Prognostic Implication of Lymphovascular Invasion Detected by Double Immunostaining for D2-40 and MITF1 in Primary Cutaneous Melanoma

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Background: Lymphovascular invasion (LVI) is associated with adverse outcomes in primary cutaneous melanoma (PCM). Detection of LVI by hematoxylin and eosin staining alone is 0%–6%, but targeting lymphovascular structures increases the detection rate.

Objective: To examine the prognostic significance of LVI detected by immunostaining for D2-40 and microphthalmia-associated transcription factor 1 (MITF1) in PCM.

Methods: The authors retrospectively analyzed 120 PCM samples. We compared the LVI detection rates of immunostaining for D2-40 only (22%), double staining for D2-40 and MITF1 (38%), and hematoxylin and eosin, and examined the association of LVI with clinicopathologic variables and clinical outcomes.

Results: Immunolabeling with both methods significantly increased the LVI detection rate. Double staining for D2-40 and MITF1 as well as D2-40–detected LVI was significantly associated with increased Breslow thickness, number of mitoses, and sentinel lymph node (SLN) metastasis. D2-40–detected LVI was also associated with ulceration. Although the difference was not significant, double staining for D2-40 and MITF1 allowed for easier detection of LVI than D2-40 alone.

Limitations: This study was conducted in a tertiary referral institution; therefore, a referral bias cannot be excluded.

Conclusions: Immunolabeling increased detection of LVI in PCM. Because LVI is a positive predictive marker for SLN metastasis, the authors propose using anti-D2-40 and anti-MITF1 in the evaluation of LVI in patients with PCM with a certain risk of SLN metastasis.

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INTRODUCTION

The detection of lymphovascular invasion (LVI) in primary cutaneous melanoma (PCM) correlates with an increased risk of relapse, lymph node metastasis, distant metastases, decreased overall survival (OS), and decreased disease-free survival (DFS).[1] Furthermore, some studies indicate that the prognostic significance of LVI could be as strong as ulceration in predicting relapse and disease-related death in melanoma.[2] However, guidelines from the College of American Pathologists and the American Joint Committee on Cancer do not currently consider LVI as a tumor-staging criterion.

The reported incidence of LVI in patients with PCM in routine hematoxylin and eosin (H&E) slides ranges from 0% to 6% and is much lower than the reported incidence of sentinel lymph node (SLN) involvement, which ranges from 17% to 33%, regardless of tumor depth.[3–10] This discrepancy may be due to the difficulties associated with accurately identifying LVI with H&E staining alone, particularly in thick melanomas, where tumoral occlusion of vascular lumina can preclude LVI identification. Also, immunohistochemical staining is routinely used for SLN evaluation but not for LVI.

Several studies have shown that immunodetection of endothelial cells can increase LVI detection.[11–13] One such monoclonal antibody is anti-D2-40, which reacts primarily with podoplanin and is expressed primarily on lymphatic endothelium. Anti-D2-40 has been shown to facilitate the detection of LVI in various malignant tumors, including melanoma.[12] In a previous study, we compared detection of LVI using double immunostaining for D2-40 and S100 versus H&E staining in 101 PCM samples. The D2-40/S100 immunostaining method increased the LVI detection rate by approximately 10 times.[14] However, clinical follow-up data for those cases were not available.

The melanoma marker microphthalmia-associated transcription factor 1 (MITF1) exclusively stains the nucleus, which is easier to interpret, whereas S100 stains both the nucleus and cytoplasm (and is also less specific).[15] MITF1 is

almost as sensitive as S100 for detecting conventional melanoma cells but has several advantages in the context of a double stain: (1) it is more specific than S100 in the context of LVI evaluation because S100 is known to highlight other types of cells in skin including dendritic cells, and (2) MITF1 is a nuclear stain, which allows easier interpretation together with a cytoplasmic stain like D2-40 than S100, which shows both nuclear and cytoplasmic positivity in melanoma. However, MITF1 may highlight other cell types (eg, histiocytes). To avoid this potential pitfall in our interpretation, we used other melanocytic markers [melanoma cocktail (MART1 + tyrosinase + HMB45) and/or SOX10] to confirm the cells, which were staining with MITF1 in our double (D2-40/MITF1)-stained slides in this study. Therefore, double staining for D2-40/MITF1 could be useful to facilitate more accurate histopathological reading of PCM lesions.

In our practice, we routinely immunostain for either D2-40 only or both D2-40 and MITF1 to detect LVI in PCM samples for prognostic purposes. In this study, we examined the prognostic value of LVI detection by immunostaining for D2-40 and/or MITF1 in PCM. We compared the immunohistochemical detection of LVI in PCM with either D2-40 alone or with the double staining method (D2-40/MITF1) to H&E staining, and we assessed the ability of LVI detected by these methods to predict SLN status and survival.

METHODS

We retrospectively reviewed the electronic medical records, surgical reports, and pathology slides of PCM specimens obtained from patients evaluated at The University of Texas MD Anderson Cancer Center (UT-MDACC) between 2010 and 2014. Inclusion criteria were a diagnosis of PCM and availability of surgical specimens that had been immunostained with D2-40 or double staining for D2-40 and MITF1 during diagnostic evaluation. Most of the cases also had SLN biopsy specimens as the main reason for referral of the patients with PCM without metastasis to UT-MDACC was for wide local excision and SLN biopsy. As our practice has used double staining for D2-40 and MITF1 since April 2012, the follow-up period for this group of patients was shorter than for the D2-40 group (starting in April 2009). Patients were separated into 2 groups, those with primary tumor specimens stained for only D2-40 during diagnostic evaluation (early in the study period; n = 64) and those who had double staining for D2-40/MITF1-stained specimens (later in the study period; n = 56). All patients (n = 120) also had H&E-stained slides available for review. Data from these groups were used to assess associations between staining results, clinicopathologic variables, and outcomes.

To assess the ability of the 2 immunostaining techniques to predict SLN metastasis, we identified the patients who had a known SLN status from the 2 groups (48 double staining for D2-40/MITF1 patients and 41 D2-40 patients). As the 2 types of D2-40 immunostaining (D2-40 only and double staining for D2-40/MITF1) were used for different sets of patients, we performed 1:1 matching between the double staining for D2-40/MITF1 and D2-40 only groups on the basis of ethnicity (white vs. other); primary tumor site (head and neck, extremity, or trunk); and ulceration status. Matching was performed to account for estimating the staining effect in the absence of randomization. We were able to match 26 patients in each group. Propensity scores were calculated to determine the probability of being in the D2-40 only or double staining for D2-40/MITF1 groups based on these variables using logistic regression. The effect of D2-40 only versus double staining for D2-40/MITF1 was assessed in the matched pairs using conditional logistic regression to predict the odds of positive SLN.

To directly compare the ability of the 2 immunostaining methods to detect LVI, we performed the double staining for D2-40/MITF1 technique retroactively by adding the MITF1 staining on the slides of 50 patients who initially had only D2-40 staining (slides were unavailable for the other 14 patients; for those, we used whole slides scanning image for review of D2-40 stain). Two of 50 slides were technically equivocal and were excluded from further evaluation. These 48 cases were examined in a blinded and randomized fashion (ie, without knowledge of the original D2-40 only results). Samples that already had D2-40/MITF1 stains could not be stained retroactively with D2-40.

We reviewed the H&E, D2-40, and double staining for D2-40/MITF1 slides when available and collected the histological findings and immunohistochemical results reported at the time of diagnosis.

From the electronic medical and surgical reports, we extracted data on patient demographics, the primary tumor site, and Clark level; Breslow thickness, growth pattern (radial growth phase or vertical growth phase), mitotic figures, and ulceration and width of any ulcers; regression, LVI (detected through H&E staining, immunostaining for D2-40, or double staining for D2-40/MITF1), perineural invasion, microsatellite lesions, mutational status (*BRAF*, *KRAS*, and *c-KIT*), SLN biopsy and lymphadenectomy results, date and type of recurrence [local (ie, on or within 2 cm of the primary melanoma excision scar), and/or distant metastases], clinical status at the most recent contact (alive without evidence of recurrence, alive after recurrence, melanoma-related death, or death caused by another disease), and follow-up interval.

All patients in this study consented to the institution's use of their residual tissue and related medical data for research, according to the MD Anderson Research Consent Database (protocol LAB03-0320). This study was approved by the MD Anderson Institutional Review Board (Protocol # PA14-0711).

Descriptive statistics for baseline characteristics are provided in percentages. Frequencies and percentages are provided for categorical variables, and medians (range)

TABLE 1. Comparison of LVI Results From H&E Staining Versus Double Staining for D2-40 and MITF1 in Tissue Samples of PCM (n = 56)

	D2-40/MITF1 LVI, n (%)		
H&E LVI	**No**	**Yes**	**McNemar *P***
No (n = 55)	34 (62)	21 (38)	<0.0001
Yes (n = 1)	0	1 (100)	
Total (n = 56)	34	22	

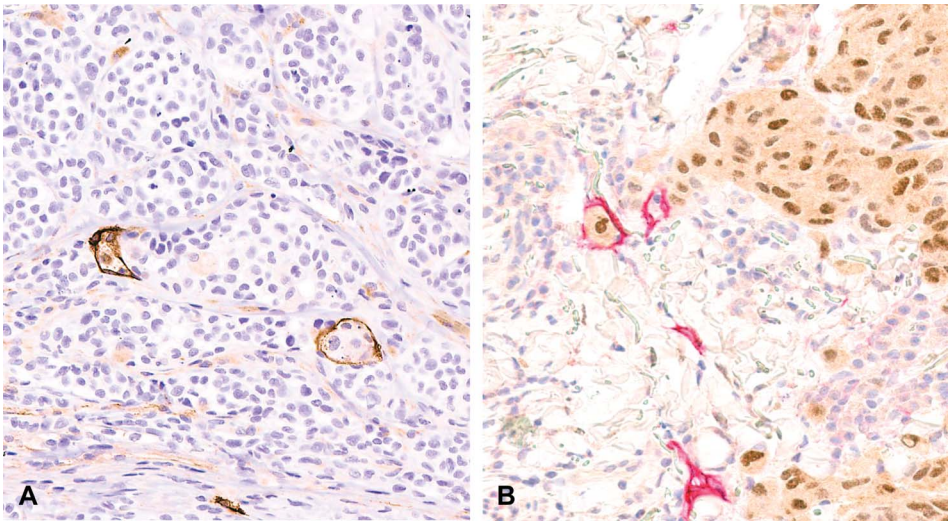

FIGURE 1. A, Representative photomicrographs of a PCM tissue sample with LVI revealed by D2-40 (brown chromogen). B, Representative photomicrograph of a PCM tissue sample with LVI detected by double staining (D2-40; red chromogen and MITF1; brown chromogen).

are provided for continuous variables. Presence of LVI was compared between staining methods in each group of patients using McNemar test for paired outcomes. Fisher exact and Wilcoxon rank-sum tests were used to assess the association between clinical variables and LVI for each staining method. All statistical analyses were performed using SAS software version 9.3. *P* values are 2-sided and significant when less than 0.05.

RESULTS

We assessed 120 PCM samples for LVI. The tumors were evaluated through double staining for D2-40/MITF1 (n = 56), D2-40 alone (n = 64), and H&E staining (n = 120). Immunohistochemistry significantly increased the rate of LVI detection. A significant percentage of patients who were LVI negative according to H&E staining were identified as LVI positive when assessed with double staining for D2-40/MITF1 (21/56, 38%; $P < 0.0001$) (Table 1 and Fig. 1A) or D2-40 (13/64, 22%; $P = 0.0003$) (Table 2 and Fig. 1B).

LVI detected by either double staining for D2-40/MITF1 or D2-40 alone was associated significantly with increased Breslow thickness ($P = 0.02$ and $P = 0.0002$, respectively) and number of mitoses ($P = 0.03$ and $P = 0.001$) (Table 3). Additionally, D2-40–detected LVI was associated with ulceration ($P = 0.0006$) and with decreased incidence of radial growth phase ($P = 0.02$) (Table 4).

The odds of SLN metastasis in LVI-positive patients were 5.2 (double staining for D2-40/MITF1) and 26 (D2-40 alone) times greater than in LVI-negative patients ($P = 0.01$ and $P = 0.0003$, respectively). We found that SLN metastasis was associated with LVI detected by either double staining for D2-40/MITF1 [13 (72%); $P = 0.02$] or D2-40 immunostaining [9 (75%); $P = 0.0001$] (Tables 5 and 6). We did not observe a significant group effect of the double staining for D2-40/MITF1 method versus D2-40 immunostaining for the prediction of SLN metastasis (Table 7).

In the D2-40 only group, 11/64 (17%) patients died. Thus, the median survival time was not reached because less than 50% of the patients died. The median follow-up time for that group was 24.6 months (range, 0.3–58.2). We did not observe a significant association between D2-40–detected LVI and OS. Because only 1 patient died in the double staining for D2-40/MITF1 group, we did not perform a survival analysis for that cohort. The median follow-up time for the double staining for D2-40/MITF1 group was 3.1 months (range, 1.0–24.3). We did not observe a significant difference between double staining for D2-40/MITF1 and D2-40 immunostaining in LVI detection when the 2 techniques were compared directly ($P = 0.4795$). However, double staining for D2-40/MITF1 identified 5 LVI-positive patients missed by D2-40 alone, whereas D2-40 identified 3 LVI patients that double staining for D2-40/MITF1 showed to be LVI negative (the intravascular cells possibly were nonmelanoma cells or were melanoma cells that did not express MITF1). The LVI detection rates for D2-40 only and double staining for D2-40/MITF1 were 23% and 27%, respectively (Fisher exact *P* value, $P = 0.814$).

TABLE 2. Comparison of LVI Results From H&E Staining Versus Immunostaining for D2-40 Alone in Tissue Samples of PCM (n = 64)

	D2-40 Only LVI, n (%)		
H&E LVI	**No**	**Yes**	**McNemar *P***
No (n = 60)	47 (78)	13 (22)	0.0003
Yes (n = 4)	0	4 (100)	
Total (n = 64)	47	17	

DISCUSSION

Our study's main objectives were to identify the histological and clinical parameters associated with LVI and to determine whether double staining for D2-40/MITF1 offered an advantage compared with D2-40 only staining.

LVI Detection

Our findings confirm the results of several studies demonstrating that immunohistochemistry for D2-40 increased

TABLE 3. Association of Clinicopathologic Variables With LVI Detected By Double Staining for D2-40 and MITF1 and LVI Detected by H&E Staining in Patients With PCM (n = 56)

	H&E LVI, n (%)*			D2-40/MITF1 LVI, n (%)*		
Variable	**No (n = 55)**	**Yes (n = 1)**	***P*†**	**No (n = 34)**	**Yes (n = 22)**	***P***
Sex						
Female	19 (100)	0	NA	12 (63)	7 (37)	0.7885
Male	36 (97)	1 (3)	NA	22 (60)	15 (41)	
Ethnicity						
Asian	1 (50)	1 (50)	NA	1 (50)	1 (50)	0.6226
White	51 (100)	0	NA	32 (63)	19 (37)	
Hispanic	3 (100)	0	NA	1 (33)	2 (67)	
Primary site						
Head/neck	13 (100)	0	NA	9 (69)	4 (31)	0.2760
Lower extr.	11 (92)	1 (8)	NA	6 (50)	6 (50)	
Trunk	19 (100)	0	NA	14 (74)	5 (26)	
Upper extr.	12 (100)	0	NA	5 (42)	7 (58)	
Clark level						
4	51 (100)	0	NA	31 (61)	20 (39)	1.000
5	4 (80)	1 (20)	NA	3 (60)	2 (40)	
RGP						
Absent	30 (97)	1 (3)	NA	17 (55)	14 (45)	0.3161
Present	25 (100)	0	NA	17 (68)	8 (32)	
VGP						
Absent	2 (100)	0	NA	2 (100)	0	0.5143
Present	53 (98.1)	1 (1.9)	NA	32 (59)	22 (41)	
Ulceration						
Absent	21 (95.5)	1 (4.5)	NA	13 (59)	9 (41)	0.8414
Present	34 (100)	0	NA	21 (62)	13 (38)	
Regression						
Absent	48 (98)	1 (2)	NA	31 (63)	18 (37)	0.4149
Present	7 (100)	0	NA	3 (43)	4 (57)	
PNI						
Absent	49 (100)	0	NA	31 (63)	18 (37)	0.4149
Present	6 (86)	1 (14)	NA	3 (43)	4 (57)	
Micro_Sat						
Absent	53 (100)	0	NA	33 (62)	20 (38)	0.5548
Present	2 (67)	1 (33)	NA	1 (33)	2 (67)	
BRAF						
Missing	36 (100)	0	NA	24 (67)	12 (33)	1.000
WT	10 (91)	1 (9)	NA	7 (64)	4 (36)	
Mut	9 (100)	0	NA	5 (56)	4 (44)	
KIT						
Missing	37 (100)	0	NA	25 (67)	12 (33)	0.7014
WT	17 (94.4)	1 (5.6)	NA	12 (67)	6 (33)	
Mut	1 (100)	0	NA	0	1 (100)	
KRAS						
Missing	38 (100)	0	NA	25 (67)	13 (33)	0.3285
WT	14 (100)	0	NA	8 (57)	6 (43)	
Mut	4 (100)	0	NA	4 (100)	0	
Age, median (range), yrs	63 (21–87)	62 (62-62)	NA	64 (31–87)	62 (21–86)	0.4553
Breslow, median (range), mm	3.2 (0.7–12)	7.8 (7.8–7.8)	NA	2.9 (0.7–12.0)	4.0 (1.3–10.2)	0.0199
Mitotic figures, median (range), mm^2	10 (1–35)	12 (12-12)	NA	8.5 (1–35)	15 (2–33)	0.0322
Ulcer width, median (range), mm^2	5.3 (0.2–28)	NA	NA	4.0 (0.2–23)	6.0 (0.5–28)	0.1260

*Unless otherwise indicated.

†*P* values for H&E are not useful with only 1 patient testing positive on H&E.

BRAF, B-Raf proto-oncogene, serine/threonine kinase; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; extr., extremity; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; Micro_Sat, microsatellite lesions; Mut, mutantated; PNI, perineural invasion; RGP, radial growth phase; VGP, vertical growth phase; WT, wild type.

TABLE 4. Association With Clinicopathologic Variables With LVI Detected By Immunostaining for D2-40 Alone and LVI Detected by H&E Staining in Patients With PCM (n = 64)

	H&E LVI, n (%)*			D2-40 Only LVI, n (%)*		
Variable	**No (n = 60)**	**Yes (n = 4)**	***P***	**No (n = 47)**	**Yes (n = 17)**	***P***
Sex						
Female	16 (89)	2 (11)	0.3131	12 (67)	6 (33)	0.4430
Male	44 (96)	2 (4)		35 (76)	11 (24)	
Ethnicity						
Asian	1 (100)	0	0.3783	0	1 (100)	0.3690
White	1 (100)	0		1 (100)	0	
Hispanic	54 (95)	3 (5)		43 (75)	14 (25)	
	4 (80)	1 (20)		3 (60)	2 (40)	
Primary site						
Head/neck	22 (96)	1 (4)	0.4151	16 (70)	7 (30)	0.2073
Lower extr.	9 (82)	2 (18)		6 (55)	5 (45)	
Trunk	17 (94)	1 (6)		16 (89)	2 (11)	
Upper extr.	12 (100)	0		9 (75)	3 (25)	
Clark level						
2	4 (100)	0	1.000	4 (100)	0	0.7962
3	4 (100)	0		3 (75)	1 (25)	
4	41 (93)	3 (7)		32 (73)	12 (27)	
5	11 (92)	1 (8)		8 (67)	4 (33)	
RGP						
Absent	12 (92)	1 (8)	0.0901	8 (61)	5 (39)	0.0171
Not evaluable	9 (82)	2 (18)		5 (45)	6 (55)	
Present	39 (97)	1 (3)		34 (85)	6 (15)	
VGP						
Absent	5 (100)	0	1.000	5 (100)	0	0.3131
Present	55 (93)	4 (7)		42 (71)	17 (29)	
Ulceration						
Absent	51 (98)	1 (2)	0.0188	43 (83)	9 (17)	0.0006
Not evaluable	1 (100)	0		1 (100)	0	
Present	8 (73)	3 (27)		3 (27)	8 (73)	
Regression						
Absent	35 (95)	2 (5)	1.000	25 (68)	12 (32)	0.2609
Present	25 (93)	2 (7)		22 (81)	5 (19)	
PNI						
Absent	48 (94)	3 (6)	1.000	38 (74)	13 (26)	0.7320
Present	12 (92)	1 (8)		9 (69.2)	4 (31)	
Micro_Sat						
Absent	59 (95)	3 (4.8)	0.1220	46 (74.2)	16 (26)	0.4638
Present	1 (50)	1 (50)		1 (50)	1 (50)	
BRAF						
Missing	47 (94)	3 (6)	0.6375	39 (78)	11 (22)	0.1667
WT	10 (91)	1 (9)		6 (54)	5 (46)	
Mut	3 (100)	0		2 (67)	1 (33)	
KIT						
Missing	52 (94)	3 (6)	0.4632	43 (78)	12 (22)	0.0734
WT	5 (83)	1 (17)		3 (50)	3 (50)	
Mut	3 (100)	0		1 (33.3)	2 (66.7)	
KRAS						
Missing	53 (94.6)	3 (5.4)	0.0656	42 (75)	14 (25)	0.3939
WT	7 (100)	0		5 (71)	2 (29)	
Mut	0	1 (100)		0	1 (100)	
Age, median (range), yrs	67 (28–96)	49 (36–83)	0.1972	64 (28–95)	75 (36–96)	0.2607

(*continued on next page*)

TABLE 4. (*Continued*) Association With Clinicopathologic Variables With LVI Detected By Immunostaining for D2-40 Alone and LVI Detected by H&E Staining in Patients With PCM (n = 64)

	H&E LVI, n (%)*			D2-40 Only LVI, n (%)*		
Variable	**No (n = 60)**	**Yes (n = 4)**	***P***	**No (n = 47)**	**Yes (n = 17)**	***P***
Breslow, median (range), mm	1.8 (0.2–29)	4.8 (2.5–5.5)	0.0690	1.3 (0.2–29)	4.8 (0.9–21)	0.0002
Mitotic figures, median (range), mm^2	2 (0–55)	5 (2–14)	0.1983	1 (0–55)	6 (2–20)	0.0010
Ulcer width, median (range), mm	9 (0.3–21)	3.0 (1.8–13.0)	0.5676	11 (3–21)	6.5 (0.3–20)	0.4236

*Unless otherwise indicated.

BRAF, B-Raf proto-oncogene, serine/threonine kinase; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; Micro_Sat, microscopic metastasis; Mut, mutantated; PNI, perineural invasion; RGP, radial growth phase; VGP, vertical growth phase; WT, wild type.

the frequency of LVI detection in PCM compared with conventional H&E staining. Niakosari et al[12] found that D2-40 immunohistochemistry identified LVI in 16% of 44 melanoma samples, while H&E staining failed to detect any cases with LVI. Fohn et al[16] showed that D2-40 immunohistochemistry increased the LVI detection rate to 22% (from 3% using only H&E staining). Xu et al[17] showed that H&E alone detected LVI in 5 (5%) of 106 PCM, whereas dual immunostaining for podoplanin and S-100 and multispectral imaging analysis identified LVI in 35 patients (33%). Rose et al[11] assessed 246 primary melanomas for LVI using D2-40 immunostaining, CD34 immunostaining, and routine histology, and found that the use of endothelial markers increased the LVI detection rate (D2-40 and/or CD34, 18%, vs. routine histology, 3%). In another study, D2-40/S-100 dual immunostaining identified LVI in 10 of 27 PCM, whereas routine histology identified only 1 patient.[10] Using immunostaining for D2-40, Peterson et al found LVI in 23% of 74 invasive melanomas.[9] Our double staining for D2-40/MITF1 method showed the highest LVI detection rate to date (38%). Taken together, these findings confirm that LVI is underestimated by H&E alone, and that immunohistochemistry is an essential ancillary diagnostic tool for LVI.

We observed that LVI detected by both double staining for D2-40/MITF1 or D2-40 alone was significantly associated with increased Breslow thickness and number of mitoses. Furthermore, LVI detected by D2-40 alone was also significantly associated with ulceration. Niakosari et al[12] also observed a trend toward an association between LVI and a deeper Clark level and increased frequency of ulceration. In a later study from the same group, D2-40–detected LVI was significantly associated with a deeper Clark level of invasion and greater Breslow tumor thickness.[13] Rose et al[11] found that D2-40–detected or CD34-detected LVI was significantly associated with more adverse clinicopathologic variables (thickness, ulceration, mitoses, and nodular subtype) compared with LVI detected by routine histology (only thickness and ulceration). Taken together, these results confirm the association between immunohistochemical detection of LVI using D2-40 with Breslow thickness, mitosis, and ulceration—3 essential prognostic parameters.

SLN Status

Our results showed an association between D2-40–detected LVI and positive SLN status, which supports the findings of recent studies. Niakosari et al[13] identified SLN metastasis in 23 (24%) of 96 cases compared with 69% (double staining for D2-40/MITF1) and 29% (D2-40 only) in our series. Through multivariate analysis, Niakosari et al[13] also found that D2-40–detected LVI, younger age, and ulceration were independent prognostic factors for SLN metastasis. Fohn et al[16] showed that D2-40–detected LVI was the most significant predictor of SLN metastasis, whereas Doeden et al[18] showed that a combination of LVI and intratumoral lymphatics had higher positive and negative predictive values for the risk of developing SLN metastasis compared with routine histology and LVI. However, Petitt et al[19] showed no association between LVI and SLN status: 8 of 10 melanomas with LVI were SLN-negative, possibly owing to the small patient sample. Our results confirm that LVI is a significant predictor of SLN metastases and can identify patients with a low risk of SLN metastasis who might be spared from invasive SLN biopsy.

Survival Outcomes

Several previous studies showed an association between LVI and distant metastasis, OS, and DFS in patients with PCM.[1,11,17] Previously, we showed that OS and disease-specific

TABLE 5. Association Between LVI Detected By Double Staining for D2-40 and MITF1 and SLN Metastasis

	Sentinel Lymph Node Metastasis, n (%)		
D2-40/MITF1 LVI	**No**	**Yes**	**Fisher Exact *P***
No (n = 30)	20 (67)	10 (33)	0.0162
Yes (n = 18)	5 (28)	**13 (72)**	
Total (n = 48)	25	33	

TABLE 6. Association Between LVI Detected By Immunostaining for D2-40 Alone and SLN Metastasis

	Sentinel Lymph Node Metastasis, n (%)		
D2-40 Only LVI	**No**	**Yes**	**Fisher Exact *P***
No (n = 29)	26 (90)	3 (10)	0.0001
Yes (n = 12)	3 (25)	9 (75)	
Total (n = 41)	29	12	

TABLE 7. Analysis of Matched Pairs of Samples of PCM With LVI Detected by Double Staining for D2-40 and MITF1 Versus Immunostaining for D2-40 Alone and the Prediction of SLN Metastasis (n = 26, SLN = 11)

	OR for SLN	95% CI for OR	*P*
D2-40/MITF1 vs. D2-40 only	2.33	0.60–9.02	0.2195

CI, confidence interval; OR, odds ratio.

survival durations were shorter for patients with LVI than for those without LVI, and that immunostaining-detected LVI and ulceration were associated significantly with OS.[9] Rose et al[11] found that immunostaining-detected LVI was a significant marker of both reduced DFS and OS. Xu et al[17] found that LVI was associated significantly with time to regional nodal metastatic disease, time to first metastasis, and melanoma-specific death.

In this study, we did not observe a significant association between D2-40–detected LVI and OS or recurrence. Additionally, neither D2-40–detected nor double staining for D2-40/MITF1-detected LVI was significantly associated with clinical outcomes, including regional nodal metastasis or satellite/local/distant metastasis. This finding might be due to the short follow-up period; during the maximum 4-year follow-up period in our study, only 11 (17%) of the 64 patients died.

Comparison Between D2-40 Only and Double Staining for D2-40/MITF1

When comparing the 26 matched patients, we did not observe a significant difference in the prediction of SLN metastasis (Table 7). When comparing D2-40 immunostaining and double staining for D2-40/MITF1 for patients who had both stains, we did not observe a significant difference in the LVI detection rates (*P* value = 0.47, n = 48). However, our double staining for D2-40/MITF1 method identified LVI in an additional 5 patients not identified by immunostaining for only D2-40. D2-40 immunostaining resulted in a false-positive LVI diagnosis for 3 patients (Fig. 1C); the double staining for D2-40/MITF1 method showed that the intravascular cells in these samples were not melanocytes (or, less likely, melanocytes that did not express MITF1 because none of these patients showed SLN metastasis and primary melanoma in these cases stained positively for MITF1) (Fig. 1D).

Limitations

This retrospective study was conducted at a single institution; therefore, misclassification and referral bias cannot be excluded. Also the shorter clinical follow-up, especially in the group with double staining for D2-40 and MITF1 is also one of the limitations. Nevertheless, the large sample size of our study and the collection of data based on electronic medical and surgical reports offer reliable information. No multivariate analysis was performed in this study to investigate if LVI is an independent prognostic marker in patients with PCM.

CONCLUSIONS

In summary, our results indicate that immunostaining with D2-40 and with double staining for D2-40/MITF1 increased the LVI detection rates in patients with PCM, and that LVI is associated significantly with known adverse clinicopathologic parameters. Although we did not observe a significant difference in LVI detection between the single immunostaining and the double staining for D2-40/MITF1 methods, double staining for D2-40/MITF1 might offer higher sensitivity and specificity than immunostaining for only D2-40. Because LVI is a positive predictive marker for SLN metastasis, we propose the use of immunohistochemical study for the evaluation of LVI in patients with PCM with a risk of SLN metastasis.

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