Melan-A and CK5/6 revealed the coexistence of melanocytes and keratinocytes, that is, presence of an epithelial collarette below the melanocytes, thus indicating that they were nests of MIS and not invasive nests (Fig. 3). Finally, the fourth case was a second opinion of an acral lentiginous melanoma with anomalous tangential, sectioning that resulted on suspicion for invasion. Dual stain corroborated frequent foci of invasive melanoma lacking brown labeling cells (ie, keratinocytes) in contrast with the in situ component with epithelial and melanocytic brown and red dyes (Fig. 4).

4. Discussion

Distinguishing MIS from thin invasive melanoma (≤1.0 mm) may be sometimes difficult on histologic grounds alone. In particular, isolated cells in the dermis, tissue fragmentation, dense lichenoid inflammatory infiltrates, or tangential sectioning may raise the possibility of invasion. Isolated atypical cells in the dermis may represent invasive melanoma, macrophages, or even reactive fibroblasts or inflammatory cells. Also important to consider is the possibility of an associated nevus (intradermal or compound) [5]. Although this study did not include any such cases, HMB-45 may help in the distinction between invasive melanoma and nevus by the observation of "maturation" (decrease labeling with HMB-45) in dermal nevus cells [6].

Obviously, change in a diagnosis from MIS to stage IA involves different surgical treatments and follow-up recommendations [7,8].

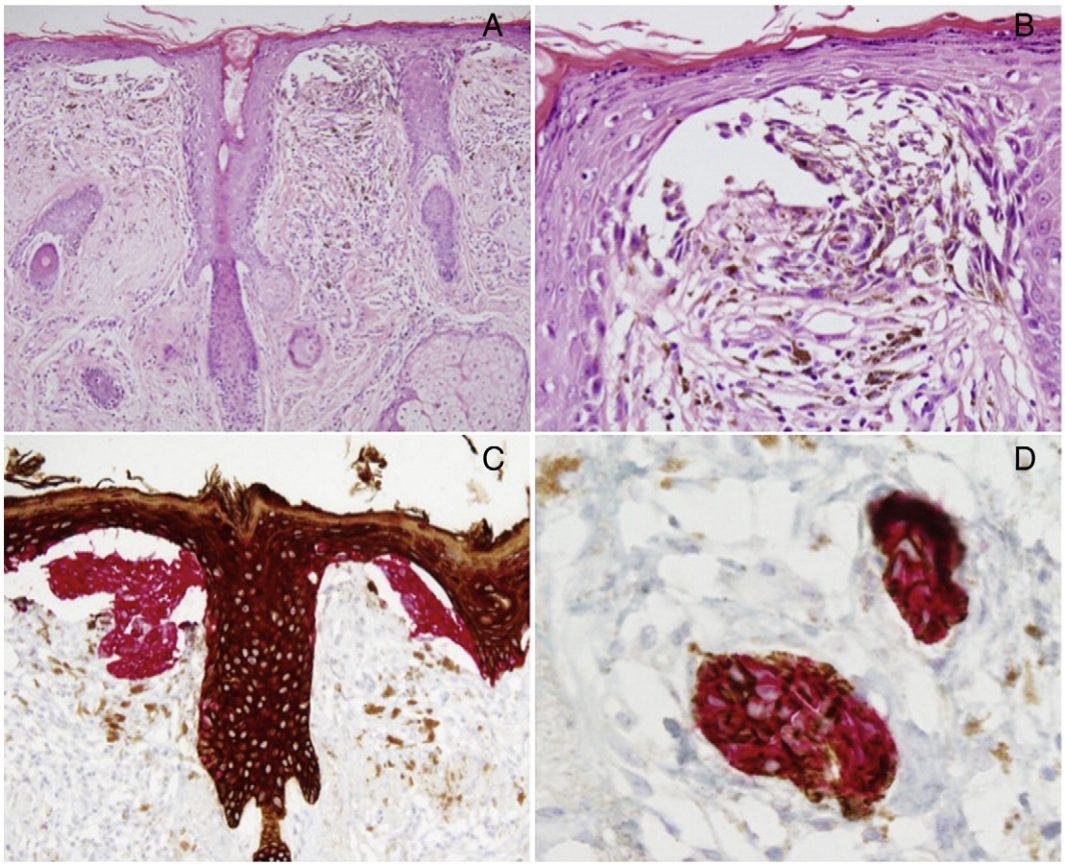

Fig. 1. (A and B) Panoramic and higher magnification of multiple voluminous intraepidermal foci of MIS with clefting and deep highly atypical hypercromatic cells in reactive dermal stroma. (C and D) In the immunostains, real cohesive MIS with some epithelial cells below clefts and aggregates of epithelial and melanocytic cells corresponding to a tangential cutting of epidermal rete. Dermal melanophages did not react with melanocytic markers.