Melanocyte Density in the Diagnosis of Melanoma In Situ in Sun-Damaged Skin

Rieke Löper, Michael P. Schön, MD, and Christina Mitteldorf, MD*

Abstract: Histologic differentiation between melanoma in situ in chronically sun-damaged skin (CSDS) [lentigo maligna (LM)] and CSDS without malignancy is difficult because signs of melanocyte activation and proliferation are found in both. A potentially reliable and quantifiable criterion is melanocyte density (MD). Here, we evaluated whether and to what extent MD allows the distinction between LM and CSDS, which is particularly relevant for the evaluation of borderline cases and surgical margins. Articles assessing MD in LM and/or CSDS were evaluated in a systematic review. The results were categorized and compared according to staining. Cutoff values were included whenever stated. Twenty articles matched the selection criteria. Six hundred forty-four samples of CSDS and 227 samples of LM were considered. In each individual study, mean MD scores were higher for LM than for CSDS. However, looking at the overall study situation, it becomes clear that the data are very heterogeneous and show overlaps. Therefore, no reliable orientation value can be derived. Only 1 article defined a cutoff value. The data of MD in LM in contrast to CSDS were sparse, and a defined cutoff value was only mentioned in 1 article for microphthalmia-associated transcription factor, which cannot yet be generalized. Especially regarding the importance for the definition of surgical resection margins, this unsatisfactory data set highlights the need for further studies. More precise diagnostic criteria could spare some patients extensive and possibly disfiguring surgery.

Key Words: lentigo maligna, chronically sun-damaged skin, melanocyte density, immunohistochemical stains

(*Am J Dermatopathol* 2024;46:358–364)

INTRODUCTION

Lentigo maligna (LM) is a melanoma in situ occurring in chronically sun-damaged skin (CSDS).[1] Dermatoscopic examination often reveals a particular involvement of the hair follicles.[2] The head and neck region of elderly patients is typically affected,[1] but LM can also occur in younger patients and in other photodamaged skin areas.[3–7] LM usually has a prolonged horizontal growth phase before it grows vertically and can become invasive (LM melanoma). It has been proposed that LM melanomas have the same prognosis than other types of invasive melanomas,[8,9] although a recent article suggests a somewhat better prognosis compared with other types of melanoma.[10]

The standard therapy is complete excision,[11,12] while radiotherapy, destructive, or topical therapies are second-line options.[13–17] Correct histologic evaluation is of paramount relevance because tumors are often extensive and located in very delicate facial anatomic sites (Fig. 1) where surgical intervention can be particularly distressing and disfiguring. Unfortunately, the histologic evaluation of surgical margins is challenging because diagnostic criteria are not well-defined.[18,19] Although some criteria, such as density of melanocytes within the epidermis [melanocyte density (MD)], nesting, pagetoid spread, and adnexal extension, have been described,[19–23] universally applicable threshold values for either of these parameters do not exist. Figure 2 shows exemplarily clear-cut histologic samples of LM and CSDS, each stained with hematoxylin and eosin (H&E) or antibodies directed against SOX-10 or Melan-A.

This systematic review evaluates MD as a diagnostic criterion in LM and/or CSDS. It also includes cutoff values, facilitating the distinction between LM and CSDS.

MATERIALS AND METHODS

Articles obtained from a systematic search in PubMed and dealing with MD in LM and/or in CSDS were reviewed. The search was performed by entering the keyword combinations "(LM) AND {[immunohistochemistry (IHC)] OR (melanocyte count) OR (density)}" and "[(sun damage) OR (photo damage)] AND [(melanocyte count) OR (melanocytic density)]." The keywords were deliberately chosen broadly to cover relevant articles as completely as possible. In addition, the bibliographies of the articles found were searched for further suitable articles. Only articles in English or German were included. Furthermore, only articles in which the MD was recorded in a defined area of epidermis were considered. Exposure to natural ultraviolet radiation was a prerequisite for the inclusion of CSDS cases. Figure 3 depicts the search algorithm in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram.

RESULTS

Twenty articles matched the search criteria. Six hundred forty-four samples of CSDS and 227 samples of LM were included. Most articles did not explicitly define chronical sun damage. Only Barlow et al[24] described that sun damage was related to solar elastosis. The results were separated

From the Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Göttingen, Germany.

The authors declare no conflicts of interest.

*These data are part of her doctoral thesis.

Correspondence: Prof. Dr. Christina Mitteldorf, MD, Department of Dermatology, Venereology and Allergology, University Medical Center Göttingen, Robert-Koch-Strasse 40, 37085 Göttingen, Germany (e-mail: christina.mitteldorf@med.uni-goettingen.de).

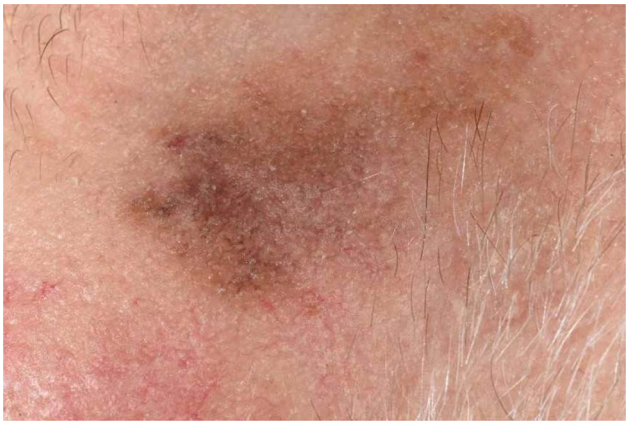

FIGURE 1. Clinical appearance of LM: irregularly configurated and in homogeneously pigmented brown macule in sun-damaged skin.

by the stains used: H&E or IHC using antibodies directed against SOX-10, Melan-A/MART-1, and microphthalmia-associated transcription factor (MITF) or, rarely, R21, Mel-5, HMB-45, S-100 protein, and MIB-1. Table 1 summarizes the results of MD in LM and CSDS. To make the data comparable, values of mean/median MD have been converted to "melanocytes per 0.5-mm epidermis" (m/0.5 mm), if the values were not already given with this unit. Within each individual study, MD values were markedly higher in LM than in CSDS. This may lead to the assumption that the mean MD values between LM and CSDS can always be clearly distinguished. In fact, however, one often finds overlaps when looking at the individual MD values of all studies. In addition, heterogeneity is apparent depending on the staining used. Therefore, a clear guideline value or generally usable cutoff cannot be determined from the literature. Only 2 articles provided direct statistical comparisons of MD densities between LM and CSDS, both of which demonstrated significant differences.[18,23]

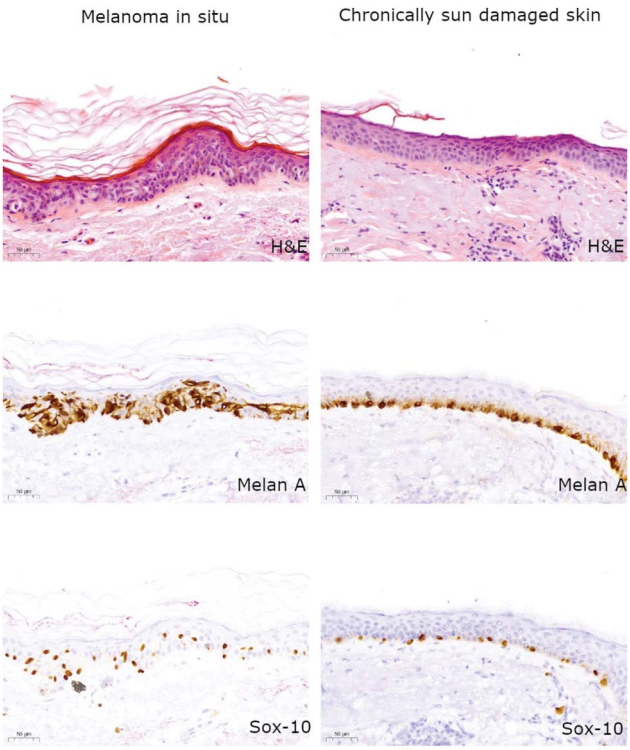

FIGURE 2. Exemplary cases of chronically sun-damaged skin in H&E, SOX-10, and Melan-A in comparison with LM.

The only article that gave a specific cutoff value was that by Black et al,[23] although the data only referred to staining against MITF. They found that ≥10 melanocytes per 200 μm of epidermis allowed the diagnosis of melanoma in situ in chronically photodamaged skin with a specificity of 100%. For the calculation used here, this would be ≥25 per 0.5 mm.

Table 2 presents an overview showing the number of studies and the total number of cases related to the 2 factors "staining method" and "type of lesion" (LM/CSDS). Articles without definite information on the staining method were not considered.[19,29,40]

Unfortunately, it was not possible to carry out a meaningful statistical evaluation regarding the comparison of the different mean values in the studies because of different proceedings used in the studies resulting in heterogeneous data.

DISCUSSION

Clear differences between the mean and/or median numbers of melanocytes in LM and CSDS were evident in all the articles examined with higher numbers in LM, regardless of the staining method used. In 2 articles, the differences were statistically significant.[18,23]

However, studies that provided ranges for both LM and CSDS showed significant overlap.[19,23] In addition, values and standard deviations usually spread over a wide range, as exemplified by the study in CSDS by Barlow et al.[24] In this study, the SD (±3.34) was about as large as the mean (3.98) and the values ranged from 0.35 to 16.7 m/0.5 mm in CSDS.

Basically, there was high interindividual variability regarding MD in all publications even with the same staining methods. This led to heterogeneous data sets and renders evaluation of individual slides in the daily routine challenging. It is obvious that a cutoff value for MD would make sense to differentiate between CSDS and LM, although it seems at least questionable that such a value can be determined at all with realistic accuracy given the "background noise" outlined. In addition, it is helpful also to consider the gradient of MD from the lesional center to the periphery as an additional criterion in daily routine examinations.

Black et al[23] set a cutoff value of ≥10 melanocytes/100 μm (≥25 melanocytes/0.5 mm), but this was only evaluated in MITF, and the study included only 14 cases. Considering that these results cannot be easily transferred to other stains, their value is limited. Interestingly, Gorman et al[41] ranked MD in H&E in relation to the recurrence risk of LM lesions with a low risk counting 0–20 melanocytes,

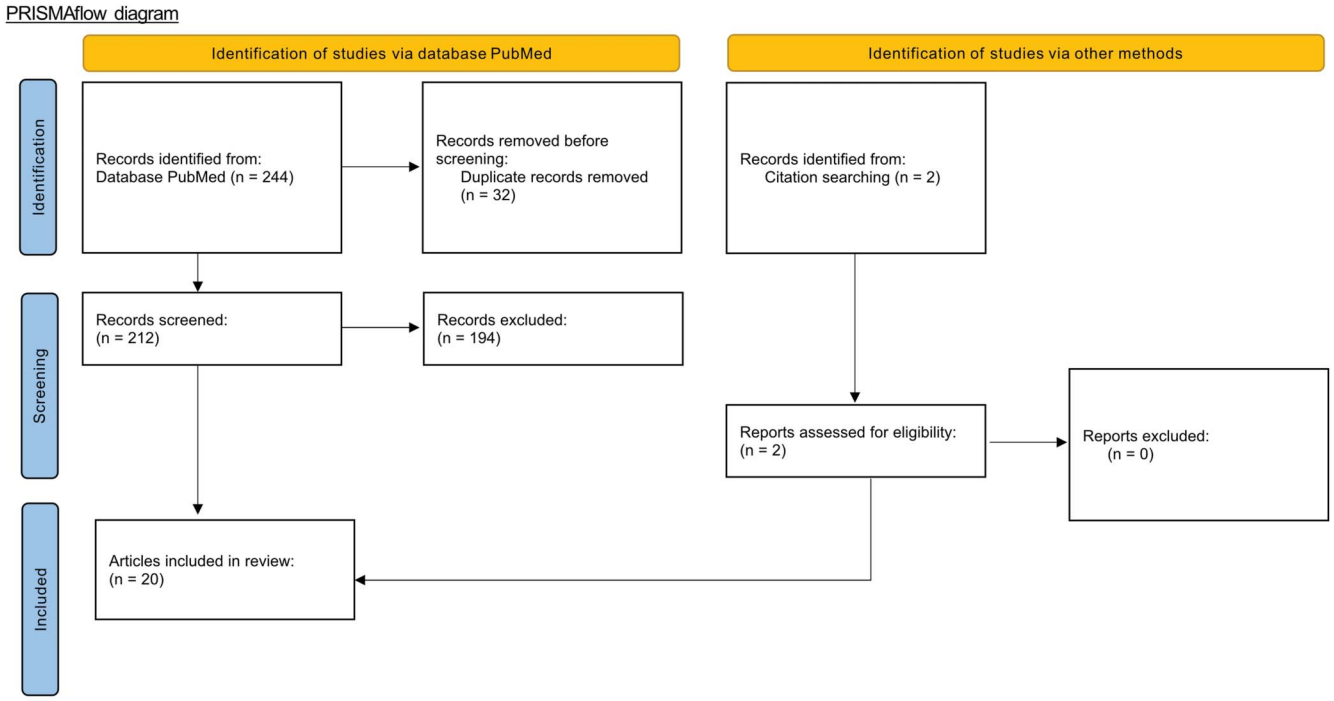

FIGURE 3. PRISMA flow diagram. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

a medium risk counting 21–30 melanocytes, and a high risk counting ≥31 melanocytes per 0.5 mm. These scores seem to be comparable with the data from Black et al, even if we would rather suspect a higher MD score in MITF than in H&E.

In summary, a generally applicable threshold value cannot yet be defined on the basis of the data pool available to date. Consequently, studies evaluating the specificity and sensitivity of such a putative value are also still lacking. However, the need for such studies is evident.[23]

Most of the studies did not compare CSDS with LM directly, but with other lesions such as solar lentigo. That was the reason why *P*-values for MD in CSDS versus LM are only reported by 2 articles.[18,23]

There are other issues that complicate the comparability of the studies. On one hand, authors used different staining techniques. Some used frozen sections, and some used permanent sections. However, Cherpelis et al[26] found no difference between frozen and permanent sections when evaluating MD. On the other hand, the source of CSDS differed and can influence MD. Even if sun damage was evident in all samples, some samples were from patients suffering from basal cell carcinomas or squamous cell carcinomas,[18,19,23,24,26,31,34–36] and others were even only mentioned as negative margins from LM lesions.[38,39] Moreover, other samples were taken as control biopsies near the lesion but from a clearly unaffected marginal area.[25,27,29,40]

The methods of melanocyte counting were also different. In several studies, more than 1 observer examined the slides,[27,29,30,32,35,38,39] while in others, there was only one.[18,19,25,26,31] Similarly, the selection of sections used for counting differed. Some articles stated they were looking for 1 representative area,[23,31,34,37–39] whereas others generated an average or median of a few areas.[6,18,24–27,29,35,36,40]

Furthermore, it was difficult to compare the articles because of the different origin and ethnicity of the participants. Sun exposure might also be variable. Hendi et al[35] demonstrated statistically significant differences regarding MD in CSDS between people who lived in Florida and Minnesota.

Another point is that the number of articles investigating MD in H&E (n = 4) and IHC (SOX-10: n = 2; Melan-A/Mart-1: n = 4; MITF: n = 3) in LM is very low. A higher number of studies dealing with IHC would make the outcome much more reliable and representative. In the daily routine, not all samples were counted for MD because it is very time consuming and should be withheld for borderline cases. In clear cases, the diagnosis can often also be made in the overview, which is much easier in IHC than in H&E alone. Immunohistochemical staining of preferentially expressed antigen in melanoma has recently shown high sensitivity and specificity in LM as compared with other stains.[42,43] It could be helpful in addition to other IHC stains, such as SOX-10.[44] Hopefully, we can look forward to more articles dealing with this special IHC stain.

In conclusion, the data about MD in CSDS and LM are sparse. To this day, no clear cutoff value could be given to differentiate CSDS and LM with certainty. Still, in unclear

TABLE 1. MD Reported in the Literature in LM and Chronically Sun-Exposed Skin

	Chronically Sun-Damaged Skin (Melanocytes per 0.5 mm of Epidermis)				Lentigo Maligna (Melanocytes per 0.5 mm of Epidermis)				
Authors in Alphabetic Order	**No. of Cases (n)**	**Anatomic Sites With Number (n)**	**Skin Phenotypes With Number (n)**		**No. of Cases (n)**	**Anatomic Sites With Number (n)**	**Skin Phenotypes With Number (n)**		***P***
Acker et al[18] 1998	18	No detailed information provided	No detailed information provided	H&E: 11.61 ± 1.98 (mean ± SD)	38	Head (19), neck (6), back (6), arm (6), leg (1)	All patients White	H&E: 37.83 ± 11.12 (mean ± SD)	$<10^{-6}$
Barlow et al[24] 2007	180	Head and neck (106) trunk (22), upper extremity (23), lower extremity (24), hands or feet (5)	Fitzpatrick skin type I (30) II (90) III (60)	H&E: 3.98 ± 3.34 (mean ± SD), 0.35–16.7 (range)	—	—	—	—	—
Black et al[23] 2011	14	Head and neck (14)	No information provided	MITF: 10 ± 3.83, 2.5–15 (mean ± SD, range)	14	Head and neck (14)	No information provided	MITF: 58.03 ± 33.63, 15–115 (mean ± SD, range)	<0.0001
Bowen et al[25] 2011	17	No information provided	All patients Caucasian	Melan-A: 25.6 ± 9.3 (mean ± SD)	—	—	—	—	—
Cherpelis et al[26] 2009	25	Head and neck (25)	All patients Caucasian; Fitzpatrick skin type I (8) II (13) III (5)	MART-1: paraffin sections: 16.7 ± 8.55 (mean ± SD), 17.5, 2.5–35 (median, range) MART-1: frozen sections: 16.8 ± 6.55 (mean ± SD), 17.5, 5–30 (median, range)	—	—	—	—	—
Christensen et al[27] 2016	16	Head and neck (15), arm (1)	All patients Caucasian	MITF-1: 9.8, 3.5–15.2 (mean, range) Melan-A: 13.7, 5.2–24.3 (mean, range)	—	—	—	—	—
Coakley et al[28] 2020	16	No information provided	No information provided	MITF: first biopsy: 13.13 (median) MITF: second biopsy: 21.38 (median)	—	—	—	—	—
Flores et al[29] 2018	52	Head (47), neck (3) Upper extremities (2)	No information provided	H&E, MART-1, SOX-10: 20.0 ± 6.2 (mean ± SD), 20.3, 9.0–36.7 (median, range)	—	—	—	—	—
Gautschi et al[30] 2016	—	—	—	—	89	Head (84) Other locations (5)	Fitzpatrick skin type I (10) II (45) III (34)	Melan-A: 16.6, 4.85–60 (median, range)	—
Glass et al[31] 2010	11	Head and neck	No information provided	MITF: permanent sections: 9.5 ± 4.0 (mean ± SD) frozen sections: 10.0 ± 2.7 (mean ± SD)	—	—	—	—	—
Gómez-Martín et al[32] 2017	12	Face	All patients white	H&E: 5.2 ± 2.8 (mean ± SD) Melan-A: 9.7 ± 3.5 (mean ± SD) MITF: 10.7 ± 3.7 (mean ± SD)	—	—	—	—	—

(*continued on next page*)

TABLE 1. (*Continued*) MD Reported in the Literature in LM and Chronically Sun-Exposed Skin

	Chronically Sun-Damaged Skin (Melanocytes per 0.5 mm of Epidermis)				Lentigo Maligna (Melanocytes per 0.5 mm of Epidermis)				
Authors in Alphabetic Order	**No. of Cases (n)**	**Anatomic Sites With Number (n)**	**Skin Phenotypes**	**With Number (n)**	**No. of Cases (n)**	**Anatomic Sites With Number (n)**	**Skin Phenotypes With Number (n)**		***P***
				SOX-10: 11.0 ± 4.6 (mean ± SD)					
Helm, Findeis-Hosey[33] 2008	—	—	—	—	20	Head/neck (12) arm (3) leg (3) Other (2)	No information provided	Melan-A: 41 ± 13.65 (mean ± SD)	—
Hendi et al[34] 2006	132	Head or neck	All patients White	MART-1: 15.60 ± 4.38 (mean ± SD) 15.0, 6–29 (median, range)	—	—	—	—	—
Hendi et al[35] 2011	100	Face and neck	Recruited in Minnesota (50) and Florida (50)	H&E: 9, 3–23 (median, range), 9.3 ± 3.7 (mean ± SD) Melan-A: 11, 3–32 (median, range), 12.0 ± 4.8 (mean ± SD)	—	—	—	—	—
Hillesheim et al[36] 2011	6	No information provided	No information provided	MITF: 9.8, 5.6–16.4 (mean, range) MART-1/Azure blue: 9.3, 5.8–12.8 (mean, range)	—	—	—	—	—
Kim et al[37] 2011	—	—	—	—	20	Head and neck (13), extremities (4) Upper back (2), clavicle (1)	No information provided	H&E: 54.3 (mean) MITF: 56 (mean) HMB-45: 55.4 (mean) Melan-A: 74.5 (mean) Mel-5: 40 (mean)	—
Mu et al[38] 2018	10	Face	No information provided	H&E: 11 (mean) MITF: 17 (mean) MART-1: 15 (mean) SOX-10: 16 (mean) R21: 9 (mean)	10	Face	No information provided	H&E: 28 (mean) MITF: 40 (mean) MART-1: 34 (mean) SOX-10: 33 (mean) R21: 27 (mean)	—
Siarov et al[39] 2021	26	No information provided	No information provided	Negative margin H&E: 7.8 (mean) SOX-10: 15.6 (mean)	26	No information provided	No information provided	Positive margin H&E: 14.8 (mean) SOX-10: 32.3 (mean)	—
Speiser et al[40] 2019	15	Head and neck (10), abdomen (1), shoulder (1), arm (3)	White/Non-Hispanic origin with Fitzpatrick skin type I	MITF/SOX-10: 16.5, 8–19 (mean, range)	—	—	—	—	—
Weyers et al[19] 1996	10	No information provided	No information provided	HMB-45, S-100 protein, MIB-1 10 ± 4,47, 3–26 (median ± SD, range)	10	No information provided	No information provided	HMB-45, S-100 protein, MIB-1: 50 ± 27.59, 11–134 (median ± SD, range)	—

TABLE 2. Overview of the Number of Studies and Cases Offering Values of MD in LM and Chronically Sun-Exposed Skin Ordered by the Most Commonly Used Stains

	CSDS		LM	
	No. of Studies (n)	No. of Cases in Total (n)	No. of Studies (n)	No. of Cases in Total (n)
H&E	6	346	4	94
SOX-10	3	48	2	36
Melan-A	8	343	4	139
MITF	7	112	3	44

cases, such criteria could help spare patients from extensive or mutilating surgery. Further studies are urgently needed. It is likely that there will ultimately be no single diagnosis-deciding criterion, but the current situation certainly leaves room for improvement.[18,19,34,40] For the diagnosis, it seems reasonable to use a combination of practicable criteria.[18,19,34,40] However, near the edge of the lesion, histologic changes, such as MD, are sometimes very subtle so that only this one criterion proves to be relevant for the decision nevertheless. In addition, other criteria, such as melanocyte nesting or proliferation down adnexal structures, accompany a higher MD in most cases. Moreover, MD is quantifiable and digital analysis or even artificial intelligence could help.

REFERENCES

1. Beltraminelli H, Shabrawi-Caelen LE, Kerl H, et al. Melan-a-positive "pseudomelanocytic nests": a pitfall in the histopathologic and immunohistochemical diagnosis of pigmented lesions on sun-damaged skin. *Am J Dermatopathol.* 2009;31:305–308.
2. Dika E, Lambertini M, Patrizi A, et al. Folliculotropism in head and neck lentigo maligna and lentigo maligna melanoma. *J Dtsch Dermatol Ges.* 2021;19:223–229.
3. Kuflik EG, Gage AA. Cryosurgery for lentigo maligna. *J Am Acad Dermatol.* 1994;31:75–78.
4. Durnick A, Stolz W, Landthaler M, et al. Lentigo maligna and lentigo maligna melanoma in young adults. *Dermatol Surg.* 2004;30:813–816.
5. Ferrara G, Ligrone L, Zalaudek I, et al. Lentigo maligna in a young adult. *Dermatology*. 2008;217:66–68.
6. Helm MF, Bax MJ, Augenblick DJ, et al. Melanoma in situ of lentigo maligna type in a young woman. *Int J Dermatol.* 2017;56:961–962.
7. Paolino G, Panetta C, Donati M, et al. Recurrent lentigo maligna in a young patient. *Ital J Dermatol Venerol.* 2021;156:89–91.
8. Robinson M, Primiero C, Guitera P, et al. Evidence-based clinical practice guidelines for the management of patients with lentigo maligna. *Dermatology*. 2020;236:111–116.
9. Koh HK, Michalik E, Sober AJ, et al. Lentigo maligna melanoma has no better prognosis than other types of melanoma. *J Clin Oncol.* 1984;2:994–1001.
10. Jasper S, Keim U, Leiter U, et al. Prognosis in stage II melanoma of the head and neck depends on the histological subtype. *J Dtsch Dermatol Ges.* 2023;21:1137–1146.
11. Hauschild A, Rosien F, Lischner S. Surgical standards in the primary care of melanoma patients. *Onkologie*. 2003;26:218–222.
12. Hauschild A, Lischner S, Christophers E. Operative und adjuvante medikamentöse Therapie des kutanen Melanoms im Kopf-Hals-Bereich. *Laryngorhinootologie*. 2000;79:428–433.
13. Farshad A, Burg G, Panizzon R, et al. A retrospective study of 150 patients with lentigo maligna and lentigo maligna melanoma and the efficacy of radiotherapy using Grenz or soft X-rays. *Br J Dermatol.* 2002;146:1042–1046.
14. Collins P, Rogers S, Goggin M, et al. Cryotherapy for lentigo maligna. *Clin Exp Dermatol.* 1991;16:433–435.
15. Naylor MF, Crowson N, Kuwahara R, et al. Treatment of lentigo maligna with topical imiquimod. *Br J Dermatol.* 2003;149(suppl 66):66–70.
16. Powell AM, Robson AM, Russell-Jones R, et al. Imiquimod and lentigo maligna: a search for prognostic features in a clinicopathological study with long-term follow-up. *Br J Dermatol.* 2009;160:994–998.
17. Räsänen JE, Neittaanmäki N, Jeskanen L, et al. Ablative fractional laser-assisted photodynamic therapy for lentigo maligna: a prospective pilot study. *J Eur Acad Dermatol Venereol.* 2020;34:510–517.
18. Acker SM, Nicholson JH, Rust PF, et al. Morphometric discrimination of melanoma in situ of sun-damaged skin from chronically sun-damaged skin. *J Am Acad Dermatol.* 1998;39:239–245.
19. Weyers W, Bonczkowitz M, Weyers I, et al. Melanoma in situ versus melanocytic hyperplasia in sun-damaged skin: assessment of the significance of histopathologic criteria for differential diagnosis. *Am J Dermatopathol.* 1996;18:560–566.
20. Juhász MLW, Marmur ES. Reviewing challenges in the diagnosis and treatment of lentigo maligna and lentigo-maligna melanoma. *Rare Cancers Ther.* 2015;3:133–145.
21. Tannous ZS, Lerner LH, Duncan LM, et al. Progression to invasive melanoma from malignant melanoma in situ, lentigo maligna type. *Hum Pathol.* 2000;31:705–708.
22. Star P, Rawson RV, Drummond M, et al. Lentigo maligna: defining margins and predictors of recurrence utilizing clinical, dermoscopic, confocal microscopy and histopathology features. *J Eur Acad Dermatol Venereol.* 2021;35:1811–1820.
23. Black WH, Thareja SK, Blake BP, et al. Distinction of melanoma in situ from solar lentigo on sun-damaged skin using morphometrics and MITF immunohistochemistry. *Am J Dermatopathol.* 2011;33:573–578.
24. Barlow JO, Maize J, Lang PG. The density and distribution of melanocytes adjacent to melanoma and nonmelanoma skin cancers. *Dermatol Surg.* 2007;33:199–207.
25. Bowen AR, Thacker BNP, Goldgar DE, et al. Immunohistochemical staining with Melan-A of uninvolved sun-damaged skin shows features characteristic of lentigo maligna. *Dermatol Surg.* 2011;37:657–663.
26. Cherpelis BS, Moore R, Ladd S, et al. Comparison of MART-1 frozen sections to permanent sections using a rapid 19-minute protocol. *Dermatol Surg.* 2009;35:207–213.
27. Christensen KN, Hochwalt PC, Hocker TL, et al. Comparison of MITF and melan-A immunohistochemistry during Mohs surgery for lentigo maligna-type melanoma in situ and lentigo maligna melanoma. *Dermatol Surg.* 2016;42:167–175.
28. Coakley A, Orlowski TJ, Muhlbauer A, et al. A comparison of imaging software and conventional cell counting in determining melanocyte density in photodamaged control sample and melanoma in situ biopsies. *J Cutan Pathol.* 2020;47:675–680.
29. Flores S, Luby NJ, Bowen GM. Comparison of melanocyte density counts in topical imiquimod-treated skin surrounding lentigo maligna vs control biopsy specimens. *Jama Dermatol.* 2018;154:482–484.
30. Gautschi M, Oberholzer PA, Baumgartner M, et al. Prognostic markers in lentigo maligna patients treated with imiquimod cream: a long-term follow-up study. *J Am Acad Dermatol.* 2016;74:81–87.e1.
31. Glass LF, Raziano RM, Clark GS, et al. Rapid frozen section immunostaining of melanocytes by microphthalmia-associated transcription factor. *Am J Dermatopathol.* 2010;32:319–325.
32. Gómez-Martín I, Moreno S, Andrades-López E, et al. Histopathologic and immunohistochemical correlates of confocal descriptors in pigmented facial macules on photodamaged skin. *Jama Dermatol.* 2017;153:771–780.
33. Helm K, Findeis-Hosey J. Immunohistochemistry of pigmented actinic keratoses, actinic keratoses, melanomas in situ and solar lentigines with Melan-A. *J Cutan Pathol*. 2008;35:931–934.

34. Hendi A, Brodland DG, Zitelli JA. Melanocytes in long-standing sun-exposed skin: quantitative analysis using the MART-1 immunostain. *Arch Dermatol.* 2006;142:871–876.
35. Hendi A, Wada DA, Jacobs MA, et al. Melanocytes in nonlesional sun-exposed skin: a multicenter comparative study. *J Am Acad Dermatol.* 2011;65:1186–1193.
36. Hillesheim PB, Slone S, Kelley D, et al. An immunohistochemical comparison between MiTF and MART-1 with Azure blue counterstaining in the setting of solar lentigo and melanoma in situ. *J Cutan Pathol.* 2011; 38:565–569.
37. Kim J, Taube JM, McCalmont TH, et al. Quantitative comparison of MiTF, Melan-A, HMB-45 and Mel-5 in solar lentigines and melanoma in situ. *J Cutan Pathol.* 2011;38:775–779.
38. Mu EW, Quatrano NA, Yagerman SE, et al. Evaluation of MITF, SOX10, MART-1, and R21 immunostaining for the diagnosis of residual melanoma in situ on chronically sun-damaged skin. *Dermatol Surg.* 2018;44:933–938.
39. Siarov J, Neittaanmäki N, Mölne J, et al. Digital quantification of melanocytic density in resection margins of lentigo maligna using SOX10 versus hematoxylin-eosin staining. *Am J Dermatopathol.* 2021;43:273–277.
40. Speiser J, Tao J, Champlain A, et al. Is melanocyte density our last hope? Comparison of histologic features of photodamaged skin and melanoma in situ after staged surgical excision with concurrent scouting biopsies. *J Cutan Pathol.* 2019;46:555–562.
41. Gorman M, Khan MA, Johnson PC, et al. A model for lentigo maligna recurrence using melanocyte count as a predictive marker based upon logistic regression analysis of a blinded retrospective review. *J Plast Reconstr Aesthet Surg.* 2014;67:1322–1332.
42. Gradecki SE, Valdes-Rodriguez R, Wick MR, et al. PRAME immunohistochemistry as an adjunct for diagnosis and histological margin assessment in lentigo maligna. *Histopathology*. 2021;78:1000–1008.
43. Lezcano C, Jungbluth AA, Nehal KS, et al. PRAME expression in melanocytic tumors. *Am J Surg Pathol.* 2018;42:1456–1465.
44. de Wet J, du Plessis PJ, Schneider JW. Staged excision of lentigo maligna of the head and neck: assessing surgical excision margins with melan A, SOX10, and PRAME immunohistochemistry. *Am J Dermatopathol.* 2023;45:107–112.