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# Nestin expression in nodular and acral lentiginous melanoma: associations with ulceration and invasion factors in Mexican patients

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## Abstract

**Introduction:** There are still many gaps in the understanding of melanoma subtypes in Mexico. Currently, there is a growing search for prognostic markers and potential therapeutic targets for melanoma treatment. Nestin has been identified as a marker of angiogenesis, invasiveness, and shortened survival in various tumor types, and therefore we evaluated nestin expression in two types of melanoma tissue to investigate its possible clinical and pathological associations.
**Methods:** Immunohistochemistry using a polyclonal antibody was performed on selected paraffin blocks. Nestin expression was assessed by two independent dermatopathologists, yielding a kappa index of 0.83 (indicating almost perfect agreement). Nestin expression levels in these types of melanoma were evaluated in relation to Breslow index, Clark level, ulceration, and mitotic rate. Statistical analysis was performed using Pearson correlation, chi-square tests, and analysis of variance (ANOVA).
**Results:** Eighty-three melanoma cases were diagnosed based on clinical and histopathological criteria: 47 were nodular melanoma, and 36 were acral lentiginous melanoma. High nestin expression was mainly found in nodular melanoma ($p = 0.0001$). This subtype was also more frequently associated with a deeper Clark level of invasion and showed a 37.5-fold increased risk of histological ulceration. A lower but relevant level of nestin expression was observed in acral lentiginous melanoma, which has rarely been reported in the literature.
**Conclusions:** Elevated nestin expression was statistically associated with nodular melanoma and a deeper Clark level of invasion. In addition, it was significantly linked to an increased risk of histological ulceration.

**Keywords:** nestin, acral lentiginous melanoma, immunohistochemistry, Breslow index, Clark level

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## Introduction

Cutaneous malignant melanoma is a neoplasm with high metastatic potential, the incidence of which has alarmingly increased in recent decades in Mexico (1). The main subtypes in Mexico are nodular melanoma (NM) and acral lentiginous melanoma (ALM) (1, 2), and these subtypes are associated with distinctive etiological factors, clinical presentations, and biological behaviors.

NM is characterized by very fast vertical growth rather than the initial radial growth of other types of melanoma (2). Consequently, NM is the most aggressive tumor and accounts for over 43% of all melanoma-related deaths (3). In general, these tumors are symmetrical, with regular borders and small diameters. Therefore, this subtype of melanoma may go undetected, resulting in devastating consequences. Moreover, NM may also be amelanotic or hypomelanotic, making diagnosis more difficult.

ALM is the second most common subtype in Mexico (2), and it usually occurs on plantar, palmar, or subungual areas without hair. The predominant presentation of ALM is on plantar sites. However, both presentations (on palms and soles) are very aggressive when the proliferative vertical phase develops. The NM and ALM subtypes are more commonly found in individuals with dark skin, Asians, and Afro-Caribbeans (4).

Nestin is a type VI protein serving as a marker for stem cells of neural origin (5, 6). Re-expression of this protein after birth mainly occurs under pathological conditions such as neoplasia (7). High levels of nestin expression have been detected in several cancer cell lines (7–9).

Several studies have found that nestin is an important marker of microvessel density (MVD). In liver metastasis, it has been proven that the tumor's potential to grow, spread, and metastasize is connected with tumor-associated angiogenesis (5). Nestin has also been reported as a marker for invasiveness, angiogenesis, and shortened survival of patients with several types of cancer. In breast cancer, for instance, the 5-year survival rate of women with tumors expressing high levels of nestin was shorter than that of women with tumors expressing low levels (10, 11).

Novel studies on molecular mechanisms of nestin in tumor progression have suggested that intermediate filaments (IF) play a prominent role in mediating cell stiffness, and that nestin increases cancer cell metastasis by reducing cell stiffness. Yamagishi et al. (12) generated nestin knockout (KO) cells by CRISPR/Cas9 genome editing in a mouse breast cancer cell line (FP10SC2), which showed high levels of motility and metastasis. They reported that the metastatic capacity of cells was eliminated in the nestin KO cells. They demonstrated that the metastatic capacity of these cells was completely abolished following nestin deletion. These findings indicate that nestin facilitates cancer cell metastasis through the modulation of cell stiffness and highlight nestin as a potential therapeutic target for preventing metastasis across multiple cancer types.

In studies of superficial spreading melanoma and NM, increased expression of nestin was associated with the depth of invasion, ulcerated lesions, tumor aggressiveness, and worse prognosis for the patients (13, 14).

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There are no published reports of nestin expression in melanoma in the Mexican population. The objective of our study was to determine the expression of nestin in histological samples corresponding to the two most common melanomas subtypes in Mexican mestizo patients and explore its possible clinical and pathological associations.

## Methods

Forty-seven patients in the sample had NM, and 36 patients had ALM (Table 1).

Nestin expression was semiquantitatively determined from patients diagnosed with NM and ALM at the dermatopathology unit by means of immunohistochemistry detection using the nestin-specific polyclonal rabbit antibody N5413 (Sigma-Aldrich, Sain Louis, MI, USA), diluted 1:50 in PBS containing 0.3% Triton-X100 and 1% bovine serum albumin. Briefly, formalin-fixed paraffin-embedded (FFPE) tissue samples were freshly cut into 5 µm thick slices and de-paraffinized in xylene and rehydrated in a graded series of isopropanol. A grade series of isopropanol was performed in citrate buffer pH 6 in a pressure cooker for 2 min under pressure before slow cooling the samples. Tissue sections were subsequently stained according to the manufacturer's instructions using the UltraVision LP Detection System (AP Polymer; Thermo Scientific, Waltham, MA, USA), with Fast Red as the chromogenic substrate (Liquid Fast Red Substrate System; Thermo Scientific, Waltham, MA, USA).

### Statistical analysis

Statistical analysis was performed to evaluate the association between nestin expression and clinicopathological variables in NM and ALM. Categorical variables were summarized using absolute frequencies and percentages, whereas continuous variables were expressed as means and standard deviations. Nestin expression was assessed semiquantitatively and analyzed as a continuous variable and, when appropriate, as a categorical variable (low vs. high expression) based on staining intensity and extent.

Interobserver agreement for nestin immunohistochemical evaluation was assessed using Cohen's kappa coefficient. Associations between categorical variables were analyzed using the chi-square ($x^2$) test or Fisher's exact test, as appropriate. Comparisons of nestin expression levels between melanoma subtypes were performed using analysis of variance (ANOVA). Pearson correlation analysis was used to assess the relationship between nestin expression and continuous prognostic variables, including Breslow index and Clark level of invasion.

A two-tailed $p$-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics software (IBM Corp., Armonk, NY, USA).

### Ethical aspects

This research, with registration number CI244//14, is classified as risk-free, according to Article 17 of the regulations in Chapter I of Title Two of the Regulations of the General Health Law on Health Research from Mexico.

## Results

This is a descriptive, cross-sectional study. Immunohistochemistry using a polyclonal antibody was performed on selected paraffin blocks. Eighty-three cases were diagnosed with melanoma by clinical criteria (Figs. 1, 2) and histopathology criteria (Fig. 3). Baseline demographic and clinical characteristics were collected for all patients, including sex, age at diagnosis, anatomical location of the primary tumor, duration of lesion evolution, lesion morphology and size, presence of palpable lymph nodes, and history of prior surgical resection. These characteristics are summarized in Table 1. Sex distribution was similar (36.1% and 34.0%). Patients' average ages were 65.7 years for ALM and 65.3 for NM. Most NMs (37/47) were classified in the highest Breslow thickness category (> 4 mm) due to marked histologic thickness and frequent ulceration or necrosis, which limited further stratification within

**Table 1** | **Clinical variables of the 83 patients.**

| Clinical characteristics | Melanoma type | | Statistical test, $p$-value |
|---|---|---|---|
| | Acral lentiginous | Nodular | |
| Male, *n* (%) | 13.0 (36.1%) | 16.0 (34.0%) | Fisher: $p = 0.78$ |
| Female, *n* (%) | 23.0 (63.8%) | 31.0 (66.0%) | |
| Age, mean (*SD*) | 65.7 (14.4) | 65.3 (14.5) | Student: $p < 0.0001$ |
| Evolution, mean (*SD*) | 26.9 (30.4) | 18.7 (23.8) | Student: $p < 0.0001$ |
| Topography | | | |
| Head, *n* (%) | 0.0 | 11.0 (23.9) | $x^2$: $p < 0.0001$ |
| Trunk, *n* (%) | 0.0 | 4.0 (8.7) | |
| Arm, *n* (%) | 0.0 | 4.0 (8.7) | |
| Hand, *n* (%) | 6.0 (16.7) | 5.0 (10.9) | |
| Thigh, *n* (%) | 0.0 | 2.0 (4.3) | |
| Leg, *n* (%) | 0.0 | 3.0 (6.5) | |
| Foot, *n* (%) | 30.0 (83.3) | 17.0 (37.0) | |
| Morphology | | | |
| Nodular, *n* (%) | 3.0 (8.3) | 36.0 (76.6) | $x^2$: $p < 0.0001$ |
| Plaque, *n* (%) | 7.0 (19.4) | 7.0 (14.9) | |
| Macular, *n* (%) | 26.0 (72.2) | 4.0 (8.5) | |
| Mean size cm (*SD*) | 2.6 (1.3) | 2.0 (1.2) | Student: $p = 0.084$ |
| Palpable nodules | | | |
| Yes, *n* (%) | 0.0 | 1.0 (2.1) | Fisher: $p = 1.0$ |
| No, *n* (%) | 36.0 (100.0) | 46.0 (97.9) | |
| Previous resection | | | |
| Yes, *n* (%) | 4.0 (11.4) | 6.0 (12.8) | Fisher: $p = 0.69$ |
| No, *n* (%) | 31.0 (88.6) | 41.0 (87.2) | |

Fisher = Fisher's exact test, Student = Student's $t$-test, $x^2$ = chi-square test, *SD* = standard deviation.

this group (Table 2). Nestin expression showed a mean of 61.48% in NM (Fig. 4) versus 27.04% in ALM ($p < 0.0001$; Fig. 5), with a staining index of 5.48 in NM and 2.85 in ALM ($p < 0.0001$; Table 3). The Pearson correlation coefficient between nestin expression and Breslow index was $r^2 = 0.029$ (indicating a small correlation), and the correlation with Clark's level of invasion was $r^2 = 0.458$ (indicating a large correlation). When comparing the presence of mitosis with elevated nestin expression, a 1.75-fold increased risk of elevated mitosis was calculated; however, this risk was not statistically significant ($p = 0.377$, confidence interval [CI] 0.53 to 5.71). Finally, the analysis of the presence of ulceration revealed that elevated nestin expression carries a 35.7-fold increased risk of histological ulceration ($p < 0.0001$, CI 4.75–296.68).

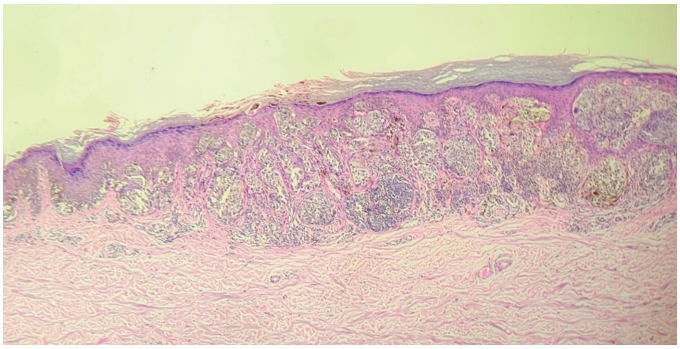

Figure 3 | Acral lentiginous melanoma, hematoxylin and eosin stain, 20×; lentiginous and nested epithelioid melanoma cells with heavy pigmentation and brisk lymphocyte infiltration throughout dermal borders.

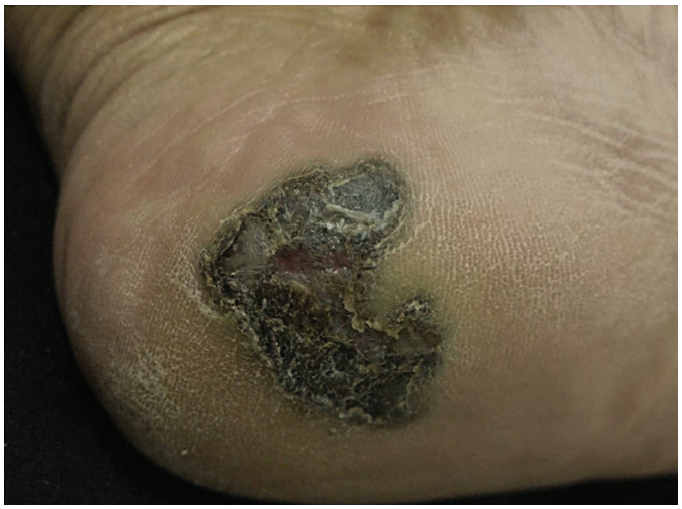

Figure 1 | Ulcerated acral lentiginous melanoma on the foot of a 79-year-old woman; Breslow not assessable, Clark level III.

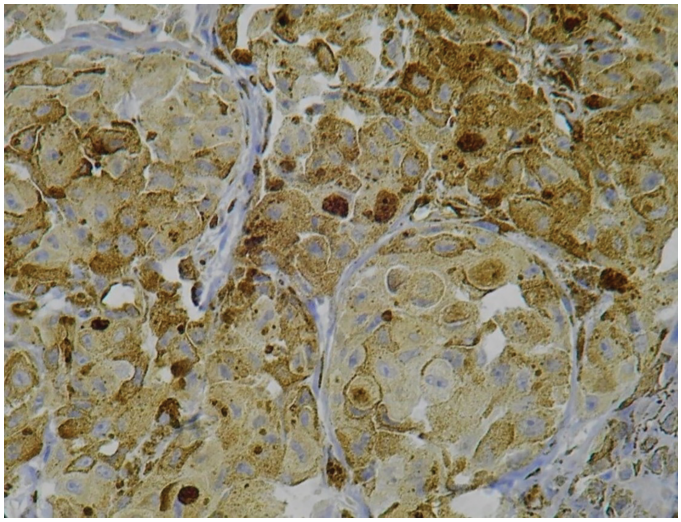

Figure 4 | Strong nestin expression in primary nodular melanoma.

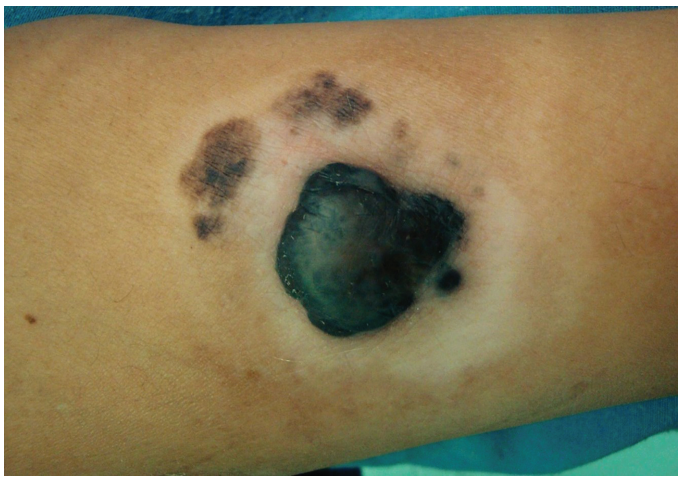

Figure 2 | Nodular melanoma on the leg of a 39-year-old man with peripheral satellite lesions; Breslow › 4 mm, Clark level V.

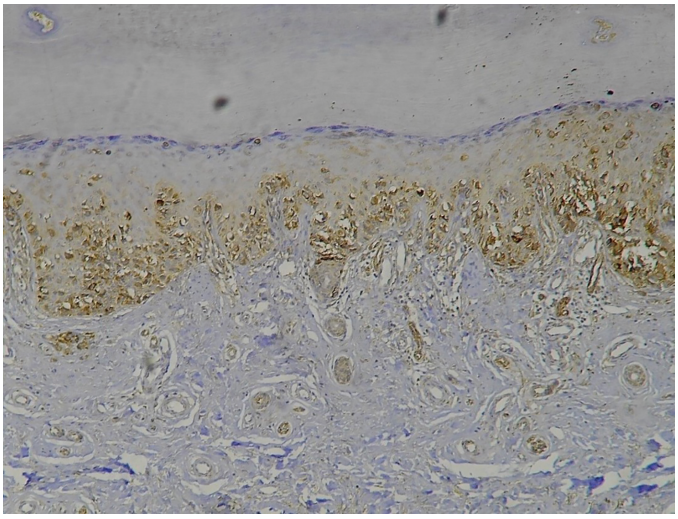

Figure 5 | High-level nestin expression in acral lentiginous melanoma in situ.

**Table 2 | Histopathological characteristics of the 83 patients (period: 5 years).**

| Histopathological characteristics | Melanoma type | | Statistical test, *p*-value |
|---|---|---|---|
| | Acral lentiginous | Nodular | |
| In situ, *n* (%) | 3.0 (8.3) | 0.0 | Fisher: $p = 0.78$ |
| Invasive, *n* (%) | 33.0 (91.7) | 47.0 (100.0) | |
| Breslow index, mm (*SD*) | 0.1 (0.6) | 2.6 (0.9) | Student: $p < 0.0001$ |
| Clark level (*SD*) | 2.1 (0.6) | 3.3 (0.6) | Student: $p < 0.0001$ |
| Mitosis, *n* per field (*SD*) | 2.2 (0.9) | 2.7 (1.3) | Student: $p = 0.134$ |
| Ulceration, *n* (%) | 5.0 (13.9) | 23.0 (48.9) | Fisher: $p < 0.001$ |
| Necrosis, *n* (%) | 5.0 (13.9) | 23.0 (48.9) | Fisher: $p < 0.001$ |
| Lymphovascular invasion, *n* (%) | 2.0 (5.6) | 5.0 (10.6) | Fisher: $p = 0.69$ |

Fisher = Fisher's exact test, Student = Student's *t*-test, *SD* = standard deviation.

**Table 3** | **Nestin expression averages and staining index for acral lentiginous melanoma, nodular melanoma, and benign nevi.**

| | *n* | *M* | *SD* | Error | 95% CI |
|---|---|---|---|---|---|
| Nestin expression | | | | | |
| Acral lentiginous melanoma | 27 | 27.04 | 25.24 | 4.85 | 17.05–37.02 |
| Nodular melanoma | 27 | 61.48 | 30.21 | 5.81 | 49.53–73.44 |
| Benign nevi | 5 | 36.00 | 20.73 | 9.27 | 10.25–61.75 |
| Total | 59 | 43.56 | 31.71 | 4.12 | 35.29–51.83 |
| Staining index | | | | | |
| Acral lentiginous melanoma | 27 | 2.85 | 2.14 | 0.41 | 2.00–3.70 |
| Nodular melanoma | 27 | 5.48 | 2.65 | 0.51 | 4.43–6.53 |
| Benign nevi | 5 | 3.20 | 2.16 | 0.97 | 0.51–5.89 |
| Total | 59 | 4.08 | 2.68 | 0.35 | 3.38–4.78 |

*SD* = standard deviation, CI = confidence interval, *M* = mean.

## Discussion

Currently, there is a comprehensive search for prognostic markers and potential therapeutic targets for melanoma treatment. Nestin is associated with an invasive phenotype and resistance to MEK and BRAF inhibitors (16). Increased nestin expression in primary uveal melanoma (PUM) is a predictor of a tumor phenotype associated with metastatic progression and reduced survival time at onset of metastasis (17). It also has been suggested as a marker of tumor angiogenesis in breast and colorectal cancers (16). Given the high resistance to chemotherapy and radiotherapy currently observed in these subtypes of cancers, early detection and complete resection remain the primary means of improving prognosis and survival in this population. ALM and NM are two subtypes known for their particularly unfavorable prognosis, often diagnosed at advanced stages, and they are among the most common melanoma subtypes found in the Mexican population.

This study found that the highest nestin expression was observed in most NMs (61.48%, staining index [SI] = 5.48) compared to benign nevi (36.0%, SI = 3.20). This is in agreement with the study conducted by Landstein et al. (14), in which SI = 8.72 for NM and SI = 2.35 for benign nevi were reported. The aggressive biological behavior of NM, characterized by fast and intense growth, may explain the elevated expression of nestin observed in the peritumoral stroma and malignant cells.

Because nestin expression persists throughout the dermal remodeling process, it is not surprising that it was also detected in the benign nevi analyzed in our study. Several authors have previously reported the expression of nestin in benign nevi (13), and even their differentiation from malignant melanoma by measuring nestin expression levels (15).

Furthermore, a lower but relevant level of nestin expression was observed in ALM (expression 27.0%, SI = 2.85), which has not been previously reported in the literature. Nevertheless, these levels are comparable to those observed in superficial spreading melanoma by Landstein et al. (SI = 2.35) (14). The initial growth phase of ALM closely resembles that of superficial spreading melanoma because it begins with lentiginous dissemination along the epidermis, followed by invasion through the basement membrane into the dermis, a pattern similar to that of superficial spreading melanoma. This resemblance in growth and invasive behavior likely accounts for the similarity in staining index between the two variants.

The Pearson correlation coefficient between nestin expression and Breslow index was low ($r^2$ = 0.029); however, this low correlation may be attributable to the fact that most cases of NM had a non-assessable histological thickness (a Breslow index greater than 4 mm) or due to the presence of ulceration.

Conversely, when comparing nestin expression to Clark's level of invasion, a moderate correlation was identified (Pearson coefficient $r^2$ = 0.458), indicating that, as the depth of melanoma invasion increased, so did nestin expression. In addition, an analysis was performed to assess the risk of increased mitotic activity and ulceration in melanomas with high nestin expression (greater than 50%). The results showed that the number of mitoses were independent of nestin expression level (odds ratio [OR] = 1.75, CI 0.53–5.71). However, elevated nestin expression was associated with a 35.7-fold increase in the risk of developing histological ulceration (CI 4.75–296.68), a poor prognosis factor. Several limitations should be considered when interpreting the results of this study. For instance, a large proportion of the melanoma tissues evaluated had a high Breslow index or were not assessable due to significant histological thickness (greater than 4 mm), as well as the presence of ulceration and necrosis. This indicates that most cases were in advanced stages, which could bias the results toward higher nestin expression associated with more aggressive melanomas, thereby limiting the applicability of the findings to early-stage melanomas. In addition, a poor correlation was observed between nestin expression and Breslow index ($r^2$ = 0.029), which may be due to the difficulty in assessing histological thickness in advanced NM, affecting the robustness of this measure as a correlative marker in this study.

Given the diagnostic challenges posed by NM and ALM—particularly in populations with darker skin tones—identifying reliable molecular markers is essential. Nestin involvement in tumor angiogenesis, invasiveness, and resistance to therapy suggests it may also have value as a future therapeutic target. Nonetheless, further research involving larger and more diverse sample populations, and including early-stage melanomas, is necessary to validate these findings and better define the prognostic and therapeutic significance of nestin in melanoma.

## Conclusions

The findings of this study demonstrate a significant correlation between elevated nestin expression and NM, the most aggressive clinical subtype observed in the Mexican population. Nestin expression was considerably higher in NM compared to ALM and benign nevi, suggesting that this intermediate filament protein may serve as a useful biomarker for tumor aggressiveness and invasive potential. A moderate correlation was also observed between nestin expression and Clark level, reinforcing its association with vertical growth and dermal invasion. The association between elevated nestin expression and histological ulceration, conferring a 35.7-fold increased risk, underscores the potential prognostic relevance of nestin in melanoma.

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