

Ablative fractional laser-assisted photodynamic therapy for lentigo maligna: A prospective pilot study

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Abstract

Background

Lentigo maligna (LM) is an in-situ form of melanoma carrying a risk of progression to invasive lentigo maligna melanoma (LMM). LM poses a clinical challenge, with subclinical extension and high recurrence rates after incomplete surgery. Alternative treatment methods have been investigated with varying results. Photodynamic therapy (PDT) with methylaminolaevulinate (MAL) has already proved promising in this respect.

Objectives

To investigate the efficacy of ablative fractional laser (AFL)-assisted PDT with 5-aminolaevulinic acid nanoemulsion (BF-200 ALA) for treating LM.

Methods

In this non-sponsored, prospective pilot study ten histologically verified LMs were treated with AFL-assisted PDT three times at two week intervals using a light dose of 90 J/cm² per treatment session. Local anaesthesia with ropivacain was used. Four weeks after the last PDT treatment the lesions were treated surgically with a wide excision and sent for histopathological examination. The primary outcome was complete histopathological clearance of the LM from the surgical specimen. Patient-reported pain during illumination and the severity of the skin reaction after the PDT treatments were monitored as secondary outcomes.

Results

The complete histopathological clearance rate was 7 out of 10 LMs (70%). The pain during illumination was tolerable, with the mean pain scores for the PDT sessions on a visual assessment scale ranging from 2.9 to 3.8. Some severe skin reactions occurred during the treatment period, however.

Conclusions

AFL-assisted PDT showed moderate efficacy in terms of histological clearance. It could constitute an alternative treatment for lentigo maligna but due to the side-effects it should only be considered in inoperable cases.

Introduction

Lentigo maligna (LM) is the most common subtype of melanoma in-situ.^{1,2} It occurs on the chronically sun-damaged skin of elderly patients, typically in the head and neck region.^{3,4} If left untreated, it can progress to invasive lentigo maligna melanoma (LMM) with an estimated lifetime risk of 5–50%.^{5,6} Due to the ageing population and high UV exposure, the incidence of LM and LMM is steadily increasing in Europe, the U.S.A. and Australia.^{2,7-10} LMM now encompasses 4–15% of all invasive melanomas.¹¹

The gold standard treatment for LM is wide surgical excision with 5–10 mm peripheral margins.³ Staged excision and Mohs micrographic surgery can be used to improve margin control and to strive for lower recurrence rates.¹² Due to the size and location of the LM and the age of the patient, surgery may sometimes be inappropriate or contraindicated, however.¹³ Alternative, non-surgical treatment modalities that have been investigated with varying results in terms of recurrence rates include cryotherapy, radiotherapy, Grenz ray therapy, topical imiquimod and photodynamic therapy (PDT).^{12,14,15} Karam et al. reported in a retrospective study that PDT with methylaminolaevulinate (MAL) achieved complete clearance in 12/15 cases,¹⁵ although admittedly only parts of the lesions were examined histologically for possible recurrence.

Clinically it can be difficult to distinguish between LM and LMM.¹¹ A novel imaging method employing a hyperspectral camera (HSC) can be used to delineate LM margins and detect dermal invasion.^{16,17}

The aim of this prospective pilot study was to investigate whether ablative fractional laser-assisted photodynamic therapy with 5-aminolaevulinic acid nanoemulsion (BF-200 ALA) is effective for treating lentigo maligna.

Materials and methods

Study design

The protocol for this non-sponsored, prospective pilot study complied with the Declaration of Helsinki and was approved by the local ethics committee of Tampere University Hospital. Informed written consent was obtained from all the participants.

Participants

Voluntary patients with a clinical suspicion of LM were enrolled from among those referred to the Department of Dermatology at Päijät-Häme Central Hospital, Lahti, Finland, between February 2016 and December 2017. Both male and female subjects aged over 18 years with a biopsy-proven LM located on the face, neck or upper body were included in the series. Exclusion criteria were: i) histologically verified invasive LMM, ii) porphyria or photosensitivity, iii) allergy to photosensitizer and iv) pregnancy or breastfeeding.

Treatment procedure

A flow-chart of the study is presented in *Figure 1*. During the first study visit the suspected LM was examined clinically with a dermatoscope (Dermlite® DL3, 3Gen, California, U.S.A.) and under Wood's light (Burton®), in order to help define the clinical borders.¹⁸ These borders were then traced on a transparent plastic sheet and photographed. The lesions were also imaged with a hyperspectral camera, prototype HSCP2 (Revenio Group, Vantaa, Finland), in order to reveal possible invasion and to provide a guide to the biopsy site.¹⁷

A 3 mm punch biopsy was taken from the darkest part of the lesion and/or from the most clearly emphasized area by HIS to confirm the histological diagnosis and to rule out invasion. The histopathological evaluations of the diagnostic samples were conducted by an experienced dermatopathologist (L.J.) who received the samples without any background information other than the location of the lesion. Only patients with a biopsy-proven LM without any invasive component were included in the series from this point in the protocol onwards.

The histologically confirmed LMs were treated with PDT three times at two week intervals, employing the following procedure. First, a 5 mm margin was drawn around the lesion and the area was anaesthetized with a local anaesthetic (Ropivacain Fresenius Kabi 7.5mg/ml, Fresenius Kabi AS, Halden, Norway). The area was then pre-treated with an ablative fractional CO₂ laser (DS-40UB Multixel, Daeshin Enterprise Co., Seoul, South Korea) to enhance the absorption of the

photosensitizer precursor. The laser settings were: Density level=5, Depth level=7 and Pulse duration=700ms, which correspond to a distance of 0.8 mm between the laser pores on the grid and a calculated pulse energy of 84mJ per pore. After the pre-treatment a 1mm-thick layer of photosensitizer precursor, 5-aminolevulinic acid nanoemulsion gel, BF-200 ALA, 78 mg/g (Ameluz®, Biofrontera AG, Leverkusen, Germany), was applied to the skin over the whole treatment area, including the margins, and occluded under a light impermeable cover for three hours. Finally, the treatment area was illuminated with an Aktilite® CL128 lamp (Galderma Nordic AB, Uppsala, Sweden) at a light dose of 90J/cm².

Four weeks after the last PDT treatment the lesions were excised surgically together with a 5 mm margin by the investigator M.G. and sent for routine histopathological examination. The lesion borders and margins were defined with the help of the pre-treatment plastic sheets.

The specimens were fixed in 4% formalin, embedded in paraffin, sectioned using the traditional vertical bread loaf technique and stained with haematoxylin-eosin (H&E). Immunohistochemistry (MART1/Melan A) was used as an aid to diagnosis where necessary.

Efficacy assessment

The primary outcome was complete histopathological clearance of the LM from the surgical specimen. For this purpose the excision specimens were evaluated by an experienced dermatopathologist (L.J.), who received the samples without any background information. The diagnosis was mainly based on routine staining with H&E, with additional immunohistochemical staining (MART-1/MelanA) if needed. The LM was considered to be histologically cleared if no sign or suspicion of atypical melanocytes could be seen, and uncured if the histological criteria for LM were still fulfilled or if there was any suspicion of atypical melanocyte proliferation.

Safety and tolerability assessment

The declared secondary outcomes of the study were pain during the PDT illumination, the severity of the skin reaction two days after the first PDT treatment, and the severity of delayed skin reactions after all the PDT sessions. The patients filled in visual analogue scales for pain (VAS 0–10) i) before the LED lamp illumination, ii) one minute after the start of the illumination, iii) in the middle and iv) at the end. To evaluate the local skin reactions (erythema, crusting, swelling), a nurse

photographed the treatment area two days after the first PDT treatment and the investigator J.E.R. assessed the severity of the skin reaction from photographs on a scale of 1–4 (1 negligible; 2 mild; 3 moderate; 4 severe). Delayed inflammatory skin reactions were also evaluated during the second and third PDT treatments by J.E.R. or M.G., in addition to which the patients were asked to report any intense or unexpected skin reactions after any of the DT sessions.

Sample size

The exact optimal sample size could not be calculated for this pilot study due to a lack of previous research data. We were aiming at a sample size of 10–15 LM lesions.

Results

Baseline characteristics

Altogether 24 patients with a total of 32 lesions were enrolled. Of these, 11 lesions were verified as LM and were included in the study. Three lesions were verified as invasive LMM, and 18 lesions as other pigmented lesions not fulfilling the criteria for LM and were thus excluded (*Fig. 1*).

Furthermore, one LM was excluded after biopsy because of difficulties in scheduling the PDT treatments according to the study protocol. Thus, altogether 10 LMs in 9 patients (one patient had two LMs) completed the study.

The patient demographics and the baseline characteristics of the LM lesions are presented in *Table 1*. None of the patients had received previous treatment (e.g. cryotherapy, surgery, PDT) in the skin areas where the LMs were located. The mean \pm SD (standard deviation) area of a LM lesion was $98 \pm 58 \text{ mm}^2$.

Primary outcome: Histopathological clearance

Seven out of the ten lesions (70%) were histologically completely cleared from the wide excision specimens, whereas the histology of three lesions demonstrated a residual LM. Example photographs and histological images of one cured and one uncured LM are shown in *Figures 2 and 3*. Details of the clinical response, dermoscopy and histological clearance of the LM lesions are

presented in *Table 2*. Of note, the numbering of the LM lesions (1–10) is uniform in *Tables 1* and *2* so that the data can be compared between the tables. In two of the three uncured lesions there was some visible and clinically detectable pigmentation left after the treatments, so that a residual was suspected. Interestingly, one uncured lesion had no visible pigmentation to be seen, so that it was apparently clinically cleared. In the histologically cleared lesions there was either no visible pigmentation left (4 lesions) or the pigmentation was almost invisible, with only a small area left (3 lesions).

Secondary outcomes: safety and tolerability

The pain VAS scores during the PDT illumination and the skin reactions two days after the first PDT are shown in *Table 3*. The maximal patient-reported pain was scored as moderate, and the highest VAS scores were reported during the second PDT session. The skin reaction was severe (swelling, pustules, intense erythema, crusting) in four LM lesions, moderate (marked erythema, crusting) in five and mild (mild erythema, scaling) in one. The delayed skin reaction two weeks after the first and second PDTs (assessed immediately before the second and third PDT treatments) was moderate in two patients, mild in five patients and negligible in two. Furthermore some unexpected adverse effects occurred after the PDT treatment (*Table 3*). Two patients experienced moderate pain for several hours after the second PDT session, one patient had a very intense skin reaction and one displayed swelling of the skin of the eyelid and neck. Likewise one patient experienced intense swelling, erythema and burning pain which led to a hospital visit on the day after the third PDT session. One patient suffered a continuous stinging pain in the excision area for four weeks after surgery, but it ceased after a second excision to increase the margins. Four weeks after the last PDT session (i.e. just before the excision) the following long-term adverse reactions of the treatment area were seen: postinflammatory erythema in five lesions, mild hypopigmentation in three lesions and mild hyperpigmentation in three lesions. None of the patients expressed visible scarring in the treatment area.

Discussion

This pilot study is to our knowledge the first prospective trial to investigate the efficacy of AFL-assisted PDT for treating lentigo maligna. The histological clearance rate appeared to be moderate,

seven out of ten LM lesions (70%) after three PDT sessions, but the treatment caused side effects and should only be considered in inoperable cases.

In an earlier retrospective study reported by Karam et al. 15 LM lesions were treated with PDT, resulting in a cure rate of 12/15 (80%).¹⁵ Methylaminolaevulinate (MAL) was used as a photosensitizer, but the light dose and the number of PDT sessions varied among the patients (in the ranges 40–90 J/cm² and 3–9 sessions). It is worth noting, however, that the clearance of LMs was assessed by clinical follow-up after variable lengths of time (18–50 months), and by means of histological examinations of multiple biopsies rather than wide excisions, which may have resulted in missed histological residuals. In our present study the lesions were excised completely after PDT for a full histopathological evaluation.

The gold standard treatment of LM is wide surgical excision which is also recommended by the current treatment consensus. However, the surgery is not always easily applicable for example if the LM lesion is large and in esthetically difficult location, or if anesthesia is contraindicated, or if the patient simply refuses the surgery. In these cases the alternative treatment options like AFL-assisted PDT could still be used. The follow-up for a possible recurrence of LM should be arranged in all non-surgical alternative therapies. It should be noted, that clinical follow-up of LM alone after PDT involves a risk that a residual could be missed. This danger exists because, even though the treatment may destroy all the visible pigment, a histological examination can still reveal a residual LM. This was the case in one of the three residual cases found here. For this reason a follow-up with histological verification is to be recommended even though the lesion may become clinically unpigmented and thus appear to be cured. We would suggest punch biopsies of the lesion in the follow-up visits taken from the previously visible center of the lesion or any visible pigmentation.

It is not known what is a sufficient PDT light dose for treating melanocytic lesions. When treating non-melanocytic lesions the photobleaching of PpIX is maximal in the initial phase of light illumination (more than 70% of PpIX is activated during the first 10 J/cm²) but the photobleaching continues slowly until the completion of the standard dose 37 J/cm².¹⁹ There is no earlier data available for the photobleaching of PpIX in melanocytic tumours but we assumed that it occurs in slower rate especially in the deeper situated melanocytes because melanin absorbs a portion of the red wavelength light. Karam et al. used higher doses of 40–90 J/cm² (on average 60 J/cm²) than for the treatment of non-melanoma skin cancers (37 J/cm²), justifying this by the fact that melanin restricts the diffusion of red light into the deep layers of the epidermis.¹⁵ In the present pilot study

we used an experimental dose of 90 J/cm² to ensure that almost all PpIX is activated also in the deeper parts of the tumour during the slow gradual photobleaching after the rapid initial phase. This was partly because we didn't want the efficacy of the PDT to be hindered due to unoptimal PDT protocol. The higher light dose lengthens the illumination time (approximately 18 min for 90 J/cm²) but causes no other disadvantage for the patient. Probably a smaller light dose would also suffice, but to confirm this further investigations are warranted with measurement of the BF-200 ALA-induced fluorescence in LM lesions during illumination.

The mean patient-reported pain during PDT illumination remained low in our study, ranging from 2.9 to 3.8 on the VAS scale (0–10). The highest average pain was experienced during the second PDT session and the maximum pain VAS value reported was 6.5. In the present instance the lesional skin was injected with a local anaesthetic (ropivacaine) 3 hours before illumination to reduce the pain to a more bearable level, taking into account the longer skin illumination time of approx. 18 min. For four out of the 10 patients the first PDT treatment provoked a severe skin reaction two days after the session. The reaction subsided within two weeks, i.e. before the second PDT session. In two patients an intense skin reaction also occurred after the second PDT session, and in one patient a very severe reaction was seen after the third PDT. The reactions were definitely more severe than those reported in PDT of non-melanocytic skin cancers.^{20,21} We assume that the stronger reactions were caused by the pre-treatment with AFL combined with the high light dose of 90 J/cm² used. The increased amounts of inflammatory cells and cytokines in the lesional skin following the first PDT might explain why the most severe side-effects were seen in relation to the second session.

The histopathological evaluation of the present excision specimens showed dermal scars in 5/10 lesions after PDT, which could be due to the earlier biopsies or to the AFL pre-treatment and not be actual reactions to the treatment. Before excision, no visible scarring could be seen in the treatment area for any of the ten LM lesions.

When treating LM non-surgically, one should note the growth of atypical melanocytes down the follicular units.²² In a histopathological review of 100 patients such follicular growth was seen in 95% of LMs, with a mean depth of 0.45 mm (range 0.1–1.1 mm).²³ For topical therapy to succeed, the topical agent such as the photosensitizer precursor should penetrate deep enough into the skin to reach all the atypical melanocytes, down to the deepest part. To ensure this and to enhance the efficacy of PDT, we considered it important to use ablative fractional CO₂-laser pre-treatment

which increases the uptake and deep penetration of ALA and MAL into the skin.²⁴ AFL pre-treatment has been shown earlier to enhance the clinical efficacy of PDT when treating non-melanoma skin cancer.²⁵⁻²⁷ The photosensitizer distribution in the deeper layers of the skin doesn't depend on the depth of the laser pores in the dermis as long as the epidermis is penetrated.²⁸ The pulse energy of the CO₂-laser used here, 84 mJ, corresponds to a channel in the skin that is approximately 200 µm deep (measured histologically from a skin biopsy of a healthy volunteer in Päijät-Häme Central Hospital, data not shown) which would be sufficient for full penetration of the epidermis, which is less than 100 µm thick except in the palms of the hands.²⁹

The failure rate of AFL-assisted PDT, i.e. the number of histopathological LM residives observed, was 3 out of 10 LM lesions (30%), whereas the reported recurrence rates for the standard surgical excision of LMs are in the range of 8–20% (mean 6.8% at 5 years), those for staged excision 0–7%, and those for Mohs micrographic surgery 0–2%.^{12,30} In a recent review of non-surgical treatments available for LM the recurrence rates were 0–31% (mean 11.5%) for radiotherapy, 4–50% (mean 24.5%) for imiquimod and 0–100% (mean 34.4%) for laser therapy, which are all inferior to those achieved with surgical methods.¹⁴ The cure rate in our present pilot study is superior to that for laser therapy, in line with imiquimod, but inferior to radiotherapy. A high recurrence rate with any treatment modality may be derived from deep follicular extension, unsuspected invasion or subclinical extension of the LM.¹² Among our three non-responders, one LM was located on the cheek (*Figure 3*) and the histopathological evaluation after PDT revealed lentigo maligna with a 1 mm deep follicular extension, so that the accumulation of protoporphyrin in the deep part of the lesion may not have been sufficient, which could explain the failure of PDT in this case. Otherwise, no correlation between demographic data or lesion baseline characteristics and histologic outcome could be found which is most likely due to small sample size.

The limitations of our study were: the small number of cases, due to the piloting nature of the study; the duration of adverse skin reactions was not recorded; and the use of the routine bread-loaf technique in the histological assessment of the lesions which could have caused us to miss some residual part of a lesion.³¹ This must partly have been offset, however, by the fact that the lesions were completely excised with 5 mm margins after the treatment. A strength of this work lies in the fact that it is the first prospective study assessing the effect of AFL-assisted PDT in the treatment of LM, offering a basis for future larger studies.

In conclusion, the present results demonstrate that ablative fractional laser-assisted PDT is an alternative effective option for treating lentigo maligna. Histopathological assessment of the wide excision specimens showed that 7 out of the 10 lesions (70%) were histologically completely cleared after three AFL-assisted PDT sessions. The patient-reported pain during PDT illumination was moderate and tolerable, although a few severe skin reactions were observed after the PDT. AFL-assisted PDT could be considered as a treatment option for non-invasive lentigo maligna in patients for whom surgery is contraindicated or as a second-line treatment for residual lesions. Further studies with larger samples are warranted to confirm these preliminary results.

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Tables

Table 1. Patient demographics and baseline characteristics of the lentigo malignas included in the series. *) Patient 9 had two lentigo malignas. AK = actinic keratosis, BCC = basal cell carcinoma, DN = dysplastic nevus, LM = lentigo maligna, LMM = lentigo maligna melanoma, MM = malignant melanoma, SCC = squamous cell carcinoma.

Patient	Gender (M/F)	Age (y)	Skin photo-type	Previous skin cancers	LM lesion	Location	Lesion size (mm)	Lesion area (mm ²)	Lesion histologically cleared (Y/N)
1	M	69	II	AK	1	forehead	8 x 10	63	No
2	M	83	II	-	2	cheek	14 x 21	231	No
3	M	62	II	BCC, SCC, DN x 2, MM x 2, LM x 2,	3	forearm	10 x 17	134	Yes
4	M	71	I	AK	4	upper back	12 x 13	123	Yes
5	M	75	II	-	5	upper thorax	11 x 13	112	Yes
6	F	77	II	-	6	forearm	9 x 12	84	No
7	M	77	II	AK, Mb Bowen, BCC	7	cheek	10 x 14	110	Yes
8	F	71	II	BCC, LMM	8	lower back	6 x 8	38	Yes
9	M	79	III	AK, kerato-acanthoma	9*	temple	8 x 8	50	Yes
9					10*	neck	6 x 8	38	Yes

Table 2. Clinical response, dermoscopy and histological clearance of the lentigo malignas four weeks after the last photodynamic therapy. *) LM 9 and 10 belonged to the same patient. IHC = immunohistochemistry, LM = lentigo maligna.

LM lesion	Clinical response	Dermoscopy	Histopathological findings in excision specimens	IHC used	Lesion histologically cleared (Y/N)
1	No visible pigment	White streaks, erythema	Atypical melanocyte proliferation/ lentigo maligna suspicion	-	<u>No</u>
2	Small pigmented area	Pigment network, erythema	Lentigo maligna	-	<u>No</u>
3	No visible pigment	White streaks, erythema	Scar and perivascular inflammation	-	<u>Yes</u>
4	No visible pigment	White streaks, erythema	Scar	-	<u>Yes</u>
5	Almost invisible pigmentation	Light diffuse pigmentation, erythema	Scar	-	<u>Yes</u>
6	Some pigmentation left, bleached	-	Lentigo maligna	-	<u>No</u>
7	Small pigmented area, mostly invisible	Small area of diffuse pigmentation, white streaks, erythema	Scar	-	<u>Yes</u>
8	Small pigmented dot in the middle	Small pigmented dot in the middle, erythema	Benign lentigo, fibrosis and inflammation	MART1, elastin	<u>Yes</u>
9*	No visible pigment	Erythema	Solar elastosis	MART1	<u>Yes</u>
10*	No visible pigment	Erythema	Scar and solar elastosis	MART1, Fontana	<u>Yes</u>

Table 3. Pain visual assessment scores (VAS) during the PDT illumination and the skin reaction two days after the 1st PDT session. *) LM 9 and 10 belonged to the same patient. LM = lentigo maligna, PDT = photodynamic therapy, SD = standard deviation.

LM lesion	Location	PDT 1 Max. pain (VAS 0–10)	PDT 2 Max. pain (VAS 0–10)	PDT 3 Max. pain (VAS 0–10)	Skin reaction 2 days after PDT 1 (0–4)	Notes
1	forehead	1.8	4.4	2.2	4	After the excision a constant stinging pain occurred in the excised area. The pain ceased after a second excision for margin control.
2	cheek	0	0.1	1.2	2	A very intense skin reaction with erythema, swelling and burning pain after the 3rd PDT session.
3	forearm	0.9	5	2.3	4	-
4	upper back	2.3	1.4	1.8	4	-
5	upper thorax	5.4	6.5	3.7	3	Moderate pain continued for several hours after the 2nd PDT session.
6	forearm	4	4.4	1.6	3	Moderate pain continued for several hours after the 2nd PDT session.
7	cheek	2.2	1.4	1.5	3	Skin swelling of the eyelid and the neck after the 2nd PDT session.
8	lower back	4.4	4.7	5.5	4	A very intense skin reaction with violaceous erythema and abundant secretion after the 2nd PDT session.
9*	temple	5	5.1	4.3	2	-
10*	neck	5	5.3	5.1	3	-
Mean ± SD		3.1 ± 1.9	3.8 ± 2.1	2.9 ± 1.6	3.3 ± 0.7	

Figures

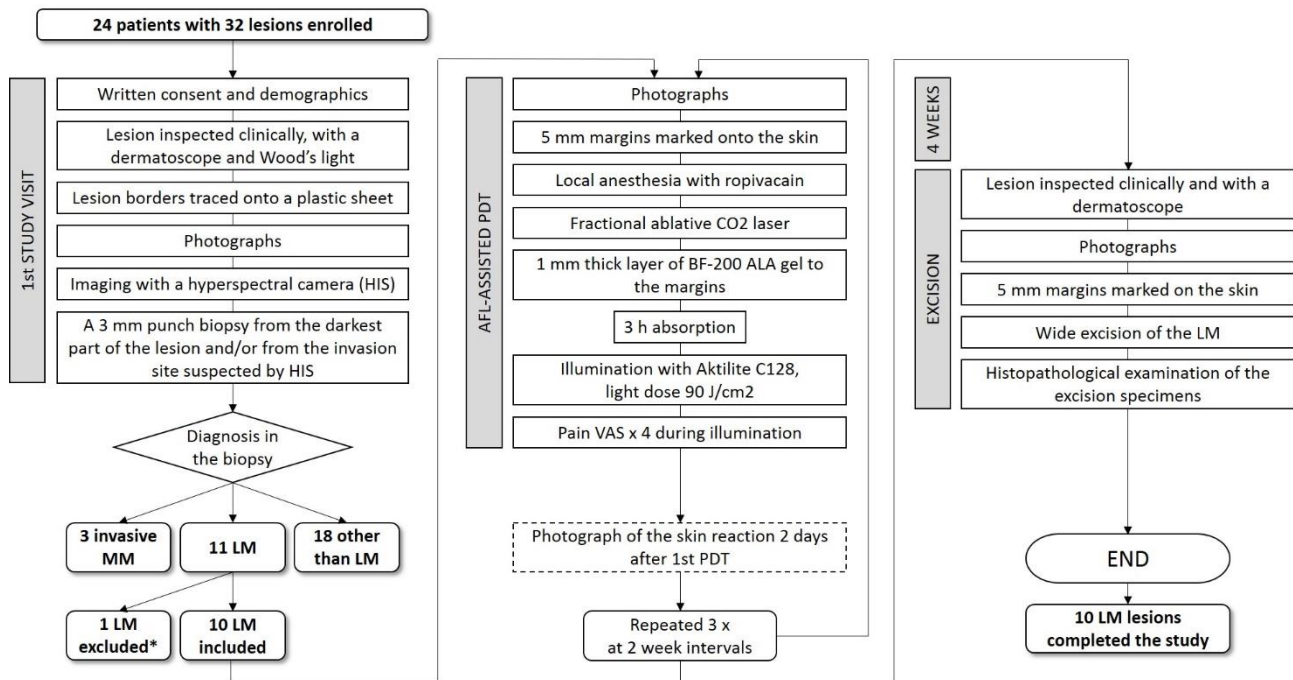


Figure 1. Flow-chart of the protocol. The histological diagnoses of the biopsied lesions were: 11 LM, 2 LMM, 1 other MM, 1 lentigo, 2 seborrheic keratoses, 3 postinflammatory hyperpigmentation, 6 pigmented actinic keratosis, 6 dysplastic nevi. *) One lentigo maligna was excluded after biopsy because of difficulties in scheduling the PDT treatments according to the study protocol. LM = lentigo maligna, LMM = lentigo maligna melanoma, MM = malignant melanoma.

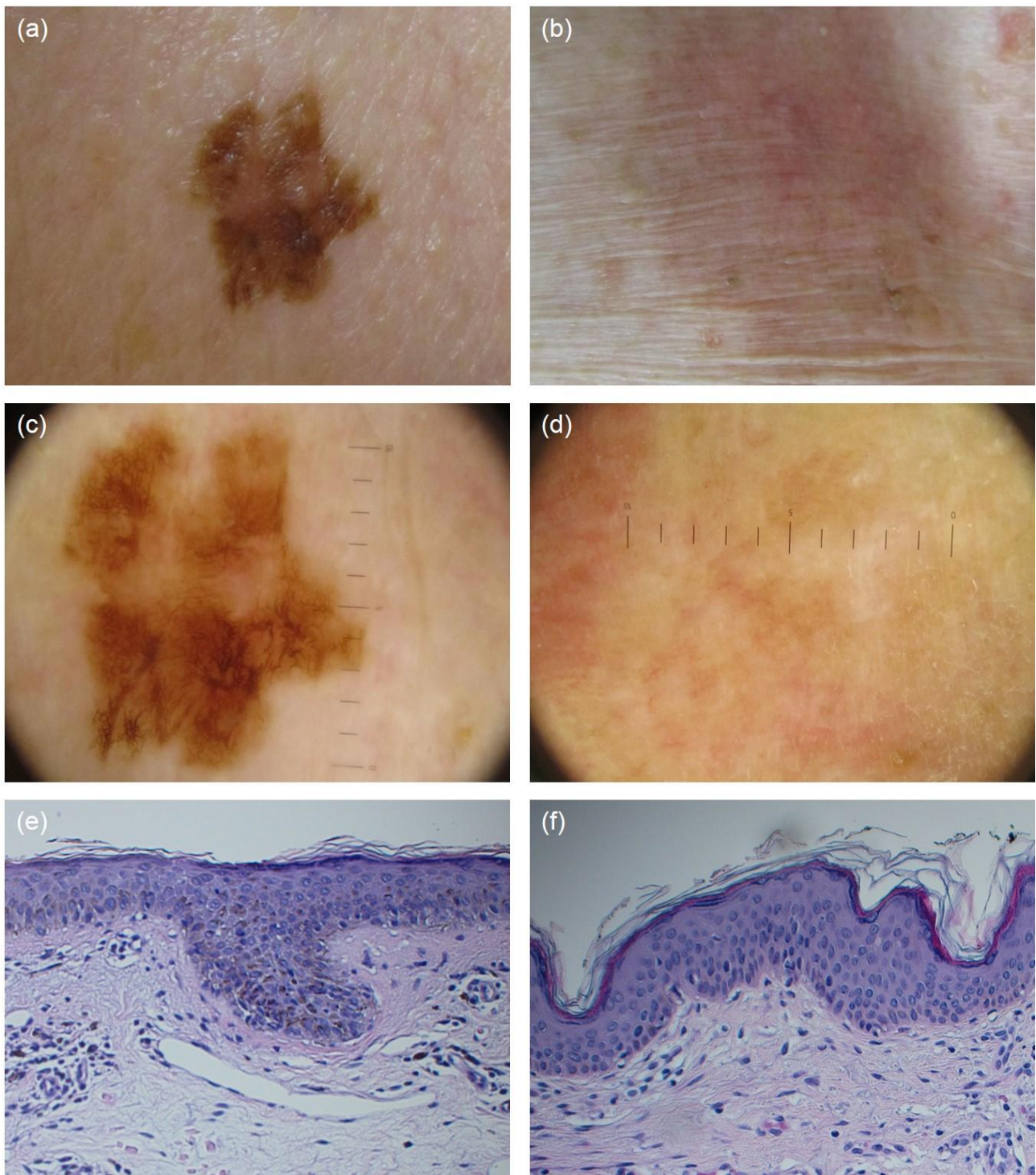


Figure 2. Clinical, dermoscopic and histological images of a cured lentigo maligna (LM) before and after photodynamic therapy (PDT). (a) Photograph of a LM located on the chest before PDT, (b) photograph four weeks after the last PDT treatment, (c) dermoscopic image before PDT, (d) dermoscopic image after PDT shows no visible pigmentation, (e) histology before PDT shows confluent atypical melanocyte proliferation at the junction, (f) histology after PDT shows scar formation in the dermis with no sign of atypical melanocytes. Magnification 20X.

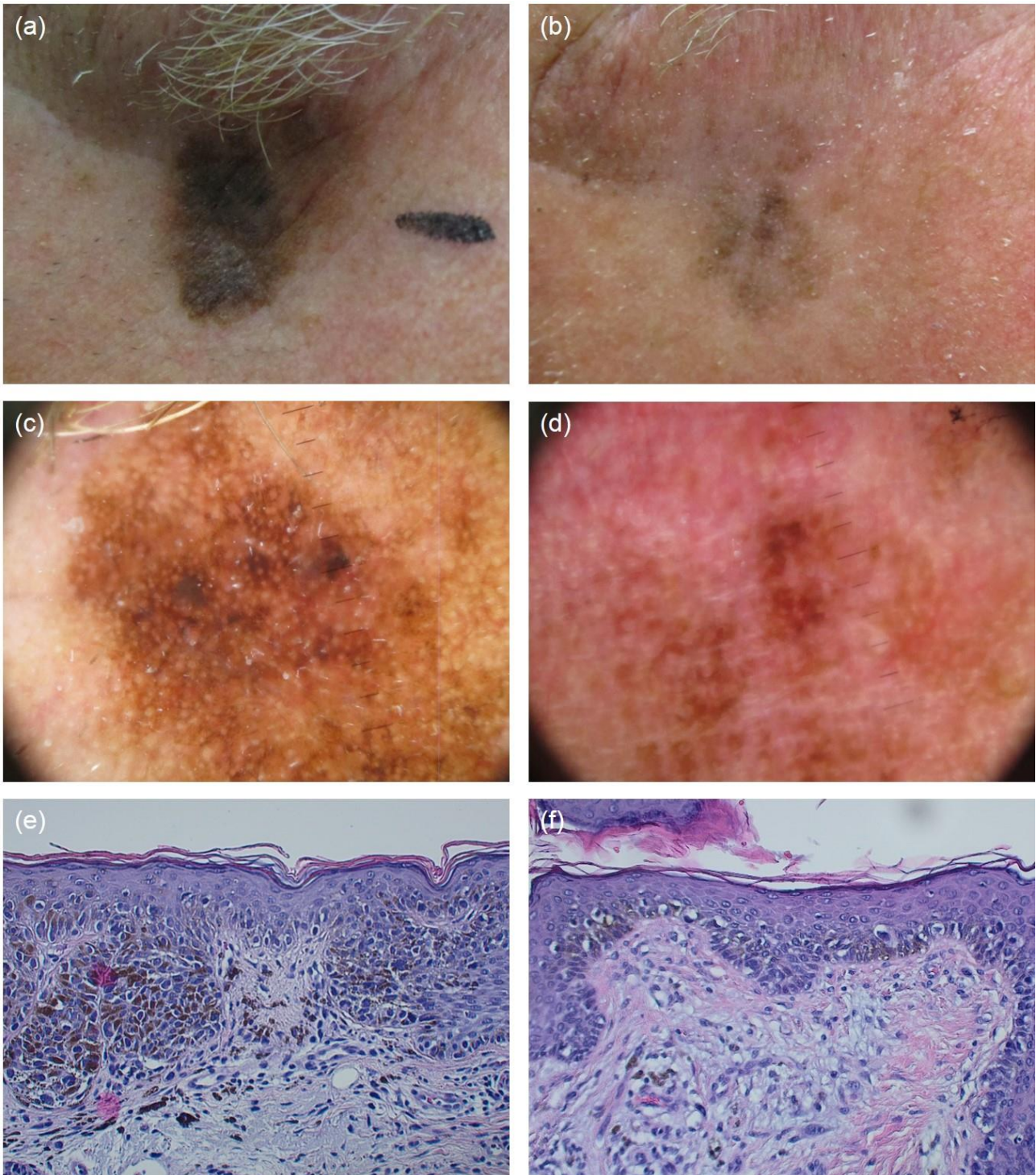


Figure 3. Clinical, dermoscopic and histological images of a non-responding lentigo maligna (LM) before and after photodynamic therapy (PDT). (a) Photograph of a LM located on the cheek before PDT, (b) photograph of the residual LM four weeks after the last PDT treatment, (c) dermoscopic image before PDT, (d) dermoscopic image after PDT, showing a residual pigment network, erythema and white streaks, (e) histology before PDT, shows islands of atypical melanocytes with poor cohesion and atypical melanocyte proliferation at the junction, (f) histology after PDT, showing lentiginous proliferation of atypical melanocytes with variations in cell shape and size. Magnification 20X.