ORIGINAL ARTICLE

Lentigo maligna – anatomic location as a potential risk factor for recurrences after non-surgical treatment

K. Greveling,[1,*,†] Th. van der Klok,[2,†] M.B.A. van Doorn,[1] V. Noordhoek Hegt,[2] E.P. Prens[1]

[1]Department of Dermatology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
[2]Department of Pathology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
*Correspondence: K. Greveling. E-mail: k.greveling@erasmusmc.nl

Abstract

Background A higher incidence of lentigo maligna (LM) recurrences on the nose was previously observed in our cohort after non-surgical treatment.

Objectives To determine histological parameters that might be related to the previously observed higher incidence of LM recurrences on the nose after non-surgical treatment.

Methods We randomly selected 22 surgical specimens of LM on the nose and 22 on the cheek. Histopathological analysis was performed on haematoxylin and eosin stained and microphthalmia transcription factor immunohistochemically stained slides. The number of pilosebaceous units (PSU) per mm, maximum depth of atypical melanocytes along the skin appendages and maximum depth of the PSU itself were determined.

Results The nose had a significantly higher density of PSU than the cheek. The atypical melanocytes extended deeper along the PSU on the nose with a mean (SD) depth of 1.29 mm (0.48) vs. a mean depth of 0.72 mm (0.30) on the cheek ($P < 0.001$). The maximum depth of the PSU on the nose was greater than on the cheek, mean (SD) depth of 2.28 mm (0.41) vs. 1.65 mm (0.82) ($P = 0.003$).

Conclusions The higher recurrence risk of LM on the nose after non-surgical treatment that we previously observed in our cohort is most likely based on a higher density of atypical melanocytes and also their deeper extension into the follicles. These results shed more light on our previous findings and learn that anatomical location is relevant for the risk of recurrence of LM after non-surgical treatment.

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Conflicts of interest

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Introduction

Lentigo Maligna (LM) is a subtype of melanoma *in situ*, and although surgical excision is the treatment of first choice, non-surgical treatment options are gaining more interest.[1–3] Deciding on the best individual treatment can be guided by the age of the patient, comorbidities, size and location of the lesion.

In a previous publication, we introduced a non-surgical combination treatment of ablative laser therapy followed by topical application of imiquimod 5% cream.[4] Recently, we found six local recurrences in our cohort of 35 patients.[5] Five of the six recurrences occurred on the nose (out of a total of 15 treated LM on the nose).

We argue that the following factors may contribute to a higher risk of LM recurrence on the nose after non-surgical treatment than on other anatomic locations. First, the downward extension of atypical melanocytes along the pilosebaceous units (PSU) may be an important factor. Non-surgical treatments mainly target the epidermis, while atypical melanocytes residing along the PSU may not be completely cleared. It is known that the atypical melanocytes of melanomas in the head and neck region tend to extend much deeper along the hair follicles than in other regions.[6] However, there are currently no data available for LM comparing the depth of atypical melanocytes along the PSU for different facial regions, or the nose in particular. Second, the density of the PSU may be highly relevant. It is known that the PSU density varies for different anatomic locations, with the nose having the highest follicle density of the

†These authors contributed equally to this work.

body.[7,8] We hypothesize that the recurrence risk of LM after non-surgical treatment may be higher for the nose than for other facial regions, because of a higher PSU density that may harbour a considerably greater load of atypical melanocytes in deeper parts of the appendages. Third, the maximum depth of the PSU itself may be of importance since this allows the atypical melanocytes to penetrate even deeper.

The aims of our study were to determine whether the PSU density, the maximum downward extension of atypical melanocytes along the PSU, and the maximum depth of the PSU itself differed between LM on the nose and LM on the cheek.

Materials and methods

Patient selection

Data on all patients who had undergone staged surgical excision for primary LM on the nose or cheek (most common location of LM) at the Erasmus Medical Center between January 2001 and December 2015 were obtained using Sympathy 2.8, a program of our pathology department that is linked to PALGA (Dutch Pathology Registry).

Inclusion and exclusion criteria

Exclusion criteria were: unavailability of the slides and corresponding formalin-fixed paraffin embedded (FFPE) tissue blocks, and presence of invasive melanoma. We decided to only use excision specimens and exclude (small) diagnostic biopsies in order to obtain adequate material for a thorough analysis and an accurate measurement of our study parameters.

SPSS Statistics 21 was used to perform random number selection and the patient numbers were put in a random order. Two lists were created, one for the nose and one for the cheek.

The corresponding surgical specimens were collected and eligibility for inclusion was determined starting at the top of each list, until 22 samples of each group were included (based on the sample size calculation below). Eligibility was based on histopathological review of haematoxylin and eosin (HE) stained slides of the surgical specimens, acquired from the pathology archive of the Erasmus MC. Specimens were not eligible if slides were cut tangential, or if there was a high presence of scar tissue (causing loss of PSU). A central slide was selected from each surgical specimen. For some slides, there was no tissue left in the corresponding FFPE block, or there was no LM left after cutting the initial HE slides. Those samples had to be excluded as well, because they could not be used for immunohistochemical staining.

Histopathological analysis

Blank slides were cut from the selected corresponding FFPE blocks at 4 μm, deparaffinized and immunohistochemically stained for microphthalmia transcription factor [MITF; mouse monoclonal, Ventana, clone C5/D5, 790-4367 (ready to use)], using the Ventana Benchmark Ultra stainer (Ventana Medical Systems, Tucson, AZ, USA). The staining procedure included pre-treatment with CC1 (Cell Conditioner 1, pH8,4) at 95 °C for 64 min, followed by primary antibody incubation at 36 °C for 16 min. Staining was visualized using the Ventana Ultraview Universal Alkaline Phosphatase Red Detection Kit (760-501). The Ventana Amplification Kit (760-080) was used, counterstaining was performed with haematoxylin. Normal skin was used as a positive control. MITF is a nuclear stain specific for melanocytes, and the most useful immunohistochemical staining to detect single epidermal melanocytes.[9] With MITF, the nuclear polymorphism of melanocytes, which is characteristic for LM, can be demonstrated as well, and these atypical melanocytes can be distinguished from the small pre-existent melanocytes found in the hair follicle, especially those in the hair bulb.

Study outcomes

The following parameters were scored: number of PSU per epidermal mm, maximum depth of downward extension of atypical melanocytes along the skin appendages and maximum depth of the PSU itself.

As the discrimination between vellus, indeterminate and terminal hairs is sometimes difficult because of the one level view of the slides, and as LM extends down into both vellus and deeper hair follicles, this distinction was not made. The total number of PSU, based on the presence of at least a part of the pilosebaceous unit (i.e. infundibulum, isthmus, bulb or sebaceous glands assumed to belong to one PSU) was scored. The number of PSU was then calculated per linear mm epidermis in one horizontal dimension. We also measured the deepest point of any of the PSU, and the maximum depth of atypical melanocytes along the skin appendages from the granular layer. If the extension was deeper in an appendage other than a PSU (i.e. sweat gland in one case), then this was counted as the maximum depth.

Statistical analysis

A sample size of 22 patients per group was estimated to provide 80% power to detect a difference in density of approximately 600 follicles (per cm^2),[8] and an estimated difference of ±0.6 mm (SD 0.7 mm) in depth of atypical melanocytes along the PSU and depth of the PSU itself, with a two-sided type I error level of 5%. A *t*-test for unpaired samples was used. In order to provide enough statistical power for all endpoints, the largest group size was chosen.

Normality of the variables was tested using a Shapiro–Wilk test. Data that were normally distributed were summarized with means and standard deviations (SD), and data that were not normally distributed, with medians and interquartile ranges. Differences between the nose and cheek group for normally distributed variables were analysed using a *t*-test for independent

samples, and for not normally distributed variables using a Mann–Whitney *U*-test.

To calculate the relative extension of atypical melanocytes down the PSU, the extension of atypical melanocytes along the PSU was divided by the depth of the PSU itself. *P*-values less than or equal to 0.05 were considered statistically significant. Additional false discovery rate control did not require significance level adjustment.[10] Statistical analysis of data was performed using SPSS Statistics 21.

Results

Between January 2001 and December 2015, 122 patients were diagnosed with a LM on the nose and 315 with LM on the cheek. Of the 122 LM on the nose, 54 slides or blocks were unavailable, 13 had an invasive component, and 16 were biopsies. Of the 315 LM on the cheek, 103 slides or blocks were unavailable, 23 had an invasive component and 58 were biopsies. After exclusion of these cases, eligibility was assessed until 44 LM cases were randomly selected; 22 located on the nose and 22 located on the cheek.

The mean (SD) age in the nose group was 76 years (14) and in the cheek group, 73 years (13). Gender distribution in the nose group was 14 female, 8 male; and in the cheek group, 15 female and 7 male.

Number of PSU per mm (density)

The median number of PSU was significantly higher on the nose than on the cheek (Table 1).

Maximum depth of atypical melanocytes along the PSU

The mean maximum depth of atypical melanocytes along the PSU was greater on the nose than on the cheek (Table 1), with a mean nose vs. cheek difference of 0.57 mm (95% CI, 0.33–0.82).

Maximum depth of PSU

The mean maximum depth of the PSU itself was greater on the nose than on the cheek (Table 1), with a mean nose vs. cheek difference of 0.63 mm (95% CI, 0.23–1.03).The atypical melanocytes extended, on average, 57% down the PSU on the nose and 49% down the PSU on the cheek (nose vs. cheek difference, 8% [95% CI, −5 to 20]; $P = 0.237$).

Figures 1–4 show histological images of HE and MITF stains of the downward extension of atypical melanocytes along the PSU and an example of occasional extension along sweat glands.

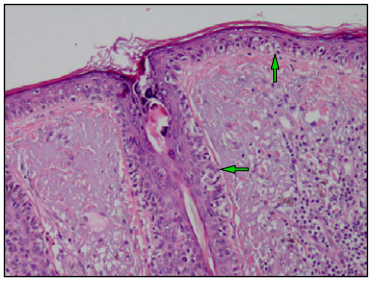

Figure 1 Lentigo maligna; continuous proliferation of atypical melanocytes along the dermo-epidermal junction. Clear extension downward, along the hair follicle is shown. In the background, severe solar elastosis. Arrows point out atypical melanocytes (haematoxylin and eosin 100×).

Discussion

Our study shows that both the density of the PSU, and the maximum downward extension of atypical melanocytes along the PSU are higher on the nose than on the cheek. This results in a considerably higher load of atypical melanocytes in deeper parts of the appendages, and may explain why LM recurrences occurred almost exclusively on the nose after our non-surgical treatment method.[5]

The maximum absolute depth of the PSU itself was found to be greater on the nose than the cheek, while we did not find a statistically significant difference in the ratio of extension of atypical melanocytes down the PSU between the nose and the cheek. This suggests that the melanocytes extended deeper on the nose than the cheek, simply because the PSU are deeper on the nose, and that there seems to be no intrinsic difference in behaviour of atypical melanocytes between these two sites.

To the best of our knowledge, anatomic location has not yet been evaluated as a potential prognostic factor for recurrences of LM after non-surgical treatments. Gautschi *et al.* assessed the maximal epidermal depth of the melanocytes (this was usually the maximal depth along the hair follicle), as well as follicle involvement (affected vs. not affected) as potential risk factors for LM recurrence after imiquimod monotherapy, but did not find statistically significant differences.[11] However, the authors did not specify the different anatomical facial regions (only 'head' or 'other locations'), which could explain the difference with the results found in this study. A factor that is considered to be a strong predictor of LM recurrence is melanocyte

Table 1 Difference between the nose and the cheek

Variables	Nose	Cheek	*P*-value
Number of PSU/mm [median (IQR)]	1.84/mm (1.53–2.63)	1.45/mm (1.07–1.64)	0.001
Maximum downward extension of atypical melanocytes along the PSU [mean (SD)]	1.29 mm (0.48)	0.72 mm (0.30)	0.001
Maximum depth of PSU itself [mean (SD)]	2.28 mm (0.41)	1.65 mm (0.82)	0.003

IQR, interquartile range; PSU, pilosebaceous units; SD, standard deviation.

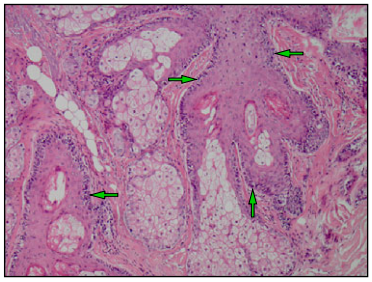

Figure 2 Very deep extension along pilosebaceous units. Arrows point out atypical melanocytes (haematoxylin and eosin 50×).

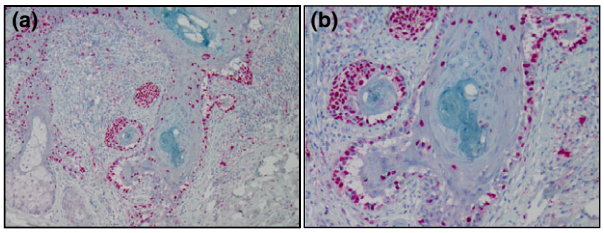

Figure 3 (a, b) Microphthalmia transcription factor (MITF) immunohistochemistry, emphasizing the number of continuous atypical melanocytes extending downward. The positive staining cells in the stroma are macrophages (a) MITF 50×, (b) MITF 100×.

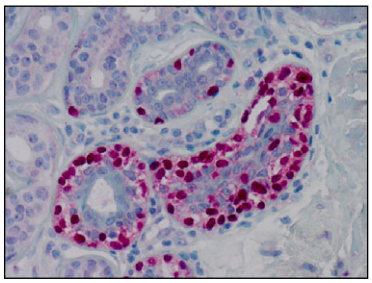

Figure 4 Microphthalmia transcription factor (MITF) immunohistochemistry. In one of the samples, the downward growth along the sweat glands was even deeper than along the pilosebaceous units (MITF 100×).

count,[11,12] which is in accordance with the higher 'load' of atypical melanocytes we found in this study, although we did not measure melanocyte count specifically. Powell *et al.* reported that the development of an inflammatory reaction to imiquimod was a strong predictor for therapeutic benefit, but did not find any histological features of prognostic significance.[13] Mora *et al.* found that cumulative dose and treatment intensity of imiquimod monotherapy was associated with tumour clearance.[14] Unfortunately, specific anatomic locations (e.g. the nose) are not mentioned in these previous publications.

A higher risk of recurrence on the nose after non-surgical treatment may influence the choice of treatment for this location. Either a surgical treatment can be chosen, which remains the treatment of first choice for LM, or in specific cases, considering the good cosmetic and functional outcomes of non-surgical treatments, the latter should be adapted for this location. In case of our combined ablative laser therapy followed by topical imiquimod cream, both the laser and imiquimod treatment protocols could be adapted.

Two types of ablative lasers are generally used, the erbium-doped yttrium aluminium garnet (Er:YAG) laser (2940 nm) and the carbon dioxide (CO_2) laser (10600 nm), both of which have been used to treat LM in our study population. The Er:YAG laser removes approximately 15–25 μm per pass, and the CO_2 laser approximately 100–150 μm per pass.[15,16] On the nose, we found a mean maximum depth of atypical melanocytes extending along the PSU of 1.29 mm. To reach this depth, approximately 10 passes of the CO_2-laser are needed, and many more using the Er:YAG laser, which in addition would be hampered by bleeding, since there is less coagulation with this type of laser.[17,18] The CO_2 laser would be more efficacious in removing a higher load of atypical melanocytes and has good coagulation enabling deeper ablation without bleeding complications. For these reasons, we propose to use the CO_2 laser as ablative laser for the treatment of LM located on the nose. However, this has to be further validated in future studies. Passes should be applied until all visible pigment has been cleared, keeping in mind that scarring should be prevented.

Mora *et al.* showed that cumulative dose (>60 total applications) and treatment intensity (>5 applications per week) of imiquimod as monotherapy affects tumour clearance.[14] The findings of Kirtschig *et al.* also suggest that a higher treatment intensity of daily applications for 12 weeks may improve the efficacy of imiquimod.[19] It should be taken into account that prolonged imiquimod treatment will significantly enhance the patients' discomfort. In our treatment protocol, we therefore reduced imiquimod applications to 6 weeks (five times per week), because the major part of the LM melanocytes were removed by laser ablation. Also, since the epidermis and reticular dermis are removed, deeper penetration of imiquimod is enabled and it is delivered closer to the target cells (the dermal dendritic cells).

Based on the findings with imiquimod monotherapy, it is conceivable that extending the duration of imiquimod treatment would achieve better tumour clearance, and this may be considered for LM on the nose, in case too many laser passes are expected to lead to a high risk of scarring.

A limitation of our study is that we could only revise vertical but not horizontal sections, because the surgical materials were treated according to the standard protocols with respect to examining LM and resection margins. Therefore, we are limited to a one-dimensional quantification of the PSU

density. Also, with our method, we were limited to two-dimensional sections, while sometimes stereology or confocal laser scanning can be used to estimate three-dimensional characteristics and allow for better understanding of the distribution.[20,21] However, since the aim of our study was to find a difference between the nose and cheek regions, and both were measured in a similar way, this would not have influenced our conclusions. Another limitation is the use of a different population to determine the histological parameters (surgical patients) than the cohort on which our hypothesis was based (laser and imiquimod patients).

In summary, we conclude that the higher recurrence risk of LM on the nose after non-surgical treatment is most likely based on the higher load of atypical melanocytes in deeper parts of the appendages. This higher load is a result of a higher PSU density on the nose, a deeper follicular extension of atypical melanocytes and a greater absolute depth of the PSU. This should be taken into account when making a choice of treatment for this specific location.

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