Prognostic markers in lentigo maligna patients treated with imiquimod cream: A long-term follow-up study

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Abbreviations used:

LM: lentigo maligna

LMM: lentigo maligna melanoma

CD: cluster of differentiation

od: once daily

CI: confidential interval

y: year(s)

Abstract.

Background: More data are needed to define factors that predict long-term success after imiquimod therapy for lentigo maligna (LM).

Objective: To determine the demographic, clinical, and histological prognostic markers of relapse-free survival in LM patients treated with imiquimod.

Methods: Single-arm, open-label, non-randomized, prospective study.

Results: Eighty-nine patients with histologically confirmed LM and a median follow-up time of 4.8 years after imiquimod treatment were included in our study. Sixteen patients (18%) relapsed. Statistically significant indicators of an increased risk of local recurrence included: the total number of melanocytes, the number of basal and suprabasal melanocytes and the number of pagetoid spreading melanocytes.

Limitations: Our study was a single-center, non-randomized study.

Conclusion: An assessment of different melanocyte fractions in the diagnostic baseline biopsy may help to predict the response of lentigo maligna to imiquimod therapy.

Key words: (6-10): prognostic marker; efficacy; imiquimod; topical immunomodulators; lentigo maligna; recurrence; follow-up

Capsule summary:

Different therapeutic modalities (surgery, radiotherapy, imiquimod) have been used in the treatment of lentigo maligna.

Imiquimod is associated with a recurrence rate of about 18%, with a higher recurrence rate being associated with an increased number of melanocytes.

Imiquimod may be an acceptable nonsurgical option after assessing melanocyte fractions in the diagnostic baseline biopsy.

Introduction

Lentigo maligna (LM), a melanoma in situ on chronically sun-damaged skin, mainly affects elderly patients and has a strong predilection for the head and neck region (1, 2). Several therapeutic modalities, such as surgery, cryotherapy (3, 4) and radiotherapy (5-7), are used. Surgical excision with safety margins (usually 5 millimeters) is the treatment of choice.

Imiquimod is a topical immune response modifier that has been recently suggested to be a valuable alternative treatment to surgery in LM, especially in cases with larger lesions located on the face in elderly and comorbid patients (1, 8-16), when surgery is not indicated or cannot be performed. It has also been proposed as a neo- or post-adjuvant treatment option to operative procedures (9, 12-14). Recurrence of LM or LMM after treatment with imiquimod has also been observed in some patients (17).

The aim of our study was to evaluate the response of LM to nonsurgical treatment with 5% imiquimod cream in a cohort of patients with long-term follow-up. Furthermore, the role of demographic, clinical and histological features for the long-term efficacy of imiquimod treatment in LM patients was assessed.

Methods.

Patients, treatments and assessments: Between 2003 and 2013, 89 patients (55 women and 34 men) with histologically confirmed LM were enrolled in the study. The patients were instructed to apply 5% imiquimod cream (Aldara®, 3M Pharma, Rueschlikon, Switzerland), with or without occlusion, to cover the macroscopically pigmented area, as described elsewhere (16), once or twice daily, until a weeping erosion developed. All patients underwent regular clinical follow-ups at the Department of Dermatology of the University Hospital of Bern, Switzerland.
The following patient data were analyzed: age at the beginning of the therapy, sex and Fitzpatrick skin phototype, side effects and clinical response during therapy, size and location of the lesion, occurrence of relapse, time from the beginning of the therapy to relapse, date of the last clinical follow-up, death and other skin malignancies.

Histological evaluation: Punch biopsies and excisional biopsies were fixed in formalin and embedded in paraffin using routine techniques. The diagnoses of LM was made by at least two board certified dermatopathologists by analyzing hematoxylin-eosin stained slides, as well as immunohistochemical stainings for melan-A antigen. The total number of melanocytes, the number of basal and suprabasal melanocytes, and the number of pagetoid spreading melanocytes (i.e., melanocytes above the basal and suprabasal layers of the epidermis) were assessed on melan-A stained tissue sections using a conventional light microscopy with an integrated millimetric scale. All of the parameters are indicated as cells/positive events per mm epidermis. As the epidermis in these mainly elderly individuals with sun damaged skin was always very thin, counting cells linearly along the length of the epidermis and indicating cells/events per mm epidermis was more reliable than counting cells/events per square mm. Furthermore, the maximal epidermal depth of the melanocytes, i.e., usually the maximal depth of the melanocytes along the hair follicle, was estimated. Similar to tumor thickness (according to Breslow), the distance between the stratum granulosum and the deepest melanocyte was measured. The maximal extent of non-invasive melanocytes was called epidermal tumor thickness.

Study design: This investigator-initiated, open-label, non-randomized, prospective study was approved by the local Research Ethics Committee of the University of Bern. Informed consent was obtained from all of the patients. All clinical investigations were conducted according to the principles of the Declaration of Helsinki.

Statistical analysis: Descriptive statistics were computed for the patients' demographic and clinical characteristics. Time to relapse was calculated from the beginning of therapy to relapse. If the patients were lost to follow-up or died before relapse, they were censored at

the time of the last visit. All p-values related to the two-sided test with an alpha level of 0.05 and R 2.13.0 were used for computations (www.r-project.org). The confidence interval (CI) of the hazard ratios for the Cox regression and survival function (for time-to-event variables) were calculated based on the cumulative hazard and point wise log (survival). Otherwise, the methods used to conduct tests (p-values) and CIs are specified separately. The two-sided exact Wilcoxon signed rank test was also used, whereas patients who were censored (lost to follow-up or death) were not included in this analysis.

Results:

Patient and tumor characteristics are shown in **Table 1**. Eighty-nine patients (55 females and 34 males) with a median age of 72.5 y (range 38.6-93.8 y) were included in the study. Only 5 of 89 LM were located outside the face (either on the arm, neck or on the shoulders). No LM was found on the trunk or lower extremities. The treatment-induced local inflammatory reaction was generally well tolerated, but one patient had to discontinue treatment after 63 days due to a generalized pruritic macular papular rash. Other side effects, including hypopigmentation, persistent erythema and telangiectasia at the treatment site, as well as malaise, hair-loss, sleeping problems, herpes labialis and swollen eyes in the morning, were observed in 16 patients (18%). Moreover, one patient developed persistent vitiligo-like white patches outside the treated area (on the dorsal surfaces of his hands).

Figure 1 shows the recurrence-free survival over time. During the follow-up period, 16 relapses were registered (18%). The median time to relapse among relapsed patients was 1.89 years (mean: 2.34 years). The majority of relapsed patients (n=10) underwent later surgical treatment. In four cases, imiquimod cream was re-used. Two patients underwent radiotherapy.

The relative risk of relapse (shown in **Table 2** and calculated with an univariable Cox regression for time to relapse) increased 1.03 times with each additional number of total melanocytes per millimeter (i.e., the more total melanocytes per millimeter the higher the risk of relapse), and the risk was statistically significant (p-value 0.0001). Similarly, each additional number of basal and suprabasal melanocytes per mm epidermis increased the relapse risk of 1.06 times (p-value<0.0001), and each additional number of pagetoid spreading melanocytes per mm epidermis is accountable for an increase in relapse of 1.05 times (p-value 0.0122).

Other variables, including the presence vs. absence of follicle involvement (HR 0.52, p-value 0.396), maximal diameter of the lesion (mm; HR 0.98, p-value 0.369), absence vs. presence of a clinically visible inflammatory reaction at the treatment site (HR 0.66, p-value 0.521), and treatment duration (months; HR 0.91, p-value 0.656), were not statistically significant in terms of LM relapse.

Figure 2 shows clinical pictures, dermatoscopic images, and histological images of hematoxylin and eosin and melan A stains before and after therapy with imiquimod in a non-relapsed and a relapsed patient.

To determine the accuracy of the bioptical assessment of LM lesions, a preliminary study was performed in ten consecutive cases. All four parameters (the number of total melanocytes, number of basal and suprabasal melanocytes, number of pagetoid spreading

melanocytes per mm epidermis and tumor thickness) were simultaneously evaluated with a punch biopsy and in completely excised LM lesions. All four parameters were higher or equal in the punch biopsies compared to the excisional specimens, indicating that the biopsies were representative and that no underestimation of the extent of the disease was made (supplementary Figure).

The total number of melanocytes, basal and suprabasal melanocytes, number of pagetoid spreading melanocytes per mm of epidermis and tumor thickness obtained from baseline biopsies were further compared between relapsed and non-relapsed patients **(Figure 3).** Interestingly, all four parameters were increased in the baseline biopsies of the relapsed patients, indicating an increased risk of recurrence. Statistical significance was observed only for the total melanocytes.

Discussion.

Lentigo maligna is the most common melanoma of the sun-damaged skin of the elderly (in our study, the median age was 72.5 y). As also confirmed by our results, it has a certain predilection for the facial region (in our study population, 84 out of 89 LM, that is 94.4%) (1, 18). The rate of progression of LM to invasive melanoma is low (<5% overall), but the exact conditions necessary for these transformations have yet to be elucidated (19, 20). Jeffrey et al. postulate that the recurrence rate following nonsurgical therapies ranges between 20% and 100%. The recurrence rate after radiotherapy ranges from 1% to 19% and is often followed by radiodermatitis, tissue destruction, and scarring and may promote the development of squamous-cell carcinomas; therefore, it is not the therapy of choice. Similarly, cryotherapy with a recurrence rate between 0 and 40% (21) is not the best therapeutic option. Surgical excision remains the treatment of choice for LM, with a recurrence rate of 3% after Mohs surgery. Nevertheless, complete surgical treatment is sometimes difficult to perform, either because of patients' comorbidities or due to the risk of cosmetic disfigurement. Its limits comprise the location and size of the lesion (9, 22-23). A good therapeutic alternative in these cases is topical imiquimod, especially when aesthetic issues are involved (9). It has been demonstrated to be a very efficient therapy with a relatively low recurrence rate (3, 15, 24, 25). According to Naylor et al, imiquimod shows no relapse in over 80% of cases (26). Imiquimod is a topical immune response modifier that acts through binding toll-like receptors 7 and 8 on dendritic cells, macrophages and neutrophils (27). In LM, imiquimod recruits CD68+ macrophages and cells involved in cytotoxic T-cell

responses, including CD 8+ T cells (28). It regulates genes involved in different aspects of the immune response, apoptosis and oncogenesis (29).

The principle aim of our study was to assess the risk factors of LM relapse after treatment with imiquimod based on an evaluation of the demographic, clinical and histological features of patients and LM lesions. As demonstrated, in relapsed patients (18% of the study population) the baseline number of the total melanocytes, as well as the basal and suprabasal, and pagetoid spreading melanocytes, per mm epidermis compared to those of non-relapsed patients, appeared to be significantly increased and might be an indicator of the risk of recurrence. To our knowledge, this is the first time that the previously mentioned typing and assessment of melanocytes in LM biopsies has been used. This easy-to-perform, inexpensive and statistically relevant method has only been validated for LM patients treated with imiquimod.

To date, visible signs of inflammation at the site of treatment and apparent lesion clearance have been considered to be predictors of a good response to/efficacy of the imiquimod treatment (12, 13). Our results confirm that the visible signs of inflammation are often correlated with successful outcomes; however, this relation was not statistically significant (95% CI, 0.18-2.37, p-value: 0.521). Also, other well established diagnostic modalities, including dermoscopy (30) and in vivo reflectance confocal microscopy (31), have appeared to be helpful in relapse estimation, but they appear not to be statistically significant. The literature suggests other predictive (histological) markers, such as CAS protein (32) or CD133 (33), that could be useful as prognostic factors, but their estimation is surely more expensive than typing of melanocytes.

Moreover, our study demonstrated (histologically and clinically) an almost a 5-year relapse-free survival in 82% of patients with LM treated with imiquimod only. The large number of study participants (n=89) strengthens the validity and significance of this outcome. Our results are compatible with the findings of Fleming et al. and Wong et al. who, respectively, identified 67% (4 out of 6 patients) and 22% (6 out of 27 patients) of positive responses after treatment with imiquimod only (10, 33). The outcomes from studies where imiquimod was used in addition to surgical treatment (9, 12, 13), ablative laser (14) or other topical treatments (e.g., tazarotene 0.1%) (15) demonstrated variable effects. To date, complete surgery remains the best therapeutic option for LM patients (34- 36).

In conclusion, in cases where surgery is not an option (due to an esthetically challenging localization or the patient’s comorbidities), imiquimod provides favorable outcomes with a low risk of relapse, is less painful and has a lower mortality rate due to the treatment. Typing and the assessment of melanocytes in a skin biopsy is a cheap and simple method that can help

predict the risk of relapse already before treatment and further contribute to the best therapeutic decision individually adjusted to each patient.

Table and figure legends:

Table 1: Lentigo maligna. Patients' and tumor characteristics.

Table 2: Lentigo maligna. The univariable cox regression for time to relapse.

Figure 1: Lentigo maligna. The Kaplan-Meier (KM) curve showing recurrence-free survival probability (± 95% confidence interval, CI).

Figure 2 : Lentigo maligna. Representative examples of two patients with imiquimod therapy. The clinical image (A), dermoscopic image (B), and histological image of hematoxylin and eosin (C) and melan A stains (D) before (1 and 3) and after (2 and 4) the therapy with imiquimod in a non-relapsed (1 and 2, upper panel) and a relapsed (3 and 4, lower panel) patient. Increased numbers of melanocytes (arrowheads) are observed preferentially in the basal and suprabasal layer in the histological images of the hematoxylin and eosin (panel 1C, 3C, and 4C) and melan A stains (panel 1D, 3D, and 4D). Pagetoid spreading melanocytes (arrows) are visible preferentially in the relapsed lesions (panel 3C, 3D, 4C, and 4D).

Figure 3: Lentigo maligna. Histological parameters without relapse (open bars) and with relapse (closed bars). The following histological parameters: A) total melanocytes per mm epidermis (p=0.0001), B) epidermal tumor thickness (p=0.4749), C) basal- and suprabasal melanocytes (p< 0.0001) and D) pagetoid spreading melanocytes (p=0.0122) were assessed for each LM. Error bars indicate the standard deviation.

Supplementary Figure: Lentigo maligna. Validation of the different histological parameters. The following histological parameters: A) total melanocytes per mm of epidermis (p=0.027), B) epidermal tumor thickness (p=0.073), C) basal- and suprabasal melanocytes per mm epidermis (p=0.02), and D) pagetoid spreading melanocytes per mm epidermis (p=0.03) were assessed in punch biopsies (open bars) and total surgical excision specimens (closed bars) from 10 consecutive patients. Error bars indicate the standard deviation.

References.

1. Ciocan D, Barbe C, Aubin F, Granel-Brocard F, Lipsker D, Velten M, et al. Distinctive features of melanoma and its management in elderly patients: a population-based study in France. JAMA dermatology. 2013;149(10):1150-7.

2. Tournillac I, Picot MC, Dereure O, Guilhou JJ, Guillot B. [Dubreuilh melanoma: and epidemiologic and prognostic study]. Annales de dermatologie et de venereologie. 1999;126(10):676-80.

3. Bichakjian CK, Halpern AC, Johnson TM, Foote Hood A, Grichnik JM, Swetter SM, et al. Guidelines of care for the management of primary cutaneous melanoma. American Academy of Dermatology. Journal of the American Academy of Dermatology. 2011;65(5):1032-47.

4. Kallini JR, Jain SK, Khachemoune A. Lentigo maligna: review of salient characteristics and management. American journal of clinical dermatology. 2013;14(6):473-80.

5. Fogarty GB, Hong A, Scolyer RA, Lin E, Haydu L, Guitera P, et al. Radiotherapy for lentigo maligna: a literature review and recommendations for treatment. The British journal of dermatology. 2014;170(1):52-8.

6. Hedblad MA, Mallbris L. Grenz ray treatment of lentigo maligna and early lentigo maligna melanoma. Journal of the American Academy of Dermatology. 2012;67(1):60-8.

7. Farshad A, Burg G, Panizzon R, Dummer R. A retrospective study of 150 patients with lentigo maligna and lentigo maligna melanoma and the efficacy of radiotherapy using Grenz or soft X-rays. The British journal of dermatology. 2002;146(6):1042-6.

8. Mora AN, Karia PS, Nguyen BM. A quantitative systematic review of the efficacy of imiquimod monotherapy for lentigo maligna and an analysis of factors that affect tumor clearance. J Am Acad Dermatol. 2015 Aug; 73 (2):205-12.

9. Ellis LZ, Cohen JL, High W, Stewart L. Melanoma in situ treated successfully using imiquimod after nonclearance with surgery: review of the literature. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2012;38(6):937-46.

10. Fleming CJ, Bryden AM, Evans A, Dawe RS, Ibbotson SH. A pilot study of treatment of lentigo maligna with 5% imiquimod cream. The British journal of dermatology. 2004;151(2):485-8.

11. Ahmed I, Berth-Jones J. Imiquimod: a novel treatment for lentigo maligna. The British journal of dermatology. 2000;143(4):843-5.

12. Ly L, Kelly JW, O'Keefe R, Sutton T, Dowling JP, Swain S, et al. Efficacy of imiquimod cream, 5%, for lentigo maligna after complete excision: a study of 43 patients. Archives of dermatology. 2011;147(10):1191-5.

13. Cotter MA, McKenna JK, Bowen GM. Treatment of lentigo maligna with imiquimod before staged excision. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2008;34(2):147-51.

14. de Vries K, Rellum R, Habets JM, Prens EP. A novel two-stage treatment of lentigo maligna using ablative laser therapy followed by imiquimod. The British journal of dermatology. 2013;168(6):1362-4.

15. Hyde MA, Hadley ML, Tristani-Firouzi P, Goldgar D, Bowen GM. A randomized trial of the off-label use of imiquimod, 5%, cream with vs without tazarotene, 0.1%, gel for the treatment of lentigo maligna, followed by conservative staged excisions. Archives of dermatology. 2012;148(5):592-6.

16. Buettiker UV, Yawalkar NY, Braathen LR, Hunger RE. Imiquimod treatment of lentigo maligna: an open-label study of 34 primary lesions in 32 patients. Arch Dermatol. 2008;144(7):943-5.

17. Woodmansee CS, McCall MW. Recurrence of lentigo maligna and development of invasive melanoma after treatment of lentigo maligna with imiquimod. Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al]. 2009;35(8):1286-9.

18. Zalaudek I, Horn M, Richtig E, Hödl S, Kerl H, Smolle J. Local recurrence in melanoma in situ: influence of sex, age, site of involvement and therapeutic modalities. Br J Dermatol. 2003;148(4):703-8.

19. Weinstock MA, Sober AJ. The risk of progression of lentigo maligna to lentigo maligna melanoma. The British journal of dermatology. 1987;116(3):303-10.

20. Tannous ZS, Lerner LH, Duncan LM, Mihm MC, Jr., Flotte TJ. Progression to invasive melanoma from malignant melanoma in situ, lentigo maligna type. Human pathology. 2000;31(6):705-8.

21. McKenna JK, Florell SR, Goldman GD, Bowen GM. Lentigo maligna/lentigo maligna melanoma: current state of diagnosis and treatment. Dermatol Surg. 2006;32(4):493-504.

22. Epstein E. Extensive lentigo maligna clearing with topical imiquimod. Arch Dermatol. 2003;139(7):944-5.

23. Powell AM, Russell-Jones R. Amelanotic lentigo maligna managed with topical imiquimod as immunotherapy. J Am Acad Dermatol. 2004;50(5):792-6.

24. Stuiber T, Paul E. [Lentigo maligna and lentigo maligna melanoma of the cheeks. Analysis of different growth patterns via photographic records]. Der Hautarzt; Zeitschrift fur Dermatologie, Venerologie, und verwandte Gebiete. 2001;52(10):873-7.

25. Möhrle M, Schippert W, Garbe C, Rassner G, Röcken M, Breuninger H. [Prognostic parameters and surgical strategies for facial melanomas]. J Dtsch Dermatol Ges. 2003;1(6):457-63.

26. Naylor MF, Crowson N, Kuwahara R, Teague K, Garcia C, Mackinnis C, et al. Treatment of lentigo maligna with topical imiquimod. Br J Dermatol. 2003;149 Suppl 66:66-70.

27. Sauder DN. Immunomodulatory and pharmacologic properties of imiquimod. Journal of the American Academy of Dermatology. 2000;43(1 Pt 2):S6-11.

28. Michalopoulos P, Yawalkar N, Bronnimann M, Kappeler A, Braathen LR. Characterization of the cellular infiltrate during successful topical treatment of lentigo maligna with imiquimod. The British journal of dermatology. 2004;151(4):903-6.

29. Urosevic M, Oberholzer PA, Maier T, Hafner J, Laine E, Slade H, et al. Imiquimod treatment induces expression of opioid growth factor receptor: a novel tumor antigen induced by interferon-alpha? Clinical cancer research : an official journal of the American Association for Cancer Research. 2004;10(15):4959-70.

30. Micantonio T, Fargnoli MC, Peris K. Usefulness of dermoscopy to monitor clinical efficacy of imiquimod treatment for lentigo maligna. Archives of dermatology. 2006;142(4):530-1.

31. Guitera P, Moloney FJ, Menzies SW, Stretch JR, Quinn MJ, Hong A, et al. Improving management and patient care in lentigo maligna by mapping with in vivo confocal microscopy. JAMA dermatology. 2013;149(6):692-8.

32. Boni R, Wellmann A, Man YG, Hofbauer G, Brinkmann U. Expression of the proliferation and apoptosis-associated CAS protein in benign and malignant cutaneous melanocytic lesions. The American Journal of dermatopathology. 1999;21(2):125-8.

33. Wong JG, Toole JW, Demers AA, Musto G, Wiseman MC. Topical 5% imiquimod in the treatment of lentigo maligna. Journal of cutaneous medicine and surgery. 2012;16(4):245-9.

34. Gambichler T, Kempka J, Kampilafkos P, Bechara FG, Altmeyer P, Stücker M. Clinicopathological characteristics of 270 lentigo maligna and lentigo maligna melanoma patients: data of a German Skin Cancer Center. Br J Dermatol. 2014.

35. Osborne JE, Hutchinson PE. A follow-up study to investigate the efficacy of initial treatment of lentigo maligna with surgical excision. Br J Plast Surg. 2002;55(8):611-5.

36. Pflugfelder A, Kochs C, Blum A, Capellaro M, Czeschik C, Dettenborn T, et al. Malignant melanoma S3-guideline "diagnosis, therapy and follow-up of melanoma". J Dtsch Dermatol Ges. 2013;11 Suppl 6:1-116, 1-26.