Lymphatic and blood vasculature in primary cutaneous melanomas of the scalp and neck

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Accepted 11 June 2014
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/hed.23801

ABSTRACT: *Background.* Scalp/neck melanomas have a poor prognosis, possibly because of a rich vascular supply that prompts tumor cells' dissemination.

Methods. We compared the accuracy of immunohistochemical (IHC) staining with morphology for the identification of lymphovascular invasion in 156 scalp/neck melanomas. We then analyzed the association of vessel invasion and density with pathological features and survival.

Results. IHC-detected lymphatic vessel invasion (LVI) and blood vessel invasion (BVI) were identified in 34.6% and 13.5% of cases, respectively. IHC increased the LVI/BVI detection compared to morphology (40.4% vs 16.6%; $p < .001$). The degree of peritumoral and intratumoral blood vessel density (BVD) was greater than lymphatic vessel density (LVD). Ulceration was the only factor independently associated with intratumoral ($p = .029$) and peritumoral ($p = .047$) BVD. Tumor thickness was the only independent predictor of survival ($p = .002$).

Conclusion. IHC allows accurate assessment of lymphovascular invasion in scalp/neck melanomas. In these tumors, we observed a high incidence of BVI, which deserves further investigations. © 2014 Wiley Periodicals, Inc. *Head Neck* **00:** 000–000, 2014

KEY WORDS: cutaneous melanoma, scalp and neck, head and neck, lymphatic vessels invasion, blood vessel invasion, lymphatic vessel density, blood vessel density

INTRODUCTION

Although cutaneous melanoma is not the most common form of skin cancer, it is recognized as the most life-threatening.[1] The seventh edition of the American Joint Committee on Cancer staging system considers features of primary tumor, and the presence of lymph node and distant metastases as essential staging criteria; however, other factors, such as patient age and sex, as well as primary tumor location, showed prognostic value.[2] Melanoma in the head and neck region represents approximately 20% of all cutaneous melanomas, and shows a greater risk of disease progression and melanoma death than other tumor locations.[3–8] Among these tumors, scalp and neck location, which represents roughly 5% of all melanomas and 35% of those arising in the head and neck region, accounts for 10% of all melanoma deaths with a 10-year survival rate of 63%, a significantly poorer prognosis compared to that of patients with melanomas of the face and ear region (10-year survival of 80%).[5,9,10]

A greater incidence of brain metastasis[11] and pitfalls in primary tumor treatment, such as narrower excision margin[12] and difficulties in the prediction of the lymphatic drainage (which lower the accuracy of sentinel lymph node biopsy[13]), are possible explanations for the risk of disease progression observed in these patients. In addition, the higher risk of disease progression observed in these patients may also be associated with a higher tumor vascularity.[14,15] However, measurement of lymphovascular invasion and density has not yet been accurately assessed in a large series of scalp and neck melanomas.

We retrospectively collected tumor samples and follow-up data from 156 patients with a primary invasive melanoma of the scalp and neck region from 16 institutions and performed double immunostaining with D2-40/CD34 antibodies in order to (1) compare the diagnostic effectiveness of double D2-40/CD34 immunohistochemical (IHC) staining with conventional hematoxylin-eosin stain

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Contract grant sponsor: This study was supported by the nonprofit association Emma Rouge.

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morphology and (2) evaluate the lymphovascular pattern and its association with clinicopathological features and patient survival.

PATIENTS AND METHODS

The study protocol was approved by the Research Committee of the Italian Melanoma Intergroup, the Italian network for melanoma treatment and research (www.melanomaimi.it), and the Italian Association of Anatomic Pathology and Diagnostic Cytopathology (www.siapec.it), the Italian branch of the International Academy of Pathology, as well as by each institution. Patients consented for the use of their specimens as well as clinicopathological and follow-up data for research purposes. Retrospective data from patients with invasive melanoma of the scalp and neck diagnosed between January 1995 and January 2012 at 16 centers were gathered in a multicenter database. Data were extracted according to the following selection criteria: (1) single invasive primary melanoma, and (2) paraffin-embedded tissue available for IHC analysis. In situ melanomas and lentigo maligna melanomas were excluded from the analysis.

IHC analysis was performed on 4-μm thick representative sections of formalin-fixed, paraffin-embedded tumor tissues. Primary antibodies anti-podoplanin (mouse monoclonal clone D2-40, ready to use, Ventana, Tucson, AZ) and anti-CD34 (mouse monoclonal clone QBEnd/10, ready to use, Ventana) were placed on the same slide for double staining, and incubated according to the IHC DS uDAB-uRED protocol suggested by Ventana automated stainer BenchMark Ultra. Diaminobenzidine and fast red were used as chromogens for podoplanin and CD34, respectively. Upon completion of the staining run, tissue sections were removed from the stainer and counterstained with Mayer's hematoxylin. Sections of lymphangioma and tonsil were used as positive controls for podoplanin and CD34, respectively. Negative controls were performed by substituting the primary antibody with a nonimmune serum at the same concentration. The control sections were treated in parallel with the samples. All sections were dehydrated and mounted with Permount.

IHC double-stained slides were reviewed independently by 3 pathologists (C.G., M.C.M., and D.M.), who were blinded to clinical outcome. Lymphatic vessel invasion (LVI) and blood vessel invasion (BVI; present or absent) were defined as the presence anywhere within the primary tumor of neoplastic cell(s) with morphologic features of melanoma cells in lumens highlighted by D2-40 (LVI) and CD34 staining (BVI), respectively. Questionable instances were discussed and disagreements were resolved by consensus reading. The location of vessel invasion was also topographically assessed as within the intratumoral or peritumoral areas. Peritumoral vessels were defined as D2-40-positive or CD34-positive vessels within an area of 500 μm from the tumor border. Intratumoral vessels were defined as D2-40-positive or CD34-positive vessels located within the tumor mass and not confined by invagination of normal tissue. Double-stained sections were digitally scanned using a D-Sight scanner (Menarini, Italy) for computer-assisted morphometric analyses. Upon identification of the "hot spot," lymphatic vessel density (LVD) and blood vessel density (BVD) were quantified in 5 adjacent fields at $\times 40$ (corresponding to 1 mm^2), both in intratumoral and peritumoral locations.

The following variables were considered for each patient: patient age and sex, primary tumor site (neck, scalp), thickness, ulceration, Clark level of invasion, tumor histotype (superficial spreading melanoma [SSM], versus others), tumor infiltrating lymphocytes (TILs), microscopic satellitosis, and hematoxylin-eosin stain detected lymphovascular invasion.

Variables Clark level, TILs, lymphovascular invasion, and satellitosis had missing values in 1 (0.6%), 8 (5.1%), 5 (3.2%), and 4 (2.5%) patients. The multiple imputation method was used to predict missing values, using variables with non-missing values as predictors (ie, age, sex, tumor thickness, ulceration, and mitotic rate).[16,17]

Diagnostic effectiveness of IHC for detecting LVI/BVI was studied with the McNemar's test. The Fisher's exact test and the chi-square test were used to investigate the association between LVI/BVI and covariates, whereas the Mann–Whitney U test and the analysis of variance were fitted to data to investigate association between intratumor/peritumoral LVD/BVD and covariates. Significant variables were tested at multivariate analysis (the logistic regression for LVI/BVI and the multivariate regression for LVD/BVD).

Disease-free survival (DFS) and melanoma-specific survival (MSS) were calculated from the time of primary melanoma diagnosis to the time of melanoma recurrence and death, respectively, or last follow-up. Univariate and multivariate survival analysis was performed with the Cox proportional hazard model (stepwise backward procedure) to study the association of covariates with DFS and MSS.

Analyses were conducted setting the alpha level of significance at 0.05 and performed with STATA SE/11.0 (College Station, TX).

RESULTS

A total of 156 patients met the inclusion criteria of this study (Table 1). IHC markers increased the detection of LVI/BVI (see Figure 1) compared to hematoxylin-eosin stain (63 patients; 40.4%; with IHC vs 26 patients; 16.6%; with hematoxylin-eosin stain; $p < .001$). IHC-detected LVI/BVI was located in the intratumoral and peritumoral areas in 18 (33.3%) and 14 (25.4%) patients, respectively. Strikingly, although IHC-detected LVI was identified in 54 patients (34.6%), BVI was diagnosed in 21 cases (13.5%).

Immunohistochemical-detected lymphatic vessel invasion and blood vessel invasion

The presence of both IHC-detected LVI and BVI was associated with primary tumors showing greater thickness ($p < .001$), ulceration ($p < .001$), mitotic rate $\geq 1/mm^2$ ($p < .001$), and histotype other than SSM ($p = .015$; Table 1). Upon multivariate analysis, greater tumor thickness (odds ratio [OR] = 1.21; 95% confidence interval [CI] = 1.07–1.37; $p = .003$) and mitotic rate $\geq 1/mm^2$ (OR = 7.35; 95% CI = 2.18–24.8; $p = .001$) were independently predictors of LVI/BVI.

Predictors of IHC-detected LVI and BVI were also separately investigated. Upon univariate analysis, LVI was

TABLE 1. Association of immunohistochemistry-detected lymphatic vessel invasion and blood vessel invasion with clinical and pathological features of 156 patients with scalp and neck cutaneous melanomas.

	LVI/BVI absent		LVI/BVI present		
Variables	No. of patients	%	No. of patients	%	*p* value
Age, y (IQR)	64 (46–75)		64 (47–75)		.531
Sex					.208
Female	23	24.7	22	34.9	
Male	70	75.3	41	65.1	
Primary tumor site					.863
Scalp	61	65.6	43	68.3	
Neck	32	34.4	20	31.7	
Tumor thickness					< .001
Median (IQR) mm	1.15 (0.7–2.8)		3.5 (1.6–6.6)		
<1.01 mm	39	41.9	10	15.8	
1.01–2.00 mm	20	21.5	11	17.4	
2.01–4.00 mm	23	24.7	13	19.0	
>4.00 mm	11	11.9	29	47.8	
Ulceration					< .001
Absent	73	78.5	30	47.6	
Present	20	21.5	33	52.4	
Mitotic rate					< .001
$<1/mm^2$	34	36.5	4	6.3	
$\geq 1/mm^2$	59	63.5	59	93.7	
Clark level of invasion					.088
II–III	81	87.1	48	76.2	
IV–V	12	12.9	15	23.8	
Tumor histotype					.015
SSM	57	61.3	26	41.3	
Others	36	38.7	37	58.7	
TILs					1.000
Absent/nonbrisk	83	89.2	56	88.8	
Brisk	10	10.8	7	11.2	
Satellitosis					.120
Absent	89	95.7	56	88.8	
Present	4	4.3	7	11.2	

Abbreviations: LVI, lymph vessel invasion; BVI, blood vessel invasion; IQR, interquartile range; SSM: superficial spreading melanoma; TILs, tumor infiltrating lymphocytes.
The underlined figures indicate statistical significance.

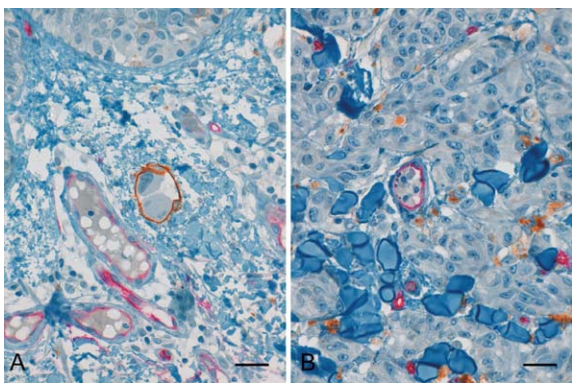

FIGURE 1. D2-40/CD34 double immunostaining in melanoma tissues. (A) A tumor embolus is observed within a D2–40 positive lymphatic channel (brown staining). Note adjacent capillary blood vessels (red staining) containing red blood cells (original magnification ×40). (B) A dilated CD34 positive blood vessel (red staining) shows melanoma cells inside its lumen (original magnification ×40). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

associated with tumors showing greater thickness ($p = .002$), ulceration ($p = .001$), mitotic rate $\geq 1/mm^2$ ($p < .001$), histotype other than SSM ($p = .029$), and satellitosis ($p = .049$). Upon multivariate analysis, ulceration (OR = 2.34; 95% CI = 1.13–4.88; $p = .023$) and mitotic rate $\geq 1/mm^2$ (OR = 2.34; 95% CI = 1.45–12.15; $p = .009$) were the independent predictors of LVI. Upon univariate analysis, BVI was associated with tumors showing greater thickness ($p < .001$), mitotic rate $\geq 1/mm^2$ ($p = .002$), Clark level IV to V ($p = .012$), and histotype other than SSM ($p = .019$). Upon multivariate analysis, greater tumor thickness (OR = 1.17; 95% CI = 1.04–1.34; $p = .011$) was the only independent predictor of BVI. Among 69 patients who underwent sentinel lymph node biopsy, 16 were found positive. Because of the limited number of patients, no statistical correlation studies were undertaken.

Lymphatic vessel density and blood vessel density

In the intratumoral area, BVD (median, $25/mm^2$; interquartile range [IQR] = $7–38/mm^2$) was greater than LVD (median, $6/mm^2$; IQR = $0–13/mm^2$). Similar findings were detected in the peritumoral area, where BVD

TABLE 2. Association of immunohistochemistry-detected peritumoral and intratumoral lymphatic vessel density and blood vessel density with clinical and pathological features.

	Intratumoral LVD		Peritumoral LVD		Intratumoral BVD		Peritumoral BVD	
Variables	Median (IQR)	*p* value	Median (IQR)	*p* value	Median(IQR)	*p* value	Median(IQR)	*p* value
Age		.166		.364		.572		.098
≤64 y	9 (2–14)		10 (6–19)		23 (15–37)		32 (22–46)	
>64 y	5 (2–10)		10 (4–18)		26 (13–42)		39 (50–32)	
Sex		.753		.682		.740		.696
Female	7 (2–12)		9 (4–20)		25 (13–44)		36.5 (26–49)	
Male	6 (2–13)		11 (5–18)		26 (13–36)		35 (22–50)	
Primary tumor site		.753		.080		.544		.642
Scalp	7 (2–14)		12 (8–20)		30 (11–42)		36.5 (24–52)	
Neck	6 (2–12)		10 (4–18)		24 (15–35)		34 (25–49)	
Breslow thickness		.086		.510		.064		.002
Continuous variable	R2 = 0.02		R2 = 0.003		R2 = 0.02		R2 = 0.06	
Ulceration		.631		.124		< .001		.002
Absent	6 (3–12)		10 (5–17)		21 (11–33)		34 (21–44)	
Present	4 (1–17)		13 (4–20.5)		31 (21–48)		41 (29–58.5)	
Mitotic rate		.139		.001		.001		.002
$<1/mm^2$	5 (1.5–9)		7.5 (3.5–10.5)		18 (8–23)		26 (15.5–39.5)	
$\geq 1/mm^2$	7 (2–14)		12 (5–20)		27.5 (15.5–42)		37 (29–53)	
Clark level of invasion		.082		.044		.136		.423
II–III	7 (2–14)		11 (5–19)		23 (13–36)		34 (24–50)	
IV–V	4 (1–9)		6.5 (3–14)		31 (17–48)		39 (29.5–49.5)	
Tumor histotype		.045		.492		.208		.368
SSM	8 (3–14)		10 (5–16)		23 (11–35)		34 (24–46)	
Others	3 (1–10)		11.5 (4–20)		26 (16–42)		38 (26.5–52)	
TILs		.716		.757		.404		.062
Absent/nonbrisk	6.5 (2–13)		10 (5–18)		26 (14–40)		36 (25–50)	
Brisk	5.5 (1.5–12)		12 (6–19.5)		21.5 (12–30.5)		25.5 (15.5–39.5)	
Satellitosis		.144		.676		.635		.844
Absent	7 (2–13)		10 (5–19)		24 (13–39)		35 (24–50)	
Present	2 (0–13)		11 (4–18)		27 (17–36)		37 (28–50)	
IHC-detected LVI		.192		.001				
Absent	5.5 (1.5–12)		9 (4–16)		NA		NA	
Present	7 (2.5–17)		14 (8–23)		NA		NA	
IHC-detected BVI						.020		.018
Absent	NA		NA		23 (12.5–35.5)		34 (24–46)	
Present	NA		NA		37.5 (22–59.5)		50 (31–55)	

Abbreviations: LVD, lymphatic vessel density; BVD, blood vessel density; IQR, interquartile range; SSM, superficial spreading melanoma; TILs, tumor infiltrating lymphocytes; IHC, immunohistochemical; LVI, lymph vessel invasion; BVI, blood vessel invasion.
The underlined figures indicate statistical significance.

(median, $35/mm^2$; IQR = $15–50/mm^2$) was greater than LVD (median, $10/mm^2$; IQR, $2–19/mm^2$).

Table 2 reports the association of patient and tumor features with LVD and BVD considering their intratumoral or peritumoral location. LVD in the intratumoral area showed only a borderline correlation with the SSM histotype ($p = .045$), whereas in the peritumoral area it was associated with mitotic rate $\geq 1/mm^2$ ($p = .001$) and Clark level of invasion IV to V ($p = .044$). The presence of IHC-detected LVI was associated with LVD surrounding ($p = .001$) but not within the primary tumor ($p = .192$). Upon multivariate analysis, peritumoral LVD was associated with Clark level II to III (correlation coefficient [CC] = −4.83; 95% CI = −8.34 to −0.76; $p = .007$), mitotic rate $\geq 1/mm^2$ (CC = 4.57; 95% CI = 1.44–7.69; $p = .004$) and LVI (CC = 4.11; 95% CI = 1.32–6.91; $p = .004$).

Interestingly, BVD in the intratumoral area was associated with ulceration ($p < .001$) and mitotic rate $\geq 1/mm^2$ ($p = .001$). Furthermore, the same pathologic features were also associated with peritumoral BVD (ulceration, $p = .002$; mitotic rate, $p = .002$) along with tumor thickness ($p = .002$). Remarkably, IHC-detected BVI was associated with both intratumoral ($p = .020$) and peritumoral ($p = .018$) BVD. Strikingly, upon multivariate analysis, ulceration was the only factor independently associated with greater intratumoral (CC = 8.43; 95% CI = 0.87–16.00; $p = .029$) and peritumoral BVD (CC = 7.16; 95% CI = 0.10–14.22; $p = .047$).

Sentinel node biopsy

Data on sentinel node biopsy were available for 69 patients, of whom 16 had a positive sentinel node (23.2%).

LVI did not predict the sentinel node status ($p = .550$), even when only tumor with pure LVI (ie, excluding melanomas showing both LVI and BVI) were considered ($p = .322$). Similarly, peritumoral ($p = .828$) and intratumoral LVD ($p = .234$) were not associated with sentinel node status.

Survival analysis

After a median follow-up of 44 months (IQR = 18–80) 50 patients (32%) had a recurrence. Twenty-five patients had local or in-transit metastasis (50%), 4 patients recurred in the regional lymph node (8%), and 21 had distant metastasis (42%) as first recurrence. Pattern of recurrence did not correlate with presence of LVI ($p = .770$) or BVI ($p = .393$).

At univariate analysis for DFS, the presence of IHC-detected LVI/BVI was associated with shorter time to recurrence (hazard ratio [HR] = 2.34; 95% CI = 1.31–4.21; $p = .004$) along with scalp primary tumor (HR = 2.48; 95% CI = 1.23–5.00), thicker (HR = 1.07; 95% CI = 1.03–1.10; $p < .001$), and ulcerated tumors (HR = 1.89; 95% CI = 1.06–3.39; $p = .032$), as well as those showing Clark level IV to V (HR = 2.61; 95% CI = 1.37–4.98; $p = .004$), mitotic rate $\geq 1/mm^2$ (HR = 3.68; 95% CI = 1.43–9.60; $p = .007$), and satellitosis (HR = 5.17; 95% CI = 2.48–10.78; $p < .0001$). LVD (intratumoral, $p = .07$; peritumoral, $p = .993$) and BVD (intratumoral, $p = .607$; peritumoral, $p = .799$) did not correlate with DFS. Upon multivariate Cox regression analysis, thicker tumors (HR = 1.06; 95% CI = 1.02–1.09; $p = .001$) and mitotic rate $\geq 1/mm^2$ (HR = 3.11; 95% CI = 1.19–8.13; $p = .02$) were independently associated with a shorter DFS, whereas IHC-detected LVI/BVI was no longer significant.

At univariate analysis for MSS, neither the presence of IHC-detected LVI/BVI ($p = .217$), nor LVD (intratumoral, $p = .184$; peritumoral, $p = .576$), nor BVD (intratumoral, $p = .382$; peritumoral, $p = .794$) were prognostic factors for patient survival. Upon multivariate Cox regression analysis, tumor thickness was the only significant predictor of MSS (HR = 1.07; 95% CI = 1.03–1.12; $p = .002$).

DISCUSSION

To our knowledge, this is the first study examining the clinical relevance of IHC-detected vascular invasion and lymphangiogenesis in a large cohort of patients with scalp and neck melanomas. We found that the use of a dual D2-40/CD34 immunostaining identified roughly 25% more tumors showing LVI/BVI than conventional hematoxylin-eosin stain staining (40.4% vs 16.6%; $p < .001$). This observation confirms previous findings[18,19–25] that lymphovascular invasion is more accurately detected by IHC than in hematoxylin-eosin stain-stained tissues.[18–20,26–30] In our study, LVI was demonstrated in 34.6% of primary tumors. Reported LVI incidence for cutaneous melanoma, irrespective of the anatomic site, ranged from 16% to 37%, whereas it has been reported in 15% to 23% of cases with head and neck melanomas.[18–20,26–30] Accurate comparison with previous studies is complicated by the fact that scalp and neck melanomas are rarely evaluated as a separate subset, being more frequently grouped together with melanoma of the face under the category of "head and neck" melanomas.

Here, IHC-detected BVI was observed in 13.5% of melanomas of the scalp and neck region, approximately 4 times higher than the 2% to 4% reported in previous studies, irrespective of the primary tumor site.[21,27,28] In the head and neck area, Storr et al[28] reported a BVI incidence of 5.8%. We also showed that the density of blood vessels was greater than the density of lymphatic vessels both in the intratumoral and peritumoral areas. Overall, these data suggest that scalp and neck melanomas have peculiar vascular characteristics in terms of higher blood vessel invasion and density, which may stimulate tumor growth and represent a potential source of early blood-borne metastases. These results may also lead to the hypothesis that this more aggressive subgroup of melanomas may potentially benefit from antiangiogenetic therapies. We also found a strong association of primary melanoma features with vessel invasion and density. The presence of ulceration significantly correlated with LVI and was the only independent predictor of intratumoral and peritumoral BVD. The association between hematoxylin-eosin stain-detected[22,23] or IHC-detected LVI[24,28] and ulceration has been previously taken into account to explain the higher propensity of ulcerated tumors to develop sentinel lymph node and distant metastasis. It was shown that the presence of both ulceration and LVI increased the risk of sentinel lymph node metastasis up to 45% and 61%, depending upon patient age.[24] In our study, because of the limited number of patients subjected to sentinel lymph node biopsy, the ability of D2-40/CD34 double IHC to predict sentinel lymph node status could not be assessed.

The relationship between ulceration and LVI is a matter of speculation. It has been suggested that ulceration in melanoma is indicative of a hypoxic state that promotes lymphangiogenesis, which, in turn, results in a larger vessel area that facilitates dissemination of melanoma cells to the sentinel lymph node and distant sites. Recent evidence shows the activation of genes involved in cell adhesion and extracellular matrix interactions in ulcerated melanomas, including osteopontin, a strong stimulator of lymphatic endothelial cell migration and lymphangiogenesis.[25,31,32] Furthermore, it would be of interest to deeply dissecting not only the ability of melanoma cells to cross the endothelial of lymphatic and blood vessels to generate lymph node and distant metastasis but also the role of the immune system in limiting this process. In fact, interferon alpha, an immunostimulatory agent, which is the only approved drug for the adjuvant treatment of patients with melanoma with lymph node metastasis,[33,34] showed greater effectiveness in ulcerated primaries,[35–37] suggesting that this immunotherapy agent may limit the angiogenetic and chemotactic potential of melanoma cells[38,39] and supporting association between interferon alpha and antiangiogenetic[40] and antilymphangiogenetic[41] treatments.

Finally, we analyzed the prognostic relevance of LVI/BVI and vessel density in scalp and neck melanoma. Although univariate analysis of IHC-detected LVI/BVI data showed a significant association with DFS, this association was no longer significant at multivariate analysis. Furthermore, in a multivariate model for MSS, in which

thickness remained the only independent predictor of survival, neither lymphovascular invasion nor density was prognostic factors. A recent systematic review[26] summarized previous studies addressing the prognostic impact of lymphatic biomarkers in melanoma (although not strictly confined to the scalp and neck area) and highlighted heterogeneous results. There is evidence that IHC-detected LVI is a predictor of sentinel lymph node metastasis and poorer survival,[14,42] but these observations are still controversial.[21,28] In addition, although the presence of a greater density of lymphatic vessels in the peritumoral area seems to be associated with melanoma spread to regional lymph nodes and distant sites,[43] the prognostic significance of LVD remains unclear.[28] Wide methodological variations, including endothelial cell markers for blood and lymphatic vessels identification, differences in study design, and sample size, have been advocated to explain these discrepancies.[44] It has also been suggested that the functionality of vessels rather than absolute vessel density may determine disease prognosis in melanoma as in other cancers.[45]

In conclusion, IHC allows accurate detection of lymphovascular invasion in scalp and neck melanomas, although our results do not support the routine assessment of IHC-detected lymphovascular invasion and density. Peculiar vascular characteristics in terms of higher BVI and density may account for the greater risk of dissemination through the hematogenous route, although vascular invasion and vessel density did not add prognostic information to the common staging features. Among them, ulceration was associated with LVI and peritumoral and intratumoral BVD, supporting the invasion of lymphatic and the blood vessels sprouting as potential mechanisms beyond the negative prognostic value of ulceration. Nevertheless, higher blood vessel supply offers a strong biological rationale to potentially explore comparisons with melanomas on other body sites as well as therapeutic strategies targeting the angiogenic axis in this subgroup of patients with melanoma.

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