Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail

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ABSTRACT

Incidence of malignant melanoma (MM) is rising rapidly throughout the Western world, and the number of melanocytic lesions removed for histological assessment has increased. MM can present with a myriad of histological appearances that make diagnosis problematic, particularly when dealing with metastatic deposits. Immunohistochemical diagnosis relies on a panel of antibodies comprising polyclonal S100 protein and the monoclonal antibodies HMB 45, MART-1, tyrosinase and, to a lesser extent, NKIC3. Confirmation of problematic cases relies on the use of polyclonal S100 protein, as its sensitivity has yet to be matched by any monoclonal antibody. The introduction of a potentially valuable pan-melanoma cocktail, composed of HMB 45, MART-1 and tyrosinase, is examined in 50 primary cutaneous malignant melanomas, five desmoplastic malignant melanomas (DMM), 35 benign naevi, 20 metastatic malignant melanomas, 10 basal cell carcinomas (BCC) and 10 squamous cell carcinomas (SCC) and compared to individual immunolabelling with S100 protein, HMB 45, MART-1 and tyrosinase. All BCCs and SCCs were negative with all antibodies. S100 protein, MART-1, tyrosinase and the pan-melanoma cocktail were positive for all cases of benign naevi. HMB 45 labelled all junctional and compound naevi, five of the eight intradermal naevi and five of the seven blue naevi. All 50 primary cutaneous MMs were positive with S100 protein, 49/50 with the pan-melanoma cocktail and tyrosinase, 47/50 with MART-1 and 46/50 with HMB 45. Of the five cases of DMM, all were positive with S100 protein and three of the five were positive with HMB 45, MART-1, tyrosinase and the pan-melanoma cocktail. In the case of metastatic MM, all 20 cases were positive with S100 protein, the pan-melanoma cocktail and tyrosinase. MART-1 was positive in 19/20 cases and HMB 45 in 17/20 cases. The pan-melanoma cocktail showed a high sensitivity for all forms of MM and should be considered a complementary marker to polyclonal S100 protein. Results confirmed that currently there is no alternative antibody available to match the sensitivity of polyclonal S100 protein for immunolabelling DMM.

KEY WORDS: Immunohistochemistry. Melanoma.

Introduction

Incidence and mortality rates for malignant melanoma (MM) in most countries, particularly throughout the Western world, have increased significantly over recent decades. The annual increase in incidence rates in the fair-skinned Caucasian population is between 3% and 7%.[1] Data produced on the cumulative lifetime risk for MM for the year 2000 showed that one in 25 individuals in Australia and approximately one in 75 in the USA are likely to develop MM during their lifetimes.[2] Although mortality rates are less dramatic (indeed, they are dropping), studies indicate that approximately 20% of all cases in the fair-skinned population across most countries in Europe, Australia and USA are likely to die from the disease.[1]

Clearly, the need for early clinical appraisal and subsequent histological evaluation of suspicious lesions is paramount in the diagnosis and management of patients with MM. In recent years, there have been substantive advances in laboratory-based investigations, with the most practically useful developments focusing on the introduction of a growing range of antibodies to assist in the histopathological interpretation of MM.

The histological spectrum of appearance, particularly in amelanotic variants of the tumour, can be highly variable, both in terms of morphological criteria and tumour architecture.[3] In addition, the diagnosis in cases of minor metastatic deposits, where a plethora of non-melanocytic tumours may enter the differential diagnosis, often pose considerable challenges to the reporting histopathologist.[4]

Increasing interest in the use of sentinel lymph node appraisal for the early detection of metastatic spread has excited great interest, both in the USA and UK, and the clear benefits in staging melanoma means that it is likely to play a continuing role in the detection of early tumour metastasis over the next few years.[5] This procedure relies heavily on histopathological, immunocytochemical and, to a lesser extent, molecular evaluation of nodal tissue. Introduction of new policies to deal with the assessment of sentinel lymph nodes, involving multiple serial sections and repeated immunocytochemical investigations at multiple levels through the tissue, will contribute significantly to increases in laboratory workload. This is likely to be exacerbated by the increasing number of primary tumours submitted for histological examination.

The quest is on, therefore, to find and introduce more sophisticated investigative techniques that will help to

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reduce workload but ensure that quality and sensitivity are not compromised.

A recent development (Biocarta Europe, Borsteler Chaussee 53, D-22453 Hamburg Germany)[6] has seen the introduction of a new pan-melanoma cocktail– the first true pan-melanoma antibody for primary histopathological screening of MM – incorporating the three most widely used monoclonal antibody markers (HMB 45, MART-1 and tyrosinase) for the assessment of MM.

This study evaluates this pan-melanoma cocktail in the assessment of benign melanocytic lesions and in primary cutaneous and metastatic tumour deposits of both nodal and cutaneous origin.

Materials and methods

All cases were retrieved from the files of St. John's Dermatopathology Department and comprised 50 primary cutaneous MMs, five desmoplastic melanomas (three with overlying lentigo maligna melanoma) and 35 benign naevi (six juctional naevi, seven blue naevi, 15 compound naevi and eight intradermal naevi). In addition, 20 cases of metastatic MM, comprising nine cutaneous metastatic deposits and 11 sentinel lymph node deposits, were also studied. To check specificity, 10 basal cell carcinomas and 10 squamous cell carcinomas were also included.

All tissues were fixed in 10% neutral-buffered formalin for 24 h and processed to paraffin wax on a standard 15-hour cycle protocol in a Leica TP 10/50 enclosed tissue processor.

Sections (4 μm) were mounted on Superfrost Plus coated slides (Merck-BDH) and dried overnight at 37°C. Serial sections were cut in all cases and haematoxylin and eosin (H&E) and Masson Fontana stain for melanin were performed in all cases. A streptavidin-biotin complex technique was employed for all antibody labelling (Biocarta). This included a universal goat-linked biotinylated secondary antibody (4 Plus detection system) and a streptavidin-HRP tertiary antibody (4 Plus detection system).

Sections were placed directly into 250 mL Biocarta Borg Decloaker solution (BD 1000MM), which is a ready-to-use one-step dewaxing and heat retrieval solution (pH 9.0), and heated in a Panasonic or Sharp microwave oven. (200-1000 W range) for 20 min at 750 W, ensuring that no excessive boiling occurred. Sections were removed from the microwave oven and allowed to cool for 10 min. Endogenous peroxidase was blocked for 10 min using 3% H_2O_2 in methanol, then placed in phosphate-buffered saline (PBS; pH 7.4).

Pan-melanoma cocktail (Biocarta CM 165 A) was prepared as a 1 in 50 dilution using Biocarta primary antibody diluent (PD 900l.) and incubated at room temperature (RT) for 1 h. Sections were washed with PBS and incubated in Biocarta universal ready-to-use biotinylated secondary antibody (GU 600H) for 10 min at RT. Sections were washed in PBS and incubated with Biocarta streptavidin-HRP ready-to-use tertiary antibody (HP 604H) for 10 min at RT. Final reaction products were visualised using the Biocarta DAB500 chromogen system (1 drop of concentrated DAB per 1 mL of buffer). DAB incubation times were predetermined and set at 5 min. All sections were counterstained with Harris' haematoxylin for 1 min.

Positive control was a known MM universally positive with all melanoma markers (HMB 45, MART-1/Melan A, tyrosinase and S100 protein.) and negative control was by substitution of the primary antibody with PBS.

Results were scored as negative, focally positive (less than 5% of all melanoma cells) and positive. In addition, cases of desmoplastic melanoma were evaluated for junctional staining and spindle cell staining.

Results obtained with the pan-melanoma cocktail were compared with those achieved using the monoclonal antibodies HMB 45 (A. Menarini, Wokingham, Berkshire UK), MART-1 (A. Menarini) and tyrosinase (Vector

Table 1. Technical details of the antibodies used in the assessment of naevi and MM

Antibody	Source	Pretreatment	Dilution
S100 protein	Dako	Trypsin digestion (ICN)	1/4000
HMB 45	A. Menarini		1/40
MART-1	A. Menarini	Microwave 0.01 mol/L sodium citrate (pH 6.0)	1/40
Tyrosinase	Vector	Microwave 1 mmol/L EDTA (pH 8.0)	1/100
Pan-melanoma cocktail	Biocarta	Borg Decloaker solution (pH 9.0)	1/50

Table 2. Antibody labelling profiles of six melanocytic markers in the melanocytic and non-melanocytic tumours

	NUMBER OF POSITIVE CASES				
Antibody	BCC/SCC	Naevi	Primary MM	DMM	Metastatic MM
S100 protein	0/20	35/35	50/50	5/5	20/20
HMB 45	0/20	30/35	46/50	3/5	17/20
MART-1	0/20	35/35	47/50	3/5	19/20
Tyrosinase	0/20	35/35	49/50	3/5	20/20
Pan-melanoma cocktail	0/20	35/35	49/50	3/5	20/20

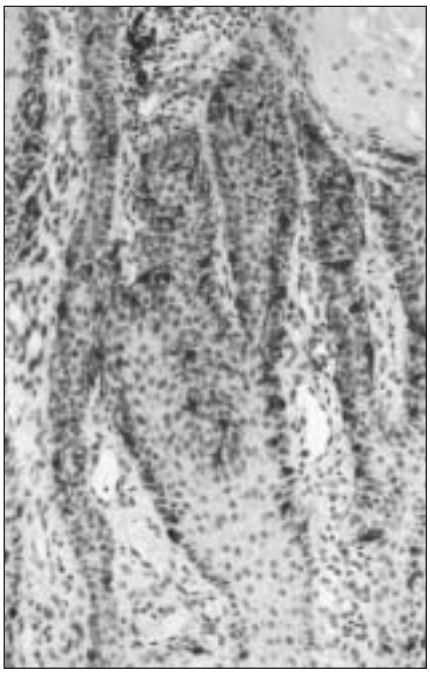

Fig. 1. Positive labelling with pan-melanoma cocktail of resting melanocytes in the epidermis, adjacent to tumour deposits in a case of basal cell carcinoma (original magnification x 50).

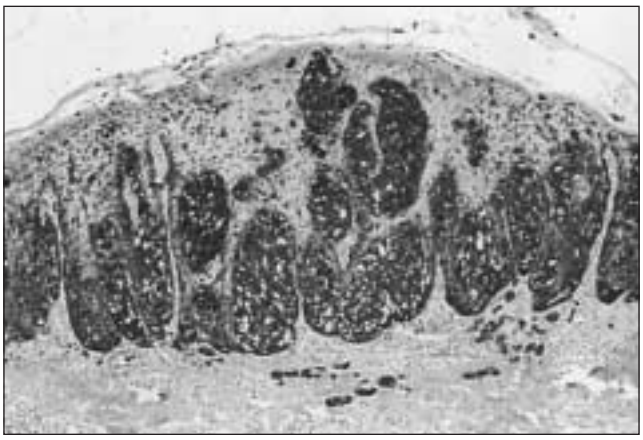

Fig. 2. Uniform labelling with the pan-melanoma cocktail in a case of a superficial spreading MM, showing tumour deposits predominantly throughout the epidermis (original magnification x10).

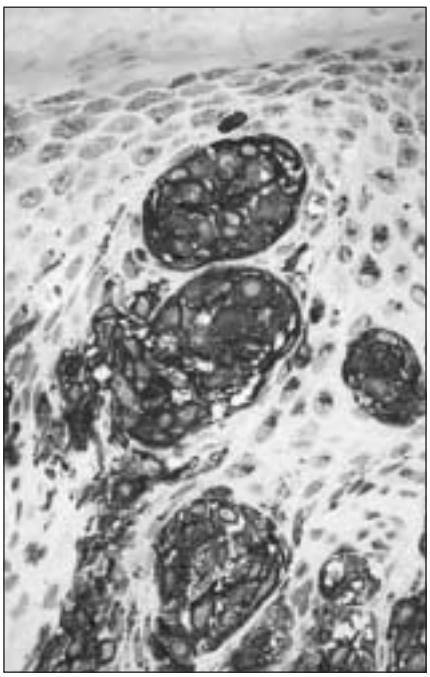

Fig. 3. Intense labelling of Pagetoid spread with pan-melanoma cocktail in a case of superficial spreading MM (original magnification x80).

Laboratories, Peterborough, UK), and polyclonal S100 protein (Dako, Ely, Cambridgeshire, UK). Antigen retrieval involved microwave heating at 700 W for 10 min in 0.01 mol/L sodium citrate at pH 6.0 (MART-1), 700 W for 12 min in 1 mmol/L EDTA at pH 8.0 (tyrosinase), or trypsin digestion (0.1 g; ICN Pharmaceuticals Basingstoke, Hants, UK) for 15 min at 37°C (polyclonal S100 protein). ChemMate streptavidin/biotin-HRP detection system (Dako, K5001) was used in all cases. Final reaction products were visualised using ChemMate DAB+ solution (Dako) and counterstained with Harris' haematoxylin for 1 min (Table 1).

Results

All immunolabelling data is presented in Table 2.

Basal cell carcinoma and squamous cell carcinoma
Immunolabelling of non-MM cases proved negative in all 20 cases with all antibodies. However, positive labelling of melanocytes was detected, mainly along the basal cell layer (junction between epidermis and dermis), with all antibodies except HMB 45 (Figure 1).

Benign melanocytic naevi
Strong immunolabelling was seen in all 35 cases examined, irrespective of naevus type, with polyclonal S100 protein, MART-1, tyrosinase and the pan-melanoma cocktail. The staining intensity was uniform in the majority of cases. Spindle cell components of blue naevi showed slightly less intense reactions. Labelling of both epidermal and dermal components of naevus cells was universally seen. HMB 45 demonstrated variable staining with blue and intradermal naevi.

Primary malignant melanoma
Of the 50 primary MM cases studied, 49 showed universal and intense, predominantly cytoplasmic, labelling of tumour cells throughout the lesion with the pan-melanoma cocktail (Figures 2-5) and tyrosinase (Figure 6). MART-1 labelled 47 cases (Figure 7) and HMB 45 labelled 46 cases. S100 protein labelled all 50 cases. The four negative cases contained only sparse or no melanin deposits, as demonstrated by Masson Fontana staining. Three cases were composed predominantly of spindle cells and the fourth was a MM composed of an epithelioid tumour cell population.

Desmoplastic malignant melanoma
The three cases with overlying lentigo maligna melanoma were strongly positive in the epidermal component with all antibodies. However, only sparse focal labelling was seen for the spindle cell component with MART-1, tyrosinase and the pan-melanoma cocktail. No labelling of the spindle cells was seen with HMB 45.

Polyclonal S100 protein was positive for both the overlying

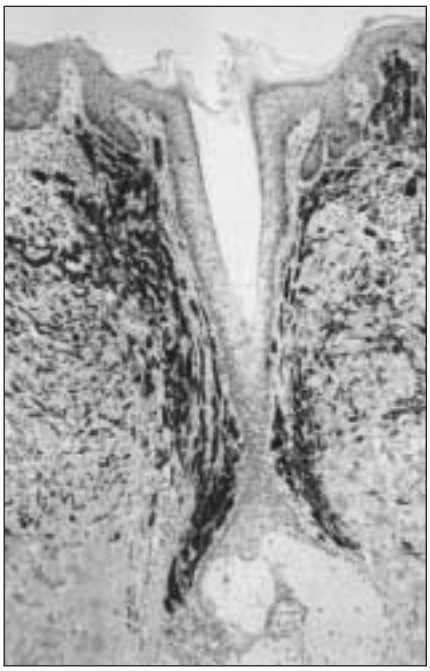

Fig. 4. Crisp, intense labelling with pan-melanoma cocktail of tumour cells sweeping down the side of a hair follicle and abutting onto the sebaceous gland, adjacent to the main tumour area, in case of superficial spreading MM (original magnification x 40).

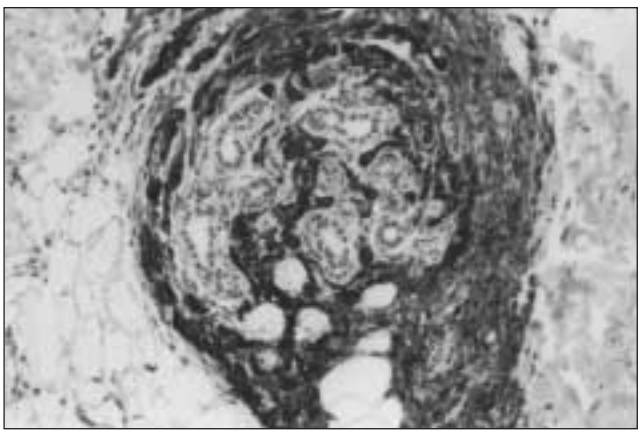

Fig. 5. Intense labelling with pan-melanoma cocktail in the deep portion of a nodular MM, showing tumour cells infiltrating and wrapping around the eccrine glands deep within the dermis (original magnification x 60).

lentigo maligna and the spindle cell population. Of the two cases in which there was no overlying lentigo maligna melanoma, showing just dermal spindle cells dissecting through the collagen, neither were labelled with any of the monoclonal antibodies or the pan-melanoma cocktail. Only S100 protein labelled the spindle cell population (Figure 8). Melanin deposition was absent from all the tumours.

Metastatic malignant melanoma

All 20 cases of cutaneous and lymph node metastasis studied showed positive labelling with polyclonal S100 protein, the pan-melanoma cocktail and tyrosinase. MART-1 was negative in one case. HMB 45 was focally positive in five cases and negative in three. In the nine cutaneous metastatic deposits, uniform labelling of the majority of tumour cells was seen in all cases with pan-melanoma cocktail, tyrosinase and polyclonal S100 protein. However, three cases were only focally positive and one was negative with HMB 45 and MART-1. The negative case was a spindle cell metastatic deposit.

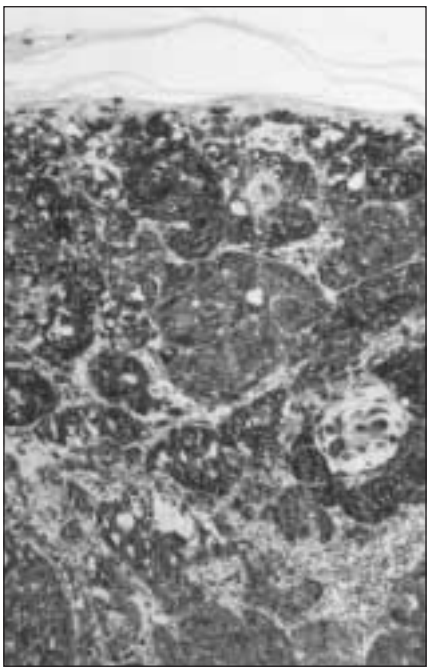

Fig. 6. Uniform labelling with tyrosinase throughout the tumour in a case of nodular MM. Note the negative labelling of inflammatory cells within the tumour mass (original magnification x50).

Of the 11 nodal metastases examined, labelling of large deposits was clearly demonstrated with all antibodies. More significantly, however, minor deposits of just one or two cells in other cases were clearly labelled and picked out from the surrounding lymphoid cells with the pan-melanoma cocktail (Figure 9), MART-1, tyrosinase (Figure 10) and polyclonal S100 protein. HMB 45 was only focally positive in two cases (Figure 11) and negative in two. Tinctorial stains for melanin demonstrated little or no melanin pigment in the large majority of cases studied.

Discussion

Application of immunocytochemistry to the evaluation of suspicious melanocytic lesions is of particular value in metastatic disease. The majority of primary cutaneous MM that exhibit characteristic histological/morphological features can be diagnosed without the need for immunocytochemistry. However, amelanotic variants of the tumour continue to pose the greatest challenge to diagnosis, mainly because of the myriad histological appearances that such tumours can present and, more significantly, the spectrum of differential diagnoses that may need to be considered.

The application of a selective panel of antibodies to confirm the nature of such tumours has always been of some value, and should include EMA, CEA, a pan-cytokeratin marker and LCA, as well as polyclonal S100 protein and at least one melanocyte-selective marker such as HMB 45, MART-1 or tyrosinase. In the large majority of cases this is adequate to reach a final diagnosis. In cases of minor metastatic tumour deposit, as often seen in sentinel lymph node biopsies from MM patients, the issue can be complicated still further by the sheer lack of tumour volume. The careful evaluation of a selective panel of markers, therefore, is of paramount importance because the issue of

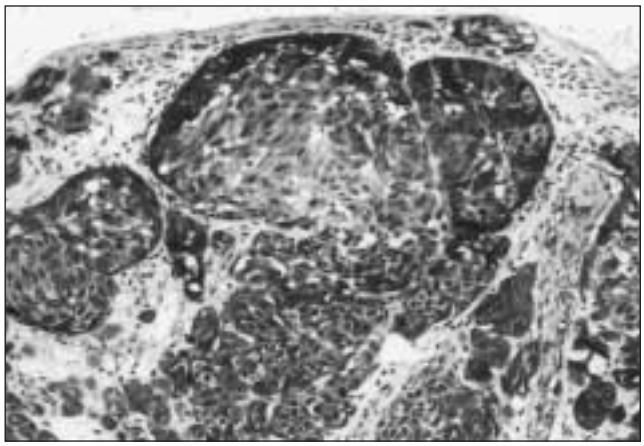

Fig. 7. Uniform labelling with MART-1 in a superficial spreading MM, showing Pagetoid spread of tumour cells (original magnification x50).

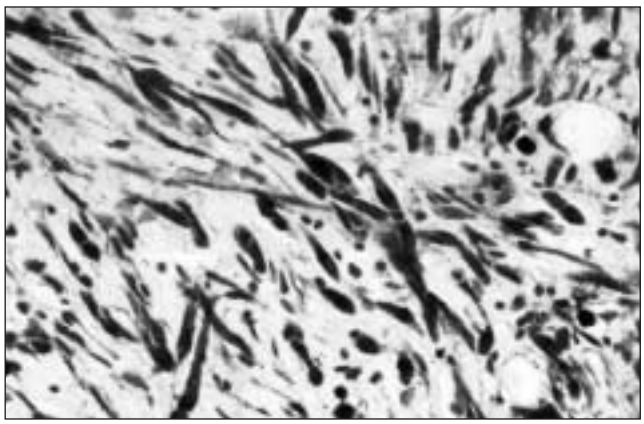

Fig. 8. Strong, crisp labelling of desmoplastic tumour cells with polyclonal S100 protein, deep in the dermis in a case of DMM (original magnification x70).

antibody sensitivity is vital if successful and reliable observations are to be made.

The 'gold standard' marker for MM is polyclonal S100 protein.[7] This antibody is reliably positive in nearly all cases of MM, irrespective of the histological variant of tumour presentation. However, although highly sensitive for MM, it has a low specificity and cross-reacts with a number of other tumour types. Thus, a melanocyte-selective monoclonal antibody is often used in conjunction with S100 protein.

However, none of the currently used markers has a sensitivity to match that of S100 protein. This is not a problem in primary cutaneous lesions, as clinical criteria may provide valuable information to assist the histological diagnosis. But this can be a more pronounced problem in cases of metastatic disease, where the origin or nature of the primary tumour is not always known. In addition, the deposits are quite often amelanotic and the histological/architectural features may not be so obvious to classify.

Application of immunocytochemistry in such cases is extremely valuable. Recent data evaluating the detection rates of antibodies to S100 protein, HMB 45, MART-1 and tyrosinase consistently imply that S100 protein is by far the most sensitive marker. A study by deVries *et al.*,[8] which

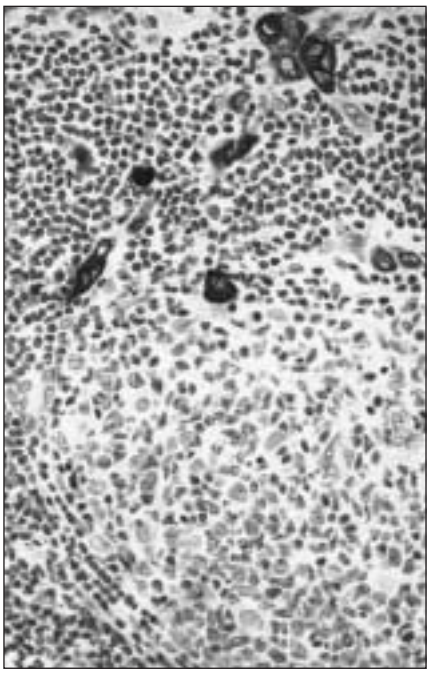

Fig. 9. Crisp, uniform labelling with pan-melanoma cocktail in a positive tumour-infiltrated sentinel lymph node, showing tumour cells invading through a lymphoid follicle and into the germinal centre (original magnification x60).

assessed positive labelling profiles of all four antibodies in 44 primary tumours, 18 locoregional metastases, 41 lymph node metastases and 27 visceral metastases from the lung, liver and brain, statistically supported the view that S100 protein was by far the most sensitive marker in all four types of lesion studied. The work also demonstrated significantly higher staining of T311 (tyrosinase) compared with HMB 45 in the primary melanomas studied; however, no significant differences were seen with the other antibodies under investigation.

A study by Hochberg *et al.*[9] of the expression of tyrosinase, MIA and MART-1 in sentinel lymph nodes of patients with MM using a reverse transcriptase-polymerase chain reaction found that both tyrosinase and MIA expression were sensitive indicators of micrometastases in sentinel lymph nodes. MIA is a 107 amino acid soluble protein described recently as an autocrine-secreted tumour cell growth inhibitor that functions as a regulator of adhesion to matrix proteins. MIA is strongly expressed by MM but is almost completely absent in naevi and normal skin.

Reports of heterogeneous labelling of metastatic melanoma deposits with HMB 45 is well documented in the literature, with one report suggesting a detection rate as low as 35%.[10] However, variations between the labelling profiles of MART-1 and tyrosinase are less striking.

The choice of which monoclonal antibody to use varies from one histopathology laboratory to another. Currently, HMB 45 is the preferred monoclonal antibody; however, it would seem logical, based on recently reported data, to use a pan-melanoma cocktail comprising HMB 45, tyrosinase and MART-1, in conjunction with polyclonal S100 protein, to improve the sensitivity of MM demonstration.

The results of the present study demonstrate that the new pan-melanoma cocktail labels all benign melanocytic lesions, irrespective of histological type. These observations also apply to MART-1, tyrosinase and polyclonal S100 protein. Universal labelling of epidermal and dermal compartments of all lesions, in addition to uninvolved non-activated

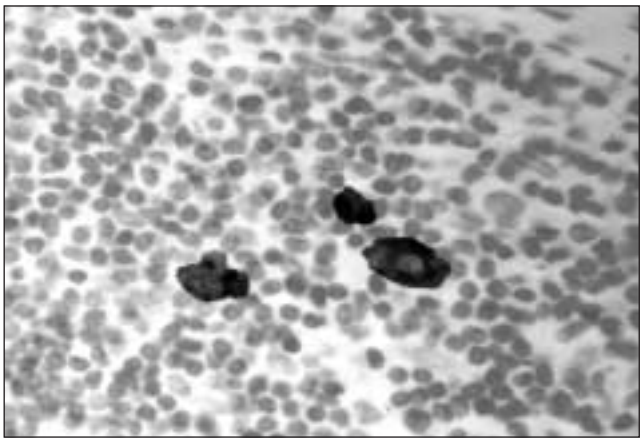

Fig.10. Three tumour cells labelled with tyrosinase invading the subcapsular sinus in a positive sentinel lymph node (original magnification x90).

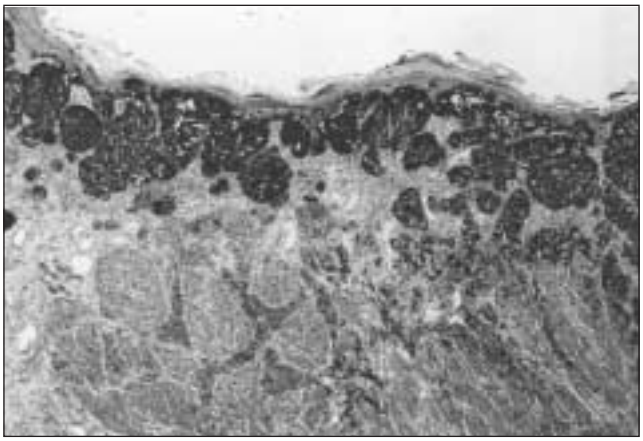

Fig.12. Characteristic HMB 45 labelling in a superficial spreading MM, showing intense labelling of the junctional component giving way to more variable staining in the dermal tumour cell population (original magnification x10).

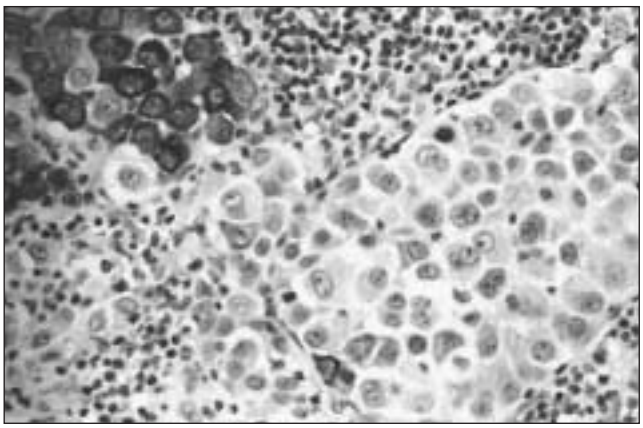

Fig.11. HMB 45 labelling a metastatic tumour deposit in a sentinel lymph node. Note the large percentage of cells on the right are negative (original magnification x60).

melanocytes, was seen in all cases. Results for HMB 45 demonstrated positive labelling of all junctional and compound naevi, but reduced labelling in both blue and intradermal naevi, neither of which have an intraepidermal component. This suggests that HMB 45 expression is largely focused at the junction between the epidermis and dermis and that its degree of expression is reduced in dermal deposits.

As expected, no positive labelling of basal cell carcinoma and squamous cell carcinoma was detected with any of the antibodies tested. However, examples of cross-reactive staining with HMB 45 and MART-1 are well documented and include tumours such as angiomyolipoma,[11] a benign tumour of the kidney. The reality, of course, is that this cross-reactive feature rarely poses problems in the differential diagnosis.

Labelling of primary cutaneous MM clearly demonstrated that S100 protein was positive in all cases, with the pan-melanoma cocktail and tyrosinase antibodies labelling 49 out of 50 cases. MART-1 and HMB 45 (Figure 12) labelled 47 and 46 cases, respectively. Staining profiles were all predominantly cytoplasmic. Those cases that were negative demonstrated little or no deposits with Masson Fontana staining, and three of the four were spindle cell melanomas and one was epithelioid.

Results for desmoplastic MM revealed that only S100 protein reliably stained all five cases, labelling both dermal spindle cells and overlying lentigo maligna melanoma. The three cases that exhibited lentigo maligna melanoma were positive in the epidermal component in all cases with all antibodies, but the dermal desmoplastic cells were only focally positive for MART-1 and tyrosinase in a single case. HMB 45 was negative in all desmoplastic cells. The remaining two cases, which solely exhibited dermal desmoplastic tumour cells, were completely negative with all markers except S100 protein – clearly indicating its value in such cases.

Desmoplastic MM is notoriously difficult to diagnose. Lesions often occur on the head and neck and may arise following excision of a conventional primary MM. In such cases, lesions may not present with an epidermal component but solely exhibit dermal spindle cells, which leads to a misdiagnosis of scar tissue or fibromatosis. S100 protein staining is essential in such cases.[12,13] One caveat to bear in mind here is that spindle cell remnants of nerve fibres damaged during primary excision of an MM will be S100-positive and may be present within the dermal mass. In cases of primary DMM, the differential diagnosis may include neurofibroma (NF), malignant peripheral nerve sheath (PNS) tumour, atypical fibroxanthoma (AFX) and even a spindle cell variant of carcinoma.[14,15]

Although S100 protein positivity can be a feature in some of these tumours, notably NF and PNS, careful scrutiny of morphological features and nuclear atypia should enable such distinctions to be made. Recently, microthalmia transcription factor (Mitf), an antibody raised against a protein encoded by the microthalmia *(mi)* gene believed to be essential as a transcription factor for the development and survival of melanocytes has proved useful in the assessment of epithelioid MM.[16] Interest in the evaluation of Mitf on spindle cell MM and DMM has produced conflicting data.

King *et al*.[14] reported just one out of 14 DMM cases to be positive with Mitf and found no positivity in spindle cell carcinoma, AFX or leiomyosarcoma. Granter *et al*.[15] reported

only four out of 21 spindle cell MM and DMM cases to be positive, but also stated that a proportion of dermatofibromas, schwannomas, leiomyomas and leiomyosarcomas studied were positive. The conclusions drawn implied that Mitf was neither a sensitive nor specific marker of spindle cell MM or DMM.

Conversely, Koch *et al*.[17] found Mitf expression in 11 out of 20 cases of spindle cell MM/DMM and in some neurofibromas, AFX, clear cell sarcomas and melanotic schwannomas. They concluded that the sensitivity and specificity of Mitf for DMM equals or exceeds that of HMB 45 or MART-1. In the author's experience, labelling profiles for Mitf demonstrate a nuclear staining pattern that labels most forms of melanocytic lesion. It is positive in normal resting melanocytes and its performance on spindle cell MM and DMM is only marginally better than that of HMB 45, and equivalent to MART-1.

An additional point worthy of note is that a recent report demonstrating positive labelling of DMM with CD34[18] suggests that it can simulate dermatofibrosarcoma protuberans (DFSP), a soft tissue tumour that is characteristically CD34-positive. This is supported by the work of Zelger *et al*.[3] on 2161 cases of MM in which 0.4% exhibited features simulating a host of soft tissue tumours, including DFSP, atypical fibroxanthoma, malignant fibrous histiocytoma, myxofibrosarcoma, malignant haemangiopericytoma and malignant peripheral nerve sheath tumour.

Data produced on labelling profiles for metastatic MM in the present study showed that polyclonal S100 protein, tyrosinase and the pan-melanoma cocktail labelled all 20 cases identically. Both HMB 45 and MART-1 were negative in one cutaneous metastatic deposit that morphologically was a spindle cell MM.

Of the 11 sentinel lymph node deposits studied, all demonstrated positive staining with polyclonal S100 protein, MART-1, tyrosinase and the pan-melanoma cocktail. HMB 45 was negative in two cases, both of which contained only a few tumour cells. Patchy or focal labelling with HMB 45 was seen in three cutaneous and two sentinel lymph node cases. Clearly, any degree of labelling with HMB 45 is sufficient to confirm the nature of the tumour cells, but when only minor deposits are present there is the possibility of a negative result. The implication from this is that HMB 45 should not be used as a primary marker for sentinel lymph node appraisal on the basis of its reduced sensitivity.

All but one spindle cell metastatic MM demonstrated a consistent degree of diffuse cytoplasmic positivity with MART-1; however, the intensity of labelling was less than that seen with the pan-melanoma cocktail, polyclonal S100 protein and tyrosinase. It would seem that the inclusion of tyrosinase in the pan-melanoma cocktail provided a more intense labelling profile on the cases studied in this series.

The main advantage to using the pan-melanoma cocktail must be its value in the assessment of sentinel lymph node tumour deposits, especially when these comprise just a handful of cells. It has a high sensitivity for all forms of MM, including metastatic deposits; however, tyrosinase exhibits similar high sensitivity, and both perform better than HMB 45 and MART-1.

Data presented here suggest that either the pan-melanoma cocktail or tyrosinase should be used to complement polyclonal S100 protein in the assessment of MM, and that the results achieved for spindle cell MM/DMM indicate that there is no alternative or complementary antibody currently available to match the sensitivity of polyclonal S100 protein in this group of tumours. □

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