Original Article

A Quantitative Immunohistochemical Evaluation of Lentigo Maligna and Pigmented Solar Keratosis

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Pigmented solar keratosis (PSK) is sometimes clinically indistinguishable from lentigo maligna, a form of malignant melanoma in situ. Occasionally histologic diagnosis is also difficult. Accurate diagnosis is essential, as the treatment and prognosis for each condition differs considerably. To determine whether there was a significant overlap in the number of melanocytes in these sun-damaged skin lesions, or whether immunohistochemistry might be helpful in the differential diagnosis, the authors examined skin biopsy specimens from 26 patients with obvious lentigo maligna and 15 patients with PSK using 3 monoclonal antibodies (HMB-45, NK1C3, and vimentin) and 1 polyclonal antibody (S-100 protein). Formalin-fixed paraffin sections were immunostained with each of the above antibodies, and immunopositive cells per mm² of epidermis were counted. The difference between lentigo maligna and PSK counts was statistically significant at a level of $P < .0001$; furthermore, there was almost no overlap between the two groups. The sensitivity for the diagnosis of lentigo maligna was high with all antibodies. However, HMB-45 had the highest sensitivity and the lowest false-positive rate and was visually most pleasing. Using a cut-off count of 60 cells per mm² of epidermis, HMB-45 had a sensitivity of 96% and a 0% false-positive rate.

In this study, lentigo maligna was easily differentiated from PSK. The real value of immunohistochemistry in the differential diagnosis of these pigmented lesions should be tested in a prospective study using cases that are difficult to diagnose by routine light microscopy. (Key words: Lentigo maligna; Pigmented solar keratosis; Quantitative immunohistochemistry; Receiver operator characteristic curves) Am J Clin Pathol 1993; 100:681–685.

Pigmented solar keratosis (PSK), a lesion clinically resembling lentigo maligna, has received scant attention in the pathology literature.[1-3] Correct diagnosis is important, as the prognosis and treatment differs considerably from lentigo maligna. Although the exact risk for invasion and metastases is unknown,[4,5] lentigo maligna (a form of malignant melanoma in situ) is potentially lethal and usually requires total excision. Pigmented solar keratosis, however, requires less rigorous ablation, often by such nonsurgical means as cryotherapy. Distinction between both conditions is made by skin biopsy and histologic examination. Atypical melanocytes aligned along the basal layer of atrophic sun-damaged skin characterize lentigo maligna. Melanization of the epidermis superimposed on the usual features of solar keratosis characterizes PSK. Usually these conditions are readily distinguished on routine histologic examination. However, recognition of atypical melanocytes is sometimes difficult; these may be scant or lightly pigmented in a small biopsy specimen. Pigmented solar keratoses is sometimes atrophic[6,7] and contains vacuolated pigmented basal keratinocytes that resemble melanocytes, making diagnosis difficult.

Immunohistochemically, a variety of antibodies help identify melanocytes. Immunohistochemistry is now the usual method of choice in distinguishing malignant melanoma from other neoplasms. Antibodies to melanocytes, keratin, and epithelial membrane antigen reliably distinguish between Paget's disease, Bowen's disease, and superficial spreading melanoma.[8,9] Using a similar immunohistochemical approach, we counted basal melanocytes in lentigo maligna and PSK to see if there was a significant overlap in the number of melanocytes in these sun-damaged pigmented lesions, and if these lesions were reliably separated by quantitative assessment of basal melanocytes.

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MATERIALS AND METHODS

Fifteen consecutive cases of PSK and 26 consecutive cases of lentigo maligna were selected from the pathology files of the Mater Hospital, Dublin, Ireland. Diagnosis was based on the histologic appearance in hematoxylin- and eosin-stained paraffin sections. Lentigo maligna sections demonstrated solar elastosis and variable epidermal atrophy with proliferation of single atypical melanocytes along the basal layer. Pigmented solar keratosis showed solar elastosis, varying degrees of epidermal atrophy or thickening, and no obvious melanocyte proliferation; keratinocyte atypia varied from minimal to moderate. Both lesions showed varying degrees of pigmentation. All cases were diagnosed clinically as either lentigo maligna or ?lentigo maligna; pigmentation varied from light to very dense. The incised biopsy specimens were 4 mm, 6 mm, or larger, taken from sun-exposed areas; all cases diagnosed as lentigo maligna on biopsy specimens were confirmed on surgical excision specimens. The patients with lentigo maligna were aged 39–84 years; those with PSK, 54–77 years. Specimens were formalin-fixed (unbuffered), paraffin-embedded, sectioned at 4 μm, and immunostained with antibodies to S-100 protein, vimentin, HMB-45, and NKIC3 using the avidin-biotin-peroxidase com-

TABLE 1. ANTIBODIES USED FOR IMMUNOHISTOCHEMICAL EVALUATION

Antibody	*Dilution*	*Source*	*Reactive Cells*	*Reference*
HMB-45	1:3000	Enzo (New York)	Malignant melanoma, spitz nevus, junctional nevus	25
S-100	1:1000	Dako (Copenhagen)	Malignant melanoma, benign nevus	26, 27
			Schwann cells	28
			Langherhan's cells	29
			Cartilage	30
NK1C3	1:10	Symbio (Uden, The Netherlands)	Malignant melanoma, benign nevus	31, 32
Vimentin	1:10	Dako (Copenhagen)	Melanoma, wide spectrum of connective tissue tumors	33

plex technique of Hsu and colleagues[10] (using diaminobenzidine as the chromogen). In previous studies, these antibodies marked melanocytes. The source of antisera, dilutions used, and main immunoreactivities are listed in Table 1.

Evaluation of Immunohistochemical Reaction

In each case, a segment of epidermis (excluding follicular epithelium) containing the greatest concentration of immunoreactive melanocytes was photographed. This area was mapped and measured from photographic prints using computer-assisted interactive morphometry with a Kontron MOP (Kontron, Munich, Germany) videoplan. Reactive cells situated on or just above the basement membrane were counted and expressed as counts per mm^2. Although area measurements were made from photographic prints, cell counts were carried out microscopically with an oil immersion objective lens (× 100 magnification, numeric aperture 1.25) and a × 10 eyepiece. It was easier to count individual cells with the microscope than from photographs. Some sections required bleaching before immunostaining, as pigmentation was intense. Bleaching did not interfere with immunostaining of HMB-45, S100, or vimentin. However, staining intensity was moderately reduced with NK1C3. Counts were confined to photographed areas.

Statistical Analysis

For each antibody the significance between lentigo maligna and PSK counts was calculated using the Mann-Whitney test; reported *P* values were two-sided. In addition, a receiver operator characteristic (ROC) curve was constructed for each antibody. An ROC curve is a method for graphically relating the sensitivity (true-positive rate) of a test to its false-positive rate (100 - specificity). When determining the presence or absence of disease (eg, lentigo maligna), if a test result is expressed in numbers on a continuous scale, the sensitivity and false-positive rate of that test changes by altering the criterion for positivity (ie, the counts). In an ROC curve the true-positive rate (sensitivity) is plotted against the false-positive rate using a range of test results.[11-13]

RESULTS

Melanocyte counts for each antibody clearly separated lentigo maligna from PSK; the differences in median counts were highly significant ($P < .0001$), with minimal overlap (Fig. 1). Receiver operator characteristic analysis showed that HMB-45 counts of 60/mm^2 identified lentigo maligna with a 96% sensitivity rate and gave no false-positive results. Very high sensitivity and low false-positive rates were also seen with the other antibodies (at higher counts). These are illustrated in Figure 2.

Although all antibodies gave similar results, HMB-45 staining was most pleasing visually and interpretation was easiest. Staining was crisp and confined to melanocytes, and produced virtually no background staining (Fig. 3). S-100 protein stained Langerhan's cells (presumably) in the epidermis and perineurial cells in the dermis in addition to melanocytes. Vimentin stained Langerhan's cells and perineurial cells in addition to other connective-tissue cells, such as endothelial cells, fibroblasts, and smooth muscle cells. NK1C3-stained sections often had an annoying degree of background dermal collagen staining. Despite these drawbacks melanocytes could be identified and counted quite well.

DISCUSSION

Immunohistochemistry is now a routine technique in most large histopathology departments, and its value in tumor differential diagnosis is well established. Many antibodies mark

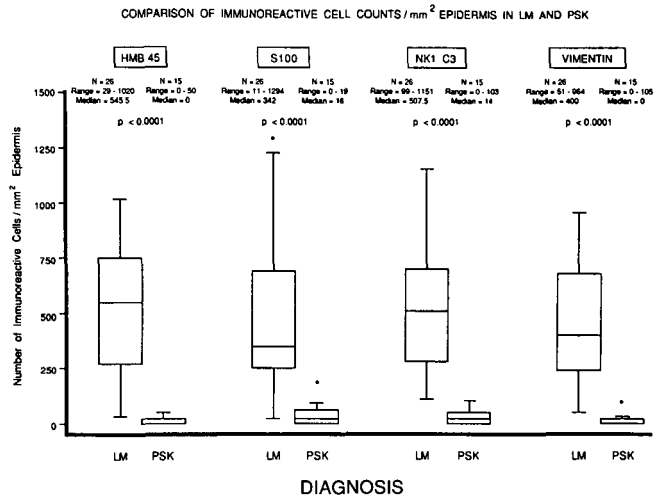

FIG. 1. Box and whiskers graph of the counts for each antibody. The central box contains 50% of the observations. The upper and lower limbs, or whiskers, represent the 90th and 10th percentiles. The rare single observations outside these limits are above the 90th percentile and below the 10th percentile. The central line in the box marks the median observation. These graphs show minimal overlap between the counts found in lentigo maligna and pigmented solar keratosis. LM = lentigo maligna, PSK = pigmented solar keratosis.

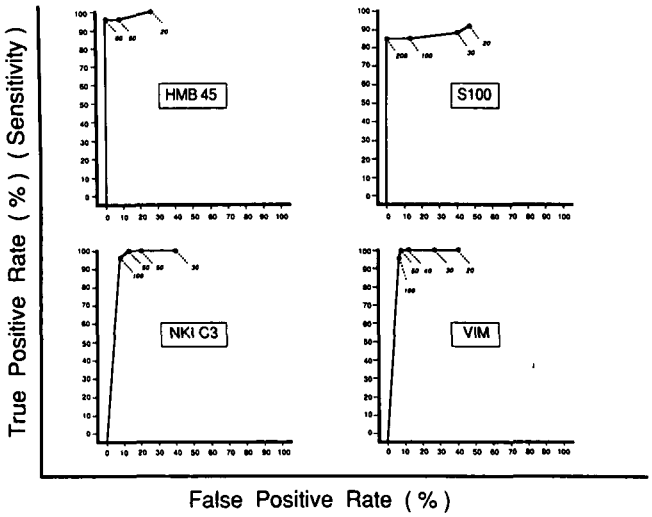

FIG. 2. Receiver operator characteristic curve for each of the antibodies used. The numbers at the end of the broken lines represent counts per mm^2 of epidermis. Each curve has a steep ascent on the left of the curve with an abrupt transition to its horizontal component. The shape of this curve is typical of a test with good sensitivity and specificity. LM = lentigo maligna. PSK = pigmented solar keratosis.

normal melanocytes, nevus cells, and melanoma cells in paraffin sections.[14-16] These will not differentiate benign from malignant tumors and are not absolutely melanocyte-specific; however, they are reliable in separating melanocytes from keratinocytes. They show varying degrees of immunoreactivity, so in general a panel of antibodies of different sensitivities and specificities should distinguish melanocytes from nonmelanocytic cells. At present HMB-45 seems to be the most useful marker.[16,17]

In this study, melanocyte counts clearly separated lentigo maligna from PSK with a high degree of statistical significance; overlap was minimal. Using ROC curve analysis, the true-positive rate (sensitivity) and the false-positive rate of various antibody counts change by setting the diagnostic counts to different thresholds.

Although our findings are clear, we encountered some practical problems early in the study. Melanin had to be distinguished from the brown immunoreactive product. In our preparations melanin is slightly green and is identified by the combination of its color and granularity. Other ploys are helpful if required: use a different staining technique with a different color reaction product, eg, red with the alkaline phosphatase peroxidase antiperoxidase technique;[18] bleach the melanin before staining;[19] or use 3-amino-9-ethylcarbazole as the chromogen instead of diaminobenzidine—the immunoreactive color product is red.

The number of positive cells in lentigo maligna varied from area to area within the same piece of tissue. We selected areas with the greatest melanocyte density. In lentigo maligna, however, there were parts of the epidermis that contained few melanocytes. A small biopsy specimen from one of these areas might be misleading or difficult to interpret. If small punch biopsy specimens are used, examination of two or more specimens from different areas should reduce the risk of this pitfall. Possible sources of variable immunoreactivity include differences in fixation, tissue preparation, and staining that vary from laboratory to laboratory.[20] This is especially true with specimens from outside clinics or hospitals. In this study, all specimens were taken by the same clinicians, in the same clinic. All were fixed in unbuffered formalin, processed, and immunostained in the same laboratory and interpreted by the same pathologist.

We did not study observer variability. All histologic quantitative or grading techniques suffer from a certain amount of observer variability. This is likely to be greatest where counts are very high and where groups of positive cells touch, overlap, or lie close to each other, and smallest where counts are low and cells are widely dispersed. From a practical point of view this is unlikely to pose a significant diagnostic problem, as very low and very high counts are easily recognized. Although we have quantified our results, in practice it was easy to differentiate the two diagnostic groups simply by looking at the slides and deciding whether there were very few or many positive cells.

Our findings raise some theoretical questions, not addressed specifically in this study. Are HMB45-immunoreactive cells present in normal epidermis or in non-PSK? What is the clini-

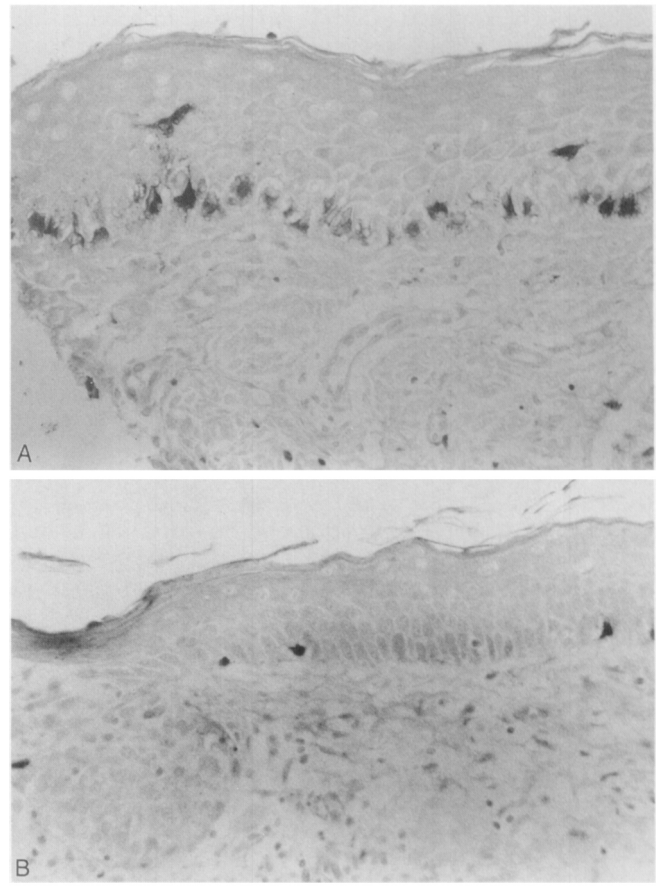

Fig. 3. A, Lentigo maligna. Numerous HMB-45 positive cells along the basal epidermis (Immunoperoxidase HMB-45). B, Pigmented solar keratosis. Epidermal hyperplasia at left of photomicrograph. Few HMB-45 positive cells in same area; most biopsy specimens of PSK show fewer immunopositive cells than illustrated here (Immunoperoxidase HMB-45, ×200). This contrast in the number of immunoreactive cells is typical of the difference between lentigo maligna and PSK.

cal significance and the natural history of a few positive HMB45-positive cells in pigmented sun-damaged skin? Do some melanomas arise in PSK?

Previous studies and our personal observations show that the normal epidermis contains vimentin-, NK1C3-, and S-100–positive cells; similar cells occur also in non-PSK. Increased melanocyte counts have also been reported in solar lentigines.[21] Recent studies by Smoller and colleagues[22,23] demonstrated HMB45-positive cells in the epidermis overlying scars and hemangiomas; they postulated that HMB45-positive cells were possibly induced by increased vascularity or by some plasma factor or endothelial-derived growth factor. We have seen rare HMB45-positive cells in non-PSK. We do not know the clinical significance of this finding and have never seen PSK progress to malignant melanoma. There is no theoretical reason why lentigo maligna and PSK may not exist in the same biopsy specimen. Progression to pigmented squamous cell carcinoma has been described.[3,24] Other studies have shown that HMB45-positive cells are absent from normal adult skin,[25] ie, normal melanocytes are nonreactive with this antibody; our own observations agree with these findings. In PSK, therefore, the HMB45-positive cells must represent either a de novo mild proliferation of abnormal melanocytes or a change in existing melanocytes.

In summary, we found a distinct immunohistochemical difference between obvious lentigo maligna and PSK. Our results confirm and quantify what is already well known to dermatopathologists and histopathologists experienced in dermatopathology, namely, that lentigo maligna contains many abnormal melanocytes and PSK contains few. However, pathologists who have little experience with skin diseases may not appreciate the extent of this difference. The cases studied were straightforward, selected on the basis of conventional light microscopic diagnosis, so the true diagnostic value of these antibodies should be evaluated prospectively in a sample of biopsy specimens presenting genuine diagnostic problems. In day-to-day practice, lentigo maligna and PSK are diagnosed in conventional hematoxylin and eosin sections. However, in difficult cases our findings suggest that immunohistochemistry might be helpful.

In cases where the diagnosis is still equivocal after immunostaining, a second biopsy should be done. At this stage, if the diagnosis is unresolved, a clinical decision must be made to either remove the pigmented lesion or observe the patient and perform another biopsy at a later date. The latter course of action is often acceptable if the lesion is large and located on the face and the patient is elderly. Such lesions usually evolve very slowly.

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