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RESEARCH

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PRAME immunohistochemistry distinguishes nodal nevi from metastatic melanoma

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Abstract

Background Preferentially expressed antigen in melanoma (PRAME) is a promising immunohistochemical marker for distinguishing benign from malignant melanocytic lesions in lymph node deposits.

Objective To evaluate PRAME expression in metastatic melanomas and nevi found in the sentinel lymph nodes of patients with primary melanoma.

Methods Thirty patients, comprising 15 nodal nevi and 15 metastatic melanomas, were immunohistochemically analyzed for PRAME expression. Nuclear expression was scored as 0–25%, > 25–50%, > 50–75% or > 75% in tumor cells. The sensitivity, specificity, and positive and negative predictive values were calculated considering nuclear expression of PRAME > 75% as positive cases.

Results Cases previously diagnosed as nodal nevi were uniformly negative for PRAME. Conversely, all cases diagnosed as melanoma showed PRAME expression in more than 50% of the cells. Twelve cases showed expression above 75% of cells and were considered positive for calculations, resulting in sensitivity and specificity rates of 80% and 100%, respectively, with corresponding positive and negative predictive values of 100% and 83%.

Conclusions A high level of PRAME immunoreactivity was identified in metastatic melanoma, suggesting that PRAME is a useful analytical tool for confirming the diagnosis of melanoma in a melanocytic nodal deposit.

Keywords PRAME, Immunohistochemistry, Metastatic melanoma, Melanocytic nevi, Sentinel lymph nodes

Introduction

Despite advances in management and treatment, malignant melanoma (MM) continues to be a very aggressive skin cancer, with 324,635 new cases and 57,043 deaths in 2020, accounting for 1.7% of all cancers worldwide and increasing in incidence. Histological diagnosis continues to be highly important in the diagnostic and therapeutic care of patients suffering from malignant melanoma (Cazzato et al. 2022; Cassalia et al. 2024).

Sentinel lymph node (SL) biopsy is routinely performed on a subset of patients diagnosed with primary cutaneous melanoma. The positivity of metastatic melanoma

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in SL is relevant, as it is the strongest predictor of recurrence in patients with MM and is part of the prognostic assessment, clinical management, and therapeutic choice (Sharouni et al. 2021).

The morphological assessment of sentinel lymph nodes alone can be considered straightforward by most pathologists. However, the assessment of nodal melanocytic deposits is challenging (See et al. 2020; Grillini et al. 2022; Constantino et al. 2023; Lezcano et al. 2021a; Plotzke et al. 2022; Innocenti et al. 2023).

The diagnosis of subcapsular and intraparenchymal nodal nevi, metastatic melanoma confined to the fibrous capsule, and the coexistence of nodal nevi and metastatic melanoma may require auxiliary tests for a definitive diagnosis. This is due to cases in which melanoma does not show marked nuclear pleomorphism but rather characteristics of nevus cells (Lezcano et al. 2020a; Sharouni et al., 2021; Lezcano et al. 2018, 2020a, b; Constantino et al. 2022; See et al. 2020).

For this reason, together with conventional histopathology, auxiliary immunohistochemistry (IHC) techniques have been introduced in an attempt to minimize diagnostic difficulties, such as the application of antibodies Melan-A (MART-1), HMB-45 (human antimelanosome clone HMB45), MITF (melanocyte-inducing transcription factor) and, more recently, PRAME (Cazzato et al. 2022; Cassalia et al. 2024).

PRAME is a tumor-associated antigen that was identified by Ikeda et al. via autologous cloning of T-cell epitopes in patients with metastatic cutaneous melanoma (Lezcano et al. 2020a, b, 2021a, b; See et al. 2020).

The regulation of PRAME gene expression is still not fully understood. However, it has been shown to be modulated by epigenetic mechanisms such as DNA methylation, being hypermethylated in most normal tissues and hypomethylated in malignant cells (Lezcano et al., 2021). Thus, the overexpression of PRAME in many malignancies appears to contribute to cellular mechanisms of tumor growth and worse overall survival by antagonizing RAR (retinoic acid receptor) signaling (Cazzato et al. 2022).

The aim of the present study was to evaluate the expression profile of PRAME in metastatic melanomas and nevi in the sentinel lymph nodes of patients with primary melanoma.

Materials and methods

This is a cross-sectional, retrospective study of sentinel lymph nodes in patients diagnosed with melanoma on the basis of archival material from a referral pathology laboratory from January 2013 to January 2023. For convenience, the nevi samples available in the period were selected, and an equal number was defined for the metastatic melanoma samples. All diagnoses were established through morphological analysis and IHC. Only samples with sufficient material for immunohistochemical analysis were selected.

The slides stained with hematoxylin and eosin (HE) were reviewed by two pathologists, as were the IHC scans previously obtained with Melan-A and HMB45 from EnVision™ FLEX DAB+Chromogen (Dako Omnis) labeling. Six sequential sections with a thickness of 4 micrometers were taken from the paraffin blocks for PRAME evaluation. These samples were then subjected to IHC examination via an Invitrogen anti-PRAME mouse monoclonal antibody (CL5148). Tests were carried out on a Dako Autostainer Link 48 platform. The detection of PRAME nuclear staining by the EnVision™ FLEX HRP Magenta Substrate Chromogen System (Dako Omnis) was designed to minimize possible difficulties in interpreting the staining of cells with cytoplasmic melanin pigment. PRAME immunostaining results were classified on the basis of the percentage of positive nuclei (0–25%, >25–50%, >50–75% and >75%) (Lezcano et al. 2021a, b).

The results are presented in terms of descriptive statistics. The sensitivity, specificity, and positive and negative predictive values were calculated considering nuclear expression of PRAME>75% as positive cases.

The research project was approved by the Research Ethics Committee, under the opinion of 6.569.902.

Results

Thirty patients were selected for this study, including 15 with nodal nevi and 15 with metastatic melanoma. All lymph nodes were from patients with known primary melanomas. The patients' ages ranged from 24 to 84 years, with an average age of 46 years, and the group consisted of 15 females and 15 males.

The nevus cell deposits in the lymph node capsule ranged in size from 0.1 to 1.5 mm in the largest dimension. Two nodal nevi were confined to perinodal or trabecular fibrous tissue. In the other 13 lymph nodes, nevus deposits were present in subcapsular areas (Fig. 1).

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Among the 15 lymph nodes derived from patients diagnosed with metastatic melanoma, HE stain revealed that the cell clusters were mainly composed of larger cells with varying degrees of pleomorphism, nuclear membrane irregularity, clumped or hyperchromatic

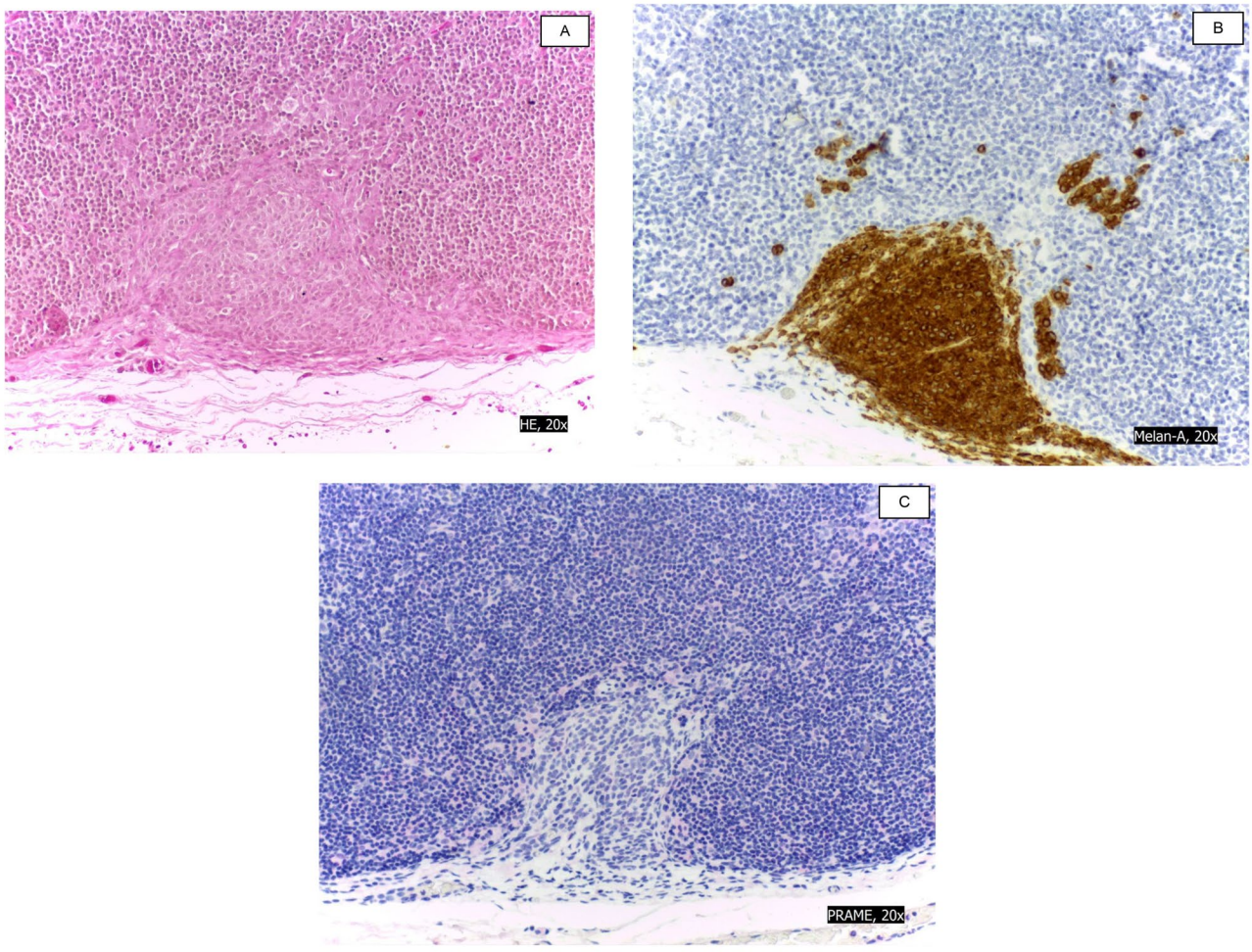

Fig. 1 Nodal nevic rests: (**A**) H&E stain; (**B**) Melan-A positive; (**C**) PRAME negative (20x)

Table 1 Clinicopathological and immunohistochemical characteristics of nevi in lymph nodes

N	Gender	Age	Site	Nodal location	Primary tumor	MELAN-A	HMB45	PRAME
1	F	61	Inguinal	S	CMM	Pos	Neg	0–25%
2	F	54	Axilla	S	CMM	Pos	Neg	0–25%
3	F	46	SLN	S	CMM	Pos	Neg	0–25%
4	M	55	SLN	S	U	Pos	Neg	0–25%
5	F	74	Axilla	S	CMM	Pos	Neg	0–25%
6	F	59	Axilla	S	CMM	Pos	Neg	0–25%
7	M	58	Axilla	S	CMM	Pos	Neg	0–25%
8	F	60	Axilla	S	CMM	Pos	Neg	0–25%
9	M	70	Axilla	S	CMM	Pos	Neg	0–25%
10	F	58	Axilla	S	CMM	Pos	Neg	0–25%
11	F	32	Axilla	F	U	Pos	Neg	0–25%
12	F	37	Axilla	S	CMM	Pos	Neg	0–25%
13	M	78	Inguinal	S	CMM	Pos	Neg	0–25%
14	F	24	Axilla	F	CMM	Pos	Neg	0–25%
15	F	75	Axilla	S	CMM	Pos	Neg	0–25%

M=male, F=female, SLN=sentinel lymph node, CMM=cutaneous malignant melanoma, ND=not done, U=unknown, Neg=negative, Pos=positive, F=fibrous tissue, S=subcapsular

chromatin, conspicuous nucleoli, granular cytoplasm or an irregular distribution of melanin pigment. There were occasional mitotic figures (Figs. 2 and 3). One had negative immunostaining for HMB-45 and Melan-A. Notably, most of these cases presented diffuse immunoreactivity for PRAME; however, twelve cases demonstrated immunoreactivity greater than 75% and were classified as positive, while three cases showed immunoreactivity between 50% and 75%, thus being considered negative (Table 2).

The sensitivity and specificity for PRAME expression were 80% and 100%, respectively, with corresponding 100% positive and 83% negative predictive values.

Discussion

The results of this study reinforce that PRAME may support or exclude the diagnosis of malignant melanocytic proliferation. The absence of PRAME expression in nevus cells strengthens the diagnosis of benign melanocytic proliferation in the lymph node capsule, and positive immunolabeling is characteristic of metastatic melanoma.

Melanocytic nevi in sentinel lymph nodes can cause diagnostic difficulties in patients with melanoma. An expert review of sentinel lymph node biopsy samples considered positive for melanoma revealed that more than 10% were incorrectly classified as melanocytic nevi (Kretschmer et al. 2022). Misdiagnosis in the distinction between nevus metastases and melanoma can result in excessive or insufficient treatment. PRAME is considered an important auxiliary tool for this assessment and therefore appears to be a suitable target for differentiating between benign and malignant melanocytic skin lesions.

After the introduction of IHC for PRAME in the diagnosis of melanocytic lesions, many researchers focused on its application in various diagnostic scenarios (Spitz lesions, lentigo maligna, acral lesions, etc.), with encouraging but also partially discordant results (Cazzato et al. 2022). Furthermore, despite the most diffuse and applied immunohistochemical score for PRAME proposed by Lezcano et al. 2018, 2020a, b), several authors have argued that alternative scores (different cutoffs and/or a combination of qualitative and quantitative assessments)

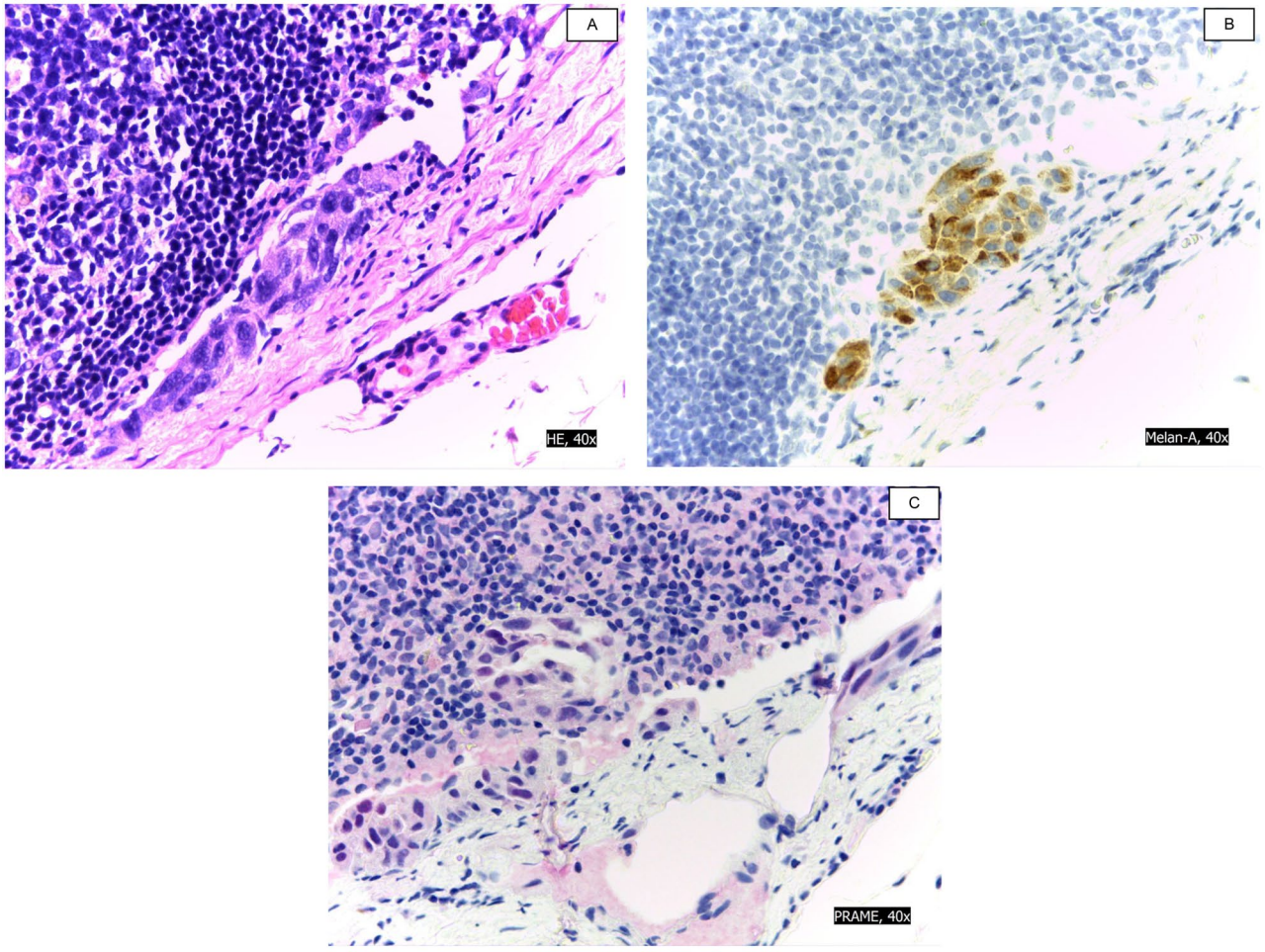

Fig. 2 Metastatic melanoma cells: (**A**) H&E stain; (**B**) Melan-A positive; (**C**) PRAME positive (40x)

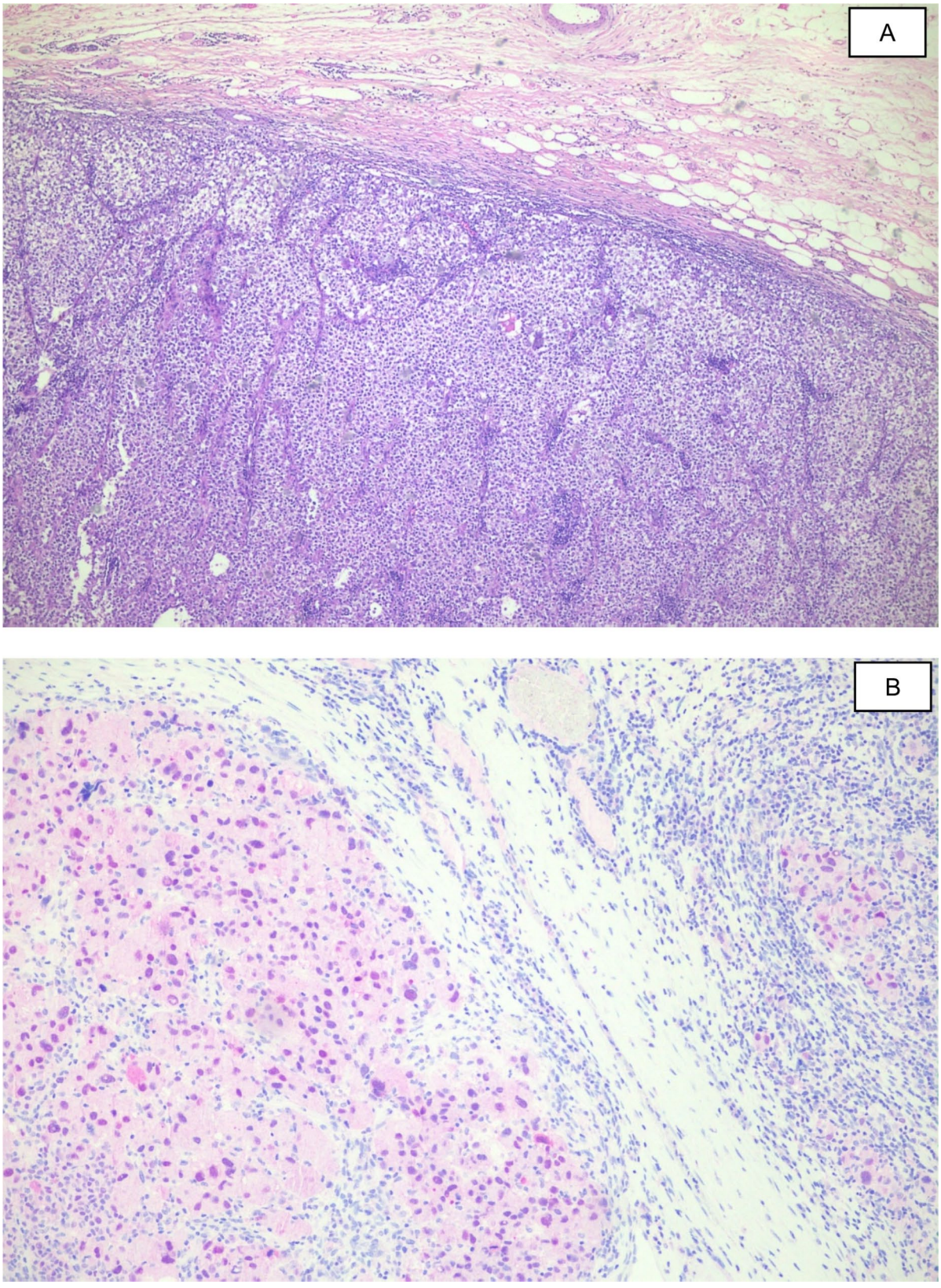

Fig. 3 Extensive melanoma (**A**, H&E, 10x) showing immunoreactivity for PRAME (**B**, 20x)

Table 2 Clinicopathological and immunohistochemical characteristics of melanoma in lymph nodes

N	Gender	Age	Site	Primary tumor	MELAN-A	HMB45	PRAME
1	M	58	Axilla	U	Pos	Pos	>75%
2	M	57	Inguinal	U	Neg	Neg	>75%
3	M	57	Axilla	U	Neg	Neg	>75%
4	M	41	Axilla	CMM	Pos	ND	>50-75%
5	M	61	Axilla	CMM	Pos	Pos	>50-75%
6	F	82	Axilla	CMM	Pos	Pos	>75%
7	M	30	Axilla	U	Pos	Pos	>75%
8	F	24	Axilla	CMM	Pos	Pos	>75%
9	M	63	Cervical	U	Pos	ND	>75%
10	M	50	Cervical	CMM	Pos	ND	>75%
11	M	65	Axilla	U	Pos	ND	>75%
12	M	55	Inguinal	U	Pos	Pos	>75%
13	F	84	Inguinal	CMM	Pos	Pos	>75%
14	F	49	Inguinal	CMM	Pos	Pos	>50-75%
15	M	65	Axilla	U	Pos	Pos	>75%

M = male, F = female, CMM = cutaneous malignant melanoma, NOS = not otherwise specified, ND = not done, U = unknown; NNC = no neoplastic cells; Neg = negative, Pos = positive

perform better for the assessment of specific histotypes of melanocytic lesions.

Lezcano et al. (2020) published a series of 30 nodal nevi, including cases with subcapsular and intraparenchymal nevus aggregates, with no PRAME immunoreactivity in any of the benign melanocytic proliferations. On the other hand, 15 lymph node melanoma metastases showed diffuse nuclear labeling. However, a subsequent study (Lezcano et al. 2021a, b) reported that the intermediate extent of PRAME staining (present in >50% but <75% of tumor cells) and/or weak immunoreactivity can be very difficult to interpret, which limits reproducibility and ultimately has little or no value in confirming a final diagnosis. In this regard, it should be noted that the 50% cutoff for positivity differs from the standard 75% cutoff typically used for PRAME expression evaluation.

Innocenti et al. 2023 analyzed 22 common melanocytic nevi, 20 cutaneous melanomas, 48 low-grade dysplastic nevi and 40 high-grade dysplastic nevi in a cohort study. PRAME immunolabeling was assessed via a five-level system (0–4+). Cutaneous melanomas scored 4+in 89% of the cases, whereas 59% of the common melanocytic nevi had negative PRAME immunolabeling. In addition, an increasing trend in PRAME expression was observed from low-grade dysplastic nevi to high-grade dysplastic nevi.

Koch et al. 2023 published a standardized computer-assisted analysis of PRAME immunoreactivity in dysplastic nevi. Histological slides stained with PRAME were digitized. The aim was to minimize intra- and interobserver variability. For these patients, an expression of PRAME greater than 100 cells/mm² should raise the suspicion of high-grade melanoma. Even so, PRAME cannot replace specialized histomorphological assessment and correlation with relevant clinical findings (Lezcano et al. 2020a; Sharouni et al., 2021; Lezcano et al. 2018, 2020a, b; Constantino et al. 2022; See et al. 2020).

In this study, we considered a cutoff score of >75% for calculating sensitivity, specificity, and positive and negative predictive values, which is consistent with the findings of most prior studies (Lezcano et al. 2018, 2020a, b, 2021a; Lezcano et al. 2021b; Lezcano et al. 2021b).

All of our cases previously classified as melanomas were positive for more than 50% of the cells. This observation aligns with findings from Gradecki et al. 2021; who noted that lymph node metastases were more likely to exhibit lower PRAME expression than were metastases in other anatomical locations.

A limitation of this study was the length of time the slides were stored, with the consequent partial loss of the original HE staining, as well for the Melan-A and HMB45 IHC slides, making it difficult to analyze cases in which the melanocytic deposits were smaller than 1 mm in the lymph node capsule. It should also be considered that the storage time of the paraffin blocks and the fixation conditions of the samples can interfere with the results of the immunohistochemical reactions. A certain number of melanomas may not have PRAME expression. We hypothesize that the sensitivity and specificity may be lower with larger case cohorts. In addition, melanocytic lesions with a spindle-shaped appearance were not included, so it is not possible to determine whether there is a difference in the immunohistochemical expression of PRAME between epithelioid and spindle-shaped cells. Finally, the blinding of the study was impaired, as the diagnosis of the cases was known, due to the general morphology and previous IHC examination confirming the nature of the nevi and melanocytic lesions.

In conclusion, PRAME immunostaining appears to be a reliable tool for distinguishing nevi from MM when

it is present in the lymph node capsule. On the basis of our results, PRAME expression indicates metastatic melanoma.

Abbreviations

SL	Sentinel lymph node
PRAME	Preferentially expressed antigen in melanoma
MM	Malignant melanoma
IHC	Immunohistochemistry
HMB-45	Human antimelanosome clone HMB45
MITF	Melanocyte-inducing transcription factor
HE	Hematoxylin and eosin
RAR	Retinoic acid receptor

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Author contributions
HGS: Data curation, Writing- Original draft preparation; HFJ: Investigation, Validation; LDSC: Investigation, Validation; JS: Investigation, Validation; RB: Investigation, Validation; CK: Investigation, Validation; BLS: Writing – review & editing; PHCF: supervision, Writing – review & editing; KMPAC: supervision, Writing – review & editing. All the authors read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The research project was approved by the Research Ethics Committee, under the opinion of 6.569.902.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

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