Optimization Strategies for Laser Therapy in Dermatology

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Optimization Strategies for Laser Therapy in Dermatology

Verbeterstrategieën voor laserbehandelingen in de dermatologie

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General introduction and aims of this thesis

LASERS

Lasers have been an ongoing field of interest and development since Albert Einstein's 'Quantum Theory of Radiation' in 1917. This contained all the necessary formulas and theoretical concepts to build a laser, but it wasn't until the 1960's that the first lasers became operational.

The word 'LASER' is an acronym for Light Amplification by the Stimulated Emission of Radiation. The explanation of the creation of laser light starts with the structure of an atom. An atom is composed of a positively charged central nucleus, with negatively charged electrons orbiting around the nucleus. Orbits closer to the nucleus have lower energy, and the closest one is called the ground state (E0; stable configuration). When an atom absorbs energy (a photon), a negatively charged electron can get excited and move to a higher energy orbit; this is called the excited state (E1; unstable configuration). After a short period of time, the excited electron will return to the stable ground state by emitting a photon equal to the energy difference (i.e. spontaneous emission). Normally, this spontaneous absorption and release of light occurs in a disorganized and random fashion and results in incoherent light [1].

Lasers are different from other light sources, as they have unique characteristics: coherence, monochromaticity, and collimation. Coherent light is light where all the waves are in phase with each other in both time and place. The coherence of laser light is due to the process of stimulated emission. In stimulated emission, an external power source creates excitation of the atoms in a medium. When more atoms are in their unstable high energy state than in their stable resting configuration, population inversion is created. This is necessary for light amplification. If an atom is already in an excited state when it collides with a photon, two photons are released in phase with one another as the electron returns to its normal, stable configuration (Fig. 1). This results in a cascade of reactions and the release of numerous photons of identical wavelength, energy, and phase. Light of a single wavelength is called monochromatic light. The third character-

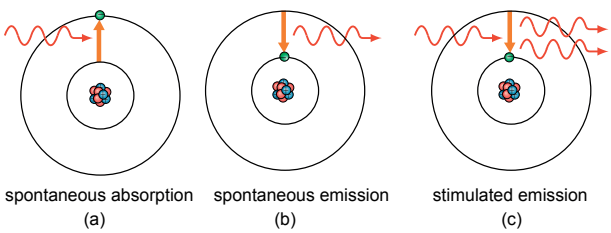

Figure 1. Electron transitions in an atom. *Reproduced with permission of the Department of Physics, The Chinese University of Hong Kong.*

istic is collimation, which refers to the parallel nature of the waves, without significant divergence.

Lasers are composed of different components: a medium, optical cavity, power supply, and a delivery system. The medium can be solid (e.g. ruby), liquid (e.g. rhodamine dye), or gas (e.g. CO_2). Each laser medium has a very characteristic photon wavelength and determines the wavelength of the emitted light. The optical cavity surrounds the medium and contains the amplification process. After the energy input by an external power source, photons start to build up with the same wavelength. The light is reflected in a chamber between two mirrors (creating collimation), and can be released out one end and delivered through the handpiece to the target.

In order for a tissue reaction to occur, the laser light needs to be absorbed by the target tissue (for example) in the skin. When light is absorbed, the radiant energy is transformed into a different form of energy by a specific interaction with the tissue. The most common reaction is a photothermal reaction, where the light energy is transformed into and dissipated as heat. Other common reactions are: photoacoustic or photomechanical (formation of shockwaves), photochemical (light energy starts a chemical reaction), and photoablation (use of light to ablate tissue). However, only when light is highly absorbed by a specific component of the skin, a precise biologic effect occurs.

Besides absorption, laser light can also interact with tissue in different ways: transmission, reflection and scattering (Fig. 2). Transmission is the passage of laser energy through a biologic tissue without producing any effect. Reflection refers to the repelling of light off the surface without any entry into the tissue. Approximately 4% to 7% of light is reflected off the skin [2]. The least amount of reflection occurs when the laser beam is directed perpendicular to the tissue. Scattering is imprecise absorption of laser energy due to the heterogeneous structure of the tissue, and spreads out the laser beam resulting in a diffuse effect on tissue. In the skin most scattering is caused by dermal collagen,

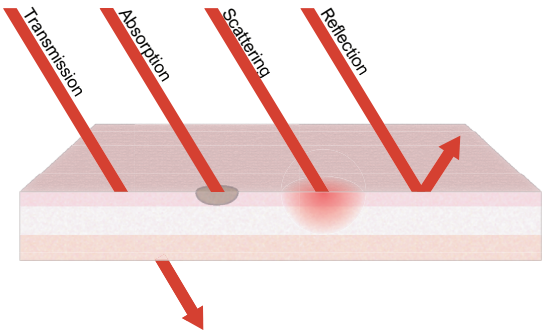

Figure 2. Laser – tissue interactions.

which limits the penetration depth [3]. The amount of scattering is inversely proportional to the wavelength of the laser, with longer wavelengths penetrating deeper into the tissue [3]. Also, larger spot sizes cause less scattering.

Anderson and Parrish introduced the theory of selective photothermolysis in 1983 [4]. The concept of selective photothermolysis states that in order to destroy a specific target, a wavelength is needed that is more absorbed by the target chromophore than any other competing chromophores in the skin. The three main components of the skin that absorb laser light are melanin, hemoglobin, and water. The absorption spectra of these different chromophores have been established and can be used to design specific lasers for specific targets (Fig. 3).

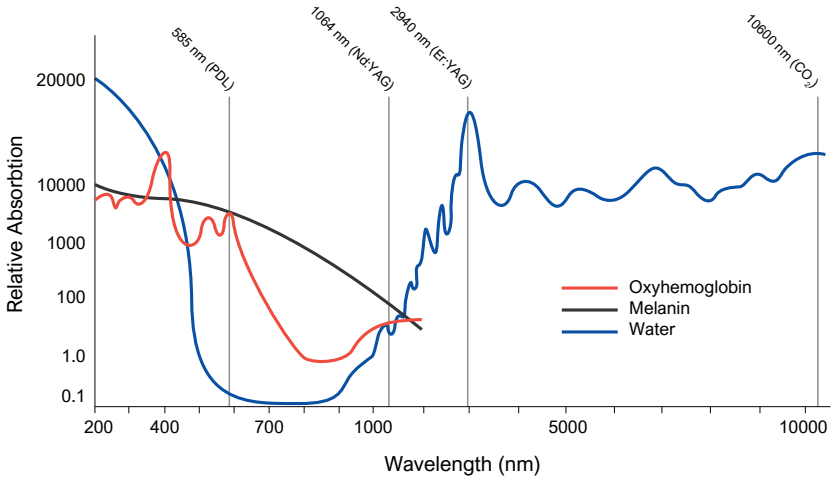

Figure 3. Absorption spectra of chromophores. Oxyhemoglobin has three main absorption peaks in the blue-green-yellow portion of the visible range (418 nm, 542 nm, 577 nm) [3, 5]. Melanin absorbs light of wavelengths from ~250 nm – 1200 nm, with decreasing absorption as the wavelength increases [6]. Water absorbs mainly infrared laser energy (700 nm – 1 mm).

The wavelength also determines the penetration depth. In general, longer wavelengths penetrate deeper into the skin than shorter wavelengths (because of less scattering). This applies to the wavelengths within the spectrum of visible light. However, the near-infrared wavelengths of light are absorbed by water in the cells at the surface of the skin, and therefore their penetration depth is decreased drastically.

The second part of the theory states that for the best selectivity, and to avoid non-selective damage, the heat should be confined to the target during the light pulse. This right amount of energy with the proper pulse duration is known as thermal relaxation

time (TRT). The TRT is the time required for the target to dissipate 50% of its heat and is based on the size of the target (Table 1) [3]. Small targets have a small TRT and need a laser with a short pulse duration. Large targets have a long TRT and therefore need lasers with a long pulse duration. The pulse duration should be shorter than the TRT to prevent the spread of thermal energy beyond the targeted chromophore.

However, Murphy and Torstensson recently suggested that not the physical dimensions of the target, but the tissue's intrinsic structure determines the time required to induce irreversible protein denaturation within the target [7].

Table 1. Thermal relaxation times of common laser targets [3, 8].

Target	Size	Thermal relaxation time
Tattoo ink particle	0.04-0.7 µm	10 ns
Melanosome	1 µm	1 µs
Erythrocyte	7 µm	20 µs
Epidermis*	50 µm	1 ms
Blood vessel	50 µm	1 ms
Ectatic blood vessel	100 µm	15 ms
Hair follicle	200 µm	20 – 100 ms

* The thickness of the epidermis, and thereby its TRT, varies for different anatomic locations (from approximately 30 - 150 µm).

Finally, sufficient energy must be delivered to the target to destroy the chromophore. Irradiance determines the ability of a laser to incise, vaporize, or coagulate tissue and is expressed in watts/cm^2 [1]. The energy fluence (EF) determines the amount of energy delivered in a single pulse and is expressed in joules/cm2. The EF can be calculated using the following formulas:

$$Intensity\ of\ \boldsymbol{power} = \frac{energy}{time} = \frac{Joules}{s} = watts$$

$$\boldsymbol{Irradiance} = \frac{intensity}{area} = \frac{Watts}{cm^2}$$

$$\boldsymbol{Fluence} = \frac{intensity\ x\ time}{area} = \frac{watts\ x\ s}{cm^2} = \frac{Joules}{cm^2}$$

The fluence necessary to destruct the target is dependent on the fraction of light absorbed by the target [5]. When the wavelength is weakly absorbed by the target, for example when the target is located deep in the dermis, or when the target contains less chromophore, higher fluences are needed to achieve the desired effect. The development and use of epidermal cooling systems minimized the non-selective epidermal

thermal injury and allowed for higher fluences to be used. Additionally, epidermal cooling reduced treatment-related pain.

Today, lasers are increasingly used for different medical and cosmetic purposes and have gained an important role in dermatology. By altering the wavelength and pulse duration of the laser, a wide variety of dermatological indications can be treated, including vascular lesions, pigmented lesions, tattoos, hairs, and (ablative and non-ablative) resurfacing.

Although laser systems have improved in the last few decades, treatments are frequently far from perfect, and patients may have unrealistically high expectations when it comes to the results of their laser treatment. The ideal laser treatment would have 100% effective treatment results, no recurrences, no side effects, a short treatment duration, no pain, a quick recovery (minimal downtime), and excellent esthetic results. As none of the currently available laser treatments can reach these goals, there is still room for improvement, and even established laser treatments can be optimized.

An example is the Pulsed Dye Laser (PDL) treatment used for port wine stains (PWS). The PDL was the first laser that was designed based on the principles of selective photothermolysis, and is today still the gold standard treatment for PWS. However, PDL treatment results are unpredictable and complete clearance is only achieved in a minority of patients [9]. Therefore, there is a clear necessity to further optimize the results of this established laser treatment.

Another, more general, optimization strategy for laser treatments is to minimize the pain experienced during treatment. Laser treatments can be painful and optimizing this discomfort can increase patient satisfaction and also treatment efficacy as pain sometimes makes it necessary to use lower laser settings.

Since lasers are one of the most rapidly advancing fields in dermatology, the opportunity to discover new laser indications is nowhere near exhausted. Therefore, innovative laser treatments for alternative indications should be further investigated, for example as an alternative non-surgical treatment option for pre-malignant skin lesions such as lentigo maligna.

In the next section of this introduction, obstacles during PWS laser therapy, the general obstacle of pain during laser treatments, and the obstacles during non-surgical treatments of lentigo maligna are further discussed.

OPTIMIZATION OF ESTABLISHED LASER TREATMENTS

Port wine stains

Port wine stains are congenital vascular malformations of the skin and occur in 0.3% - 0.5% of the population [10]. Unlike capillary hemangiomas, PWS do not involute spontane-

ously. Clinically, PWS appear as pink macules in infancy, and over time develop a deeper red or purple color due to underlying progressive vascular ectasia (Fig. 4.)

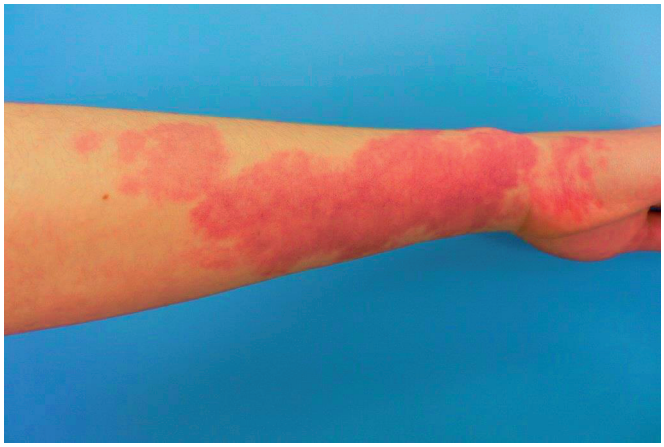

Figure 4. Clinical presentation of a port wine stain.

Nodular components and hypertrophy of the underlying soft tissue occur in 65% of the people by the age of 50 [11]. Port wine stains can cause significant psychological problems as most lesions occur in the head and neck region [12, 13].

The pathogenesis of PWS remains largely unknown. Histologically, PWS are characterized by ectatic capillaries and post-capillary venules in the papillary and mid-reticular layers of the dermis [14]. The diameter of the vessels can increase over time. A hypothesis for the pathogenesis of PWS is a decreased nerve density and an increased vessel-to-nerve ratio, resulting in an abnormal neural regulation of blood flow [15]. As a result, the blood vessels are unable to constrict normally and remain permanently dilated.

Mutations in RASA1 and vascular endothelial growth factor (VEGF) expression also appear to play a role in the pathogenesis of PWS [16, 17]. Recently, a somatic activating mutation in the GNAQ gene was found to cause Sturge-Weber syndrome and PWS [18].

Most PWS occur in isolation and are not associated with other health problems. However, PWS can be associated with Sturge-Weber syndrome, Klippel-Trenaunay syndrome, Cobb syndrome, von Hippel-Lindau syndrome, and cutis marmorata teleangiectatica congenital.

Laser treatment of PWS

Laser therapy is the treatment of first choice for PWS. The target tissues in PWS are 10-500 µm dilated capillaries and postcapillary venules in the papillary and reticular dermis [19]. The target chromophore is hemoglobin, which absorbs light strongly at 400 - 600 nm, while absorption is less at 700 - 1100 nm [20]. When laser light is absorbed by hemoglobin

it causes photocoagulation and aggregation of erythrocytes through a photothermal reaction, and ultimately necrosis of the endothelial cells [21]. The highest absorption peak for hemoglobin is at 418 nm, however the penetration of this wavelength is limited to the dermal-epidermal junction (about 100 µm).

The first laser that was commonly used for the treatment of PWS was the argon laser [1]. The argon laser emitted light at wavelengths of 488 - 514 nm, which is preferentially absorbed by oxyhemoglobin and melanin. The pulse duration varied from 50 - 200 ms. This continuous wave laser with relatively long pulse widths had an exposure time that was too long for selective photohermolysis and resulted in nonspecific thermal damage (textural alteration, scarring, pigmentary changes) to the surrounding tissue [5]. Also, treatment with this laser was painful and infiltration anaesthesia was often needed.

Continuous wave dye lasers were developed with longer wavelengths of light (and therefore a deeper penetration), which were thought to be more suitable for the treatment of PWS. These lasers operated at a wavelength of 577 nm, which coincides with the beta absorption peak of hemoglobin. However, the light was emitted in a continuous fashion and pulse durations were still quite long (50 – 200 ms), which resulted in nonspecific thermal damage, and was the major disadvantage of these lasers. The operator could move the beam across the surface to reduce exposure time, but these results were very dependent on the skills of the operator. Alternatively, automated robotic scanning devices were used to create shorter pulse durations, uniformity of energy placement and faster treatment.

The first laser that was designed based on the concept of selective photothermolysis was the PDL. The active medium is rhodamine dye selected to produce yellow light at 577-595 nm. The first generation PDLs produced light at 577 nm, which coincides with the hemoglobin absorption peak while having a lower absorption of melanin than the 488 nm and 514 nm wavelengths of the argon laser [22]. However, the 577 nm PDL did not penetrate deep enough for successful treatment of PWS [14], and therefore the 585 nm PDL was developed. Additionally, this wavelength had a lower melanin-to-hemoglobin absorption coefficient than the 577 nm PDL. It was shown that penetration depth with the 577 nm PDL increased from 0.5 mm to 1.2 mm with the 585 nm PDL in a pig skin model [23]. However, the penetration depth remained too shallow for the deeper vessels, and since the absorption of light by oxyhemoglobin is greater at 577 nm than 585 nm, clearance was not significantly improved [14]. Subsequently, the modern 595-600 nm PDLs were developed further enhancing penetration depth.

The first generation PDLs used a pulse duration of 0.45 milliseconds. Short pulse durations can cause vaporization of blood, local rupture of microvessels, and purpura. These purpura can last for 7-10 days and are often cosmetically unacceptable. The second generation PDLs were developed with longer pulse widths. This longer pulse width was thought to be more appropriate for larger caliber vessels, based on the estimated TRT

of PWS vessels at 0.45 – 10 ms [4, 24]. These longer pulse durations produced little or no immediate purpura [25] and resulted in a better treatment outcome [14].

With all these wavelengths, there is, besides absorption of oxyhemoglobin and deoxyhemoglobin, also absorption of melanin. Therefore, skin cooling is needed to protect the epidermis from thermal damage, and to allow for the use of higher fluences without increasing the risk of epidermal damage.

The development of the PDL highly increased the effectiveness of PWS treatment. However, treatment results remain unpredictable, and complete clearance is only achieved in a minority of patients [9]. A commonly occurring limitation of PDL treatment is recurrence of the PWS after treatment, which occurs in 16.3% – 50% of patients as early as five years after treatment [26, 27].

Obstacles in the treatment of PWS

The efficacy of selective photothermolysis and completeness of photocoagulation of the blood vessels depends on the extent of epidermal pigmentation, optical shielding by blood and superimposed vessels, and PWS vascular anatomy and morphology [14]. A high melanin content, superimposed vasculature, increased PWS size, vessel depth, and skin thickness reduce light penetration and result in a decreased treatment efficacy [14, 28]. Vessels of moderate size are correlated with a better clinical response to PDL treatment than small (< 20 μm) vessels [29].

Also, it has been suggested that treatment failure of PWS, or recurrence of PWS after laser treatment, is in part due to regeneration and revascularization of photocoagulated vessels [30]. After successful laser photothermolysis, the blood supply to the skin is significantly reduced due to vessel photocoagulation. This results in the induction of local hypoxia. The normal wound healing response of the skin detects hypoxia and initiates appropriate defense mechanisms such as vasodilation and angiogenesis. In order to achieve PWS clearance, hyperdilated PWS vasculature has to be replaced by normal lower-volume capillaries. However, in therapy-resistant PWS, neo-angiogenesis may occur too extensively during the short-term vascular remodeling phase and hampers the reduction in dermal blood content [30].

Options for treatment-resistant PWS

Over the years, different approaches have been undertaken to optimize the laser treatment of therapy-resistant PWS. For example, longer wavelength lasers, such as the 755 nm Alexandrite and 1064 nm neodymium-doped yttrium aluminium garnet (Nd:YAG), may be especially useful for hypertrophic and nodular PWS lesions as they penetrate deeper [14]. However, since there is also a decreased absorption by hemoglobin, higher fluences are often needed, which are associated with a higher risk of adverse events. Non-coherent light sources, like the Intense Pulsed Light (IPL), appear to be less effec-

tive than PDL treatment [31], but may be considered as an alternative treatment for PDL-resistant PWS [14]. Other techniques that have been used to improve PWS clearance are different spotsizes, multiple passes, pulse stacking, and dual laser approaches. However, Chen et al. performed a review of literature between 1990 and 2012, and concluded that, in this period, no substantial progress had been made in PWS laser treatment clearance [14]. They showed that 12% - 85% of patients achieved less than 50% clearance, regardless of treatment modality. Therefore, there is a clear necessity for the continued development of optimization strategies for refractory PWS.

Different experimental treatments or techniques to improve the outcome of laser treatment of PWS include photo dynamic therapy (PDT), pneumatic skin flattening (PSF), fractional ablative resurfacing, indocyanine green-augmented diode laser therapy, and anti-angiogenic agents (e.g. sirolimus).

Sirolimus

Mammalian target of rapamycin (mTOR) signaling is an important component of the cellular response to hypoxia through upregulation of hypoxia-inducible factor 1-alpha (HIF-1a), which increases the expression of hypoxia-responsive genes. Sirolimus (rapamycin [RPM]) is a specific inhibitor of mTOR and has a long history of use as an immunosuppressive agent [32]. Because of its ability to inhibit mTOR-mediated functions, such as protein synthesis, cell proliferation and tumor angiogenesis, the use of sirolimus as an anticancer drug has been investigated [33, 34]. As an anti-angiogenic agent, sirolimus may also enhance the therapeutic efficacy of PWS laser therapy. Sirolimus inhibits the

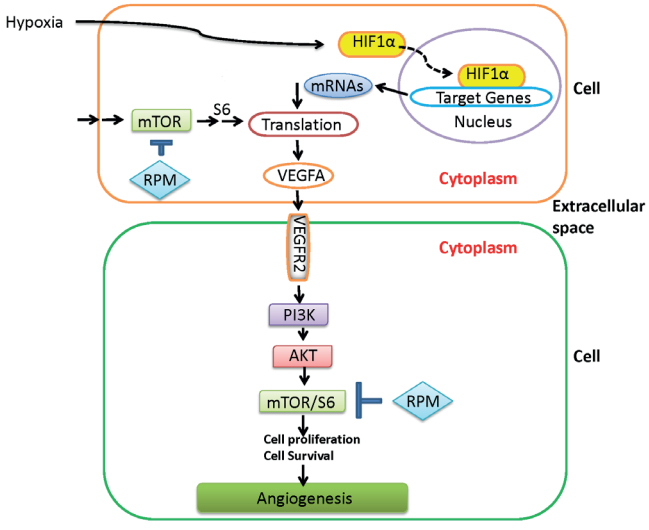

Figure 5. Schematic diagram of RPM-mediated inhibition of PDL-induced angiogenesis pathways [36].
Figure reproduced from Tan et al. Lasers Surg Med. 2012 with permission, © 2012 Wiley Periodicals, Inc.

proliferation of vascular endothelial cells driven by vascular endothelial growth factor (VEGF) and suppresses the induction of HIF-1a [35]. Therefore, sirolimus potentially may inhibit the growth of vascular endothelial cells and smooth muscle cells, which are critical elements for new blood vessel formation (Fig. 5.).

OPTIMIZING LASER TREATMENTS IN GENERAL

Pain

Pain is a frequently occurring side-effect of laser treatments and can decrease patient satisfaction. Pain can also decrease treatment efficacy as it may force the treating physician to use lower laser settings.

Invasive anaesthetics can be used to reduce treatment-related pain. However, these injectable anaesthetics are often painful to administer, and patients can be needle phobic. Alternatively, non-invasive anaesthetic methods can be used, which are painless to administer. Non-invasive anaesthetic methods for pain reduction during dermatological laser treatments include skin cooling, PSF, and topical anaesthetic drugs.

Different skin cooling methods are available, including cold air, dynamic skin cooling (cryogen spray), application of cold gels, and contact cooling. Cooling lowers the skin temperature and thereby decreases pain sensation and discomfort. Secondly, skin cooling protects the epidermis from thermal injury, as melanin in the epidermis is an unwanted site for absorption of light, except when treating epidermal pigmented lesions [5]. Skin cooling may be applied before, during and/or after laser treatment. The depth of cooling that is desired differs with different laser applications.

Pneumatic skin flattening utilizes a vacuum chamber, covered by a transparent sapphire window, to generate negative pressure. This activates tactile and pressure skin

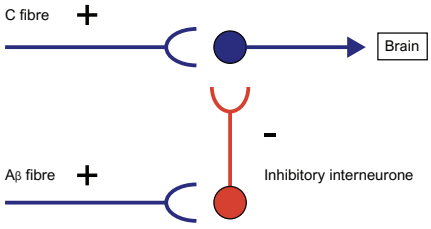

Figure 6. Gate control theory of pain. "Slow" C-fibres transmit pain from the periphery. "Fast" Aβ-fibres transmit mechanical stimulation impulses. Competitive activation by "fast" fibres activates the inhibitory neurone (closing the "gate"), and modulates the transmission and subsequent cortical perception of pain.

receptors, which block the transmission of pain, based on the gate control theory of pain (Fig. 6) [37, 38]. The laser light can be delivered through the sapphire window, and the vacuum is released at the end of the treatment pulse.

Topical anaesthetic drugs inhibit sodium influx through voltage-gated sodium channels and prevent nerve cell depolarization and input to the central nervous system [39]. Cocaine was the first topical anaesthetic drug that was discovered [40]. Later, ester anaesthetics (e.g. tetracaine) were developed, however, allergic contact dermatitis frequently occurred as an adverse effect [41]. Subsequently, amide anaesthetics (e.g. lidocaine, prilocaine) were created and became the preferred anaesthetic. Epinephrine may be added to topical anaesthetic drugs to prolong the anaesthetic effect and decrease systemic absorption through vasoconstriction. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) can be used topically. They reduce pain by inhibiting cyclo-oxygenase enzymes, and thereby the synthesis of prostaglandins. Today, various prescription and over-the-counter topical anaesthetics and combinations are available.

Which of these non-invasive methods for anaesthesia provides the best pain reduction during different dermatological laser procedures has never been systematically investigated.

DEVELOPMENT OF INNOVATIVE LASER TREATMENTS

Lentigo maligna

Lentigo maligna (LM) is the most common subtype of melanoma *in situ*, histologically showing atypical melanocytes confined to the epidermis and appendageal epithelium (Fig. 7).

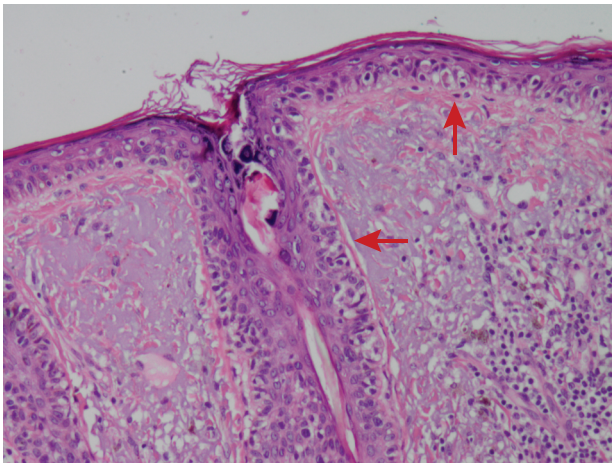

Figure 7. Histopathology of lentigo maligna. Continuous proliferation of atypical melanocytes along the dermo-epidermal junction, with downward extension along the hair follicle. In the background severe solar elastosis. Arrows point out atypical melanocytes (HE 100x).

The clinical appearance of LM is characterized by a slowly growing pigmented macular lesion on chronically sun-exposed skin of middle-aged or elderly individuals (Fig. 8). Most lesions are located on the head and neck region [42]. Lentigo maligna has the potential for dermal invasion and progression to lentigo maligna melanoma (LMM). However, this risk of progression is not well known. One epidemiological study from 1987 estimated the lifetime risk of LM progressing to LMM to vary from 2.2% to 4.7% [43].

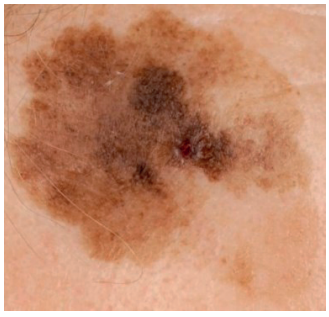

Figure 8. Clinical presentation of a lentigo maligna.

The primary treatment goal is to madicate the lesion completely and prevent recurrences. However, since LM occurs mainly in the head and neck region [44], important secondary goals are to minimize functional impairments and cosmetic deformities as well.

Surgical excision is the accepted standard of treatment for LM [45]. The main advantages are that the atypical melanocytes in both the surface epidermis and appendages can

be completely removed, and that it allows for careful histological examination, staging, and the assessment of margin clearance. The treatment of first choice, according to the current guidelines, is wide local excision with 5 mm margins [46, 47]. However, as clinical margins are often difficult to delineate, this may be considered inadequate, and a form of staged surgical excision might be more appropriate [48, 49].

Surgical excision is not always reasonable and appropriate, especially for large lesions in cosmetically sensitive areas or elderly patients. In these cases, alternative non-surgical therapies, or even a wait-and-see policy may be considered [45].

Non-surgical treatments that are available for LM include laser therapy, topical therapies such as imiquimod, cryotherapy and radiation therapy.

Laser therapy

Both lasers targeting pigment and lasers targeting water have been used in the treatment of LM. When using pigment lasers, the target chromophore is epidermal and appendageal melanin. Melanin absorbs a wide range of wavelengths of 250 – 1200 nm. Therefore several lasers and intense pulsed light sources can effectively treat pigmented lesions. When light of q-switched lasers is absorbed by melanin, it causes a photoacoustic or photomechanical reaction and selectively ruptures the skin melanosomes. The rate of local heating and rapid material expansion can be so severe that structures are torn apart by shock waves, cavitation, or rapid thermal expansion [1]. Infrared lasers, on the other hand, are highly absorbed by water, converting the light energy to heat and vaporizing the tissue (e.g. LM lesion). The first laser that was used in the treatment of LM was the argon laser in 1981 [50]. More recently, the Q-switched ruby, Q-switched Alexandrite, Q-switched Nd:YAG, and ablative lasers such as the carbon dioxide laser have been used for the treatment of LM.

The potential advantages of laser treatment are rapid treatment time, tolerable side effect profile, reduced post-treatment care requirements, wide treatment margins, and good functional and cosmetic outcomes. However, limitations are the lack of histopathological control, the poor complete response rates (ranging between 54.5% to 100%), and recurrence rates up to 100% [51]. Read at al. pooled data of all patients undergoing laser treatments (n=61), and generated a mean recurrence rate of 34.4% (and statistical heterogeneity of $I^2 = 67.9\%$) [51]. These poor response and high recurrence rates may be due to incomplete eradication of malignant cells, primarily in the appendageal structures and possibly at the periphery of the lesion.

Imiquimod

The topical toll-like receptor (TLR) 7 and 8 agonist imiquimod can induce an inflammatory immune response that clears atypical melanocytes. Also, a direct apoptotic effect has been described of melanocytes that were treated with imiquimod [52, 53]. Ten studies

(including 245 patients) investigated imiquimod for the treatment of LM [51]. Initial clinical response rates ranged from 50% to 93%, and the calculated mean recurrence rate was 24.5% (with a heterogeneity of I^2 = 62.6%). Benefits are the excellent functional and cosmetic outcomes, and the use of wide margins. Limitations are the lack of histopathological control, the side effects (e.g. flu-like symptoms), and the long treatment duration.

Cryotherapy

Cryotherapy induces low temperatures that can destroy the epidermis, including the atypical melanocytes (-4°C to -7°C) [54] . Evidence is limited to case series and case reports. Recurrences rates are described from 0 – 40% [55]. Benefits are the ease of use and rapid treatment time. Limitations are the lack of histopathological control, and the side effects that can frequently occur (i.e. hypopigmentation, atrophy, hyperpigmentation).

Radiotherapy

Read et al. systematically reviewed the available evidence for radiotherapy of LM. Ten studies, including 454 patients, demonstrated complete response rates of 87% - 100%, and a mean recurrence rate of 11.5% (with a statistical heterogeneity of I^2 = 72.7%) [51]. Benefits are that radiotherapy is well tolerated, cosmetic results are good, and wide margins can be used. Limitations are the lack of histopathological control, the numerous treatments and subsequent hospital visits and costs, and the (long-term) side effects that can occur when using radiation therapy, including telangiectasia's, pigmentary changes, radiation necrosis, squamous cell carcinoma, and erythema [55].

Combination treatments

Non-surgical treatments (e.g. imiquimod, radiotherapy) are often used as an adjuvant to surgical excision. However, another strategy may be to combine two non-surgical treatments. In this way, the benefits of non-surgical treatment modalities are preserved, and it may result in higher clearance and lower recurrence rates than either treatment alone.

Aiming to find a non-surgical treatment with low recurrence rates and excellent cosmetic and functional outcomes, we have introduced a novel treatment combination of ablative laser therapy followed by topical application of imiquimod 5% cream. This combination treatment resulted in the absence of recurrences in 12 patients after a mean follow-up of 22 months, and with good-to-excellent functional and cosmetic results [56]. However, patient numbers and follow-up time need to be increased to obtain more information about this experimental treatment.

AIMS OF THIS THESIS

The aim of this thesis was to develop and evaluate several optimization strategies for laser therapy in dermatology. Three different strategies were chosen: optimizing an established laser treatment, optimizing laser treatment-related pain in general, and optimizing laser treatment as an alternative for a surgical treatment indication.

The aim of **Chapter 2** was to enhance the standard of care Pulsed Dye Laser (PDL) treatment of port wine stains. Anti-angiogenic agents have been proposed to optimize treatment efficacy. In **Chapter 2.1** we evaluated the efficacy and safety of PDL treatment followed by topical application of sirolimus (with and without laser ablation of the stratum corneum) in a randomized controlled trial. **Chapter 2.2** describes a case of allergic contact dermatitis to sirolimus.

The aim of **Chapter 3** was to optimize laser treatments in general by minimizing pain and discomfort during these treatments. In **Chapter 3.1** we summarized the available data on non-invasive anaesthetic methods for dermatological laser treatments by means of a systematic review. Since we found that there was a lack of high-quality studies, and especially head-to-head studies, we performed two randomized controlled trials. In **Chapter 3.2** we compared the efficacy of two commonly used local anaesthetics, lidocaine/prilocaine cream (EMLA®) versus lidocaine/tetracaine cream (Pliaglis®) in reducing self-reported pain during two painful laser indications: acne keloidalis nuchae and tattoo removal.

The aim of **Chapter 4** was to evaluate the efficacy, safety and feasibility of an innovative non-surgical combination treatment of ablative laser therapy and topical imiquimod for the treatment of lentigo maligna (LM). In **Chapter 4.1** we determined the incidence rates of LM and lentigo maligna melanoma (LMM) in The Netherlands and estimated the risk of progression of LM to LMM. In **Chapter 4.2** we increased the size of our cohort and the follow-up time of LM patients treated with the two-stage treatment of ablative laser therapy followed by topical imiquimod application, and determined the recurrence rates and patient satisfaction. Since we found that recurrences of LM after our non-surgical combination therapy occurred almost exclusively on the nose, we investigated histological parameters that might be related to a higher recurrence risk on this anatomic location in **Chapter 4.3**. In **Chapter 4.4** we described micrographically controlled staged surgical excision for LM, and determined the recurrence rates after this treatment, as a surgical perspective to our non-surgical treatment.

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Chapter 2

Port wine stains

Chapter 2.1

Treatment of port wine stains using Pulsed Dye Laser, Erbium YAG Laser, and topical rapamycin (sirolimus) – a randomized controlled trial

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ABSTRACT

Background and objective

Pulsed Dye Laser (PDL) is currently the gold standard treatment for port wine stains (PWS), although the degree of lesion blanching is variable and often unpredictable. This appears to be due to reformation and reperfusion of blood vessels. Rapamycin has shown potential as an anti-angiogenic agent and may prevent the revascularization after PDL treatment.

The objective of this study was to evaluate the efficacy of adjuvant use of (commercially available) topical rapamycin after PDL treatment in patients with PWS.

Materials and Methods

We conducted a prospective, intra-patient, randomized study. Four treatment areas of 1 cm^2 were created in each PWS. PDL-only treatment was compared to the following three treatments: PDL + rapamycin, PDL + Erbium YAG laser ablation of the stratum corneum + rapamycin, and rapamycin monotherapy. We also compared PDL + Erbium YAG + rapamycin with PDL + rapamycin. The primary endpoint was the percentage clearance assessed colorimetrically at six months follow-up. Secondary outcomes were photographic evaluation by an expert panel, patient satisfaction, treatment-related pain, and safety.

Results

Fourteen patients completed the treatment protocol. The highest percentage clearance was achieved with PDL-only treatment (mean [SD] 16% [34]), but there were no statistically significant differences between treatments. The best photographic evaluation and highest patient satisfaction were also achieved with PDL-only treatment, but only the difference between PDL-only and rapamycin monotherapy was statistically significant. The treatment-related pain was well tolerated. Application-site pruritus was a frequent occurring adverse event. Allergic contact dermatitis to rapamycin occurred in one patient. There were no serious adverse events.

Conclusion

Topical application of the commercially available solution of rapamycin (Rapamune® 0.1%) as an adjuvant to PDL treatment does not appear to improve PWS blanching.

INTRODUCTION

Port wine stains (PWS) are congenital (micro)vascular malformations of the skin. They occur in 0.3%- 0.5% of the population [1], are progressive and do not involute spontaneously. Nodular components and hypertrophy of the underlying soft tissue occur in 65% of the people by the age of 50, resulting in spontaneous bleeding and dysmorphism [2]. Most lesions occur in the head and neck region, causing significant psychological problems [3, 4].

The Pulsed Dye Laser (PDL) is currently the treatment of first choice for PWS. However, the degree of lesion blanching after PDL treatment is variable and unpredictable and complete blanching is achieved in only 0-22% [5-9]. This appears to be (partly) due to reformation and reperfusion of blood vessels, through hypoxia-inducible factor 1-alpha (HIF-1α) and vascular endothelial growth factor (VEGF)-mediated pathways [10, 11].

Sirolimus (also known as rapamycin) is a specific inhibitor of the mammalian target of rapamycin (MTOR) and shows potential as an anti-angiogenic agent [12-14]. PDL combined with oral rapamycin showed promising results in one patient compared to PDL monotherapy [15]. However, systemic use of rapamycin is associated with a wide range of possible adverse effects, and a topical formulation could minimize these potential risks associated with systemic exposure. So far, only one clinical trial has evaluated the efficacy of PDL combined with topical rapamycin in 23 PWS patients with Sturge-Weber syndrome, and it appeared to be an effective treatment [16]. In this study, a formulation of 1% rapamycin cream was fabricated, however, this cream is not commercially available. On the other hand, the commercially available oral solution of rapamycin 0.1% (Rapamune®) used as a topical formulation has not been formally evaluated in the treatment of PWS patients thus far.

When using topical therapeutics, the stratum corneum is the most important barrier for transdermal drug delivery. To optimize topical delivery to the vasculature, the stratum corneum can selectively be removed by ablative lasers and better penetration and distribution of topically applied drugs can be facilitated [17-19].

The aims of this study were to evaluate the efficacy of PDL treatment followed by – commercially available – topical rapamycin (with and without laser ablation of the stratum corneum), and of rapamycin monotherapy in PWS patients.

MATERIALS AND METHODS

Study design and setting

This prospective, intra-patient randomized controlled trial was approved by the medical ethical review board of the Erasmus Medical Center (MEC-2014-022), and registered at

ClinicalTrials.gov (NCT02214706). This was a single center study and all patients were treated at the Erasmus University Medical Centre in Rotterdam, The Netherlands.

Study population

Patients (age ≥18 years) with an extra-facial, homogenous PWS were eligible. Key exclusion criteria included a nodular or hypertrophic component in the treatment area, pregnant or breast-feeding women, and laser treatment of the PWS in the last three months. All patients provided written informed consent before participating in the study.

Interventions

Four treatment areas of 1 cm^2 were created in each PWS to compare the following four interventions:

1. PDL treatment.
2. PDL treatment followed by topical rapamycin application.
3. PDL treatment followed by topical rapamycin application after erbium yttrium-aluminum-garnet (Er:YAG) laser ablation of the stratum corneum.
4. Topical rapamycin monotherapy.

A template (of matte finish metal) with squares of 1 cm^2 was used to create four equal treatment areas in each PWS. All patients received a total of five treatments with two-week intervals. Two tattoo ink points were placed in the PWS at baseline to assure an identical position of the template during each treatment (Fig. 1).

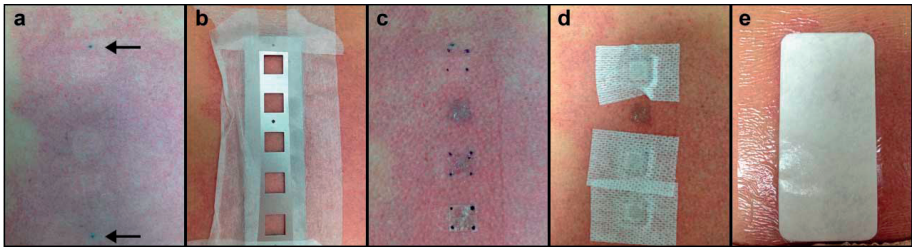

Figure 1. Treatment method: (a) Two tattoo points are used to mark the placement of the template; (b) the template is placed on the PWS and secured with tape; (c) the squares which are randomized to receive rapamycin are marked before the template is removed; (d) rapamycin is placed on the marked squares in 1 cm^2 chambers, (e) the PWS is covered with water-resistant bandages and left *in situ* for seven days.

Laser treatment

Laser treatments were performed with a 585 nm PDL (Cynosure Cynergy, Westford, MA). The following parameters were used: fluences between 7 and 11 J/cm^2, pulse durations of 0.5-2ms, and a spotsize of 7 mm. Five slightly overlapping passes were applied per

treatment square. Standardized air-cooling (Zimmer air cooler) was administered during treatment for thermal protection of the epidermis.

Ablative laser treatments were performed with a 2,940 nm Er:YAG laser (Alma Lasers, Burane XL). A spotsize of 5 mm and fluence of 4 J/cm^2 were used. One or two passes were applied to remove the stratum corneum.

Topical treatment

Rapamycin (Rapamune® 1mg/ml oral solution) was applied topically under occlusion, using 1 cm^2 square chambers (van der Bend Chambers®), with 40 µl Rapamune® per 1 cm^2, and left *in situ* for seven days.

Colorimetric assessment

The colours of the four treatment areas and of the contralateral normal skin were measured with a colorimeter (CR-300, Konica Minolta). The colorimeter was calibrated before each measurement. The colours were expressed by the Commission Internationale de l'Eclairage (CIE) colour measurement system: L* (lightness), a* (values from green to red) and b* (values from blue to yellow).

The color difference (ΔE) between the PWS lesion and contralateral normal skin was determined according to: $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$, where ΔL*, Δa* and Δb* represent the differences in the respectively measured L*, a* and b* values between the PWS lesion and its contralateral site [5, 20, 21].

The blanching effect (i.e. percentage clearance) was then calculated based on the colour differences before and after treatment (at six months follow-up) according to: blanching rate (%) = $1 - (\Delta E_{\text{after treatment}} / \Delta E_{\text{before treatment}}) \times 100$ [20].

Photographic evaluation by an expert panel

Standardized digital photographs were taken by a medical photographer at pre-treatment and at six months follow-up. Blanching of the PWS lesion was assessed by three independent experts for each treatment area. Clearance scores were graded as: five, excellent (>75% clearance); four, good (51-75% clearance); three, fair (25-50% clearance); two, poor (<25% clearance); and one, no clearance.

Randomization

The sequence of the four treatments was randomly assigned to the four squares, using a standard generated randomization list provided by the department of Biostatistics.

Questionnaires

Patient satisfaction was evaluated six months after the last treatment for each of the four treatment areas, using a 0-100 scale.

Pain during and after the laser treatments was assessed with a 0-100 scale for each of the four treatment areas.

Safety

Blood samples were taken regularly to assess safety parameters. The serum concentration of rapamycin was measured approximately 24 hours after the fifth treatment to evaluate potential systemic absorption. This time point was chosen as the most likely moment to measure any possible (cumulative) systemically absorbed rapamycin. The assay used to measure the rapamycin drug concentrations had a lower limit of detection of 0.1 µg/L.

Study outcome

The primary objective was the percentage clearance assessed colorimetrically at six months follow-up (compared to baseline). Four different hypotheses were tested: PDL was compared to the three "experimental" treatments: PDL + rapamycin, PDL + Er:YAG + rapamycin, and rapamycin monotherapy, and to determine the effect of stratum corneum removal PDL + Er:YAG + rapamycin was also compared to PDL + rapamycin.

Secondary outcomes were photographic evaluation by an expert panel, patients satisfaction, pain during and following treatment, adverse effects and systemic rapamycin exposure.

Sample size

A sample size of 20 patients was estimated to provide 80% power to detect an improvement of 20% (SD 22%) in the "experimental" treatments compared to the gold standard treatment, with a two-sided type I error level of 1.25% (Bonferroni correction (α=0.05/4)). A t-test for paired samples was used.

Statistical analysis

Normality of the variables was tested using a Shapiro-Wilk test. At six months follow-up, the differences in therapeutic response (determined colorimetrically, by photographic evaluation, and patient satisfaction) between two treatments were analysed using a t-test for paired samples (if the variables were normally distributed) or a Wilcoxon signed-rank test (if the variables were not normally distributed). Normally distributed data were summarized with means and standard deviations (SD), and not normally distributed data with medians and interquartile ranges (IQR). Agreement between the three independent experts was determined using the intraclass correlation coefficient (ICC two-way mixed model). *P*-values < 0.0125 were considered statistically significant. All analyses were performed using SPSS Statistics 21.

RESULTS

Patients

Between August 2014 and January 2015, a total of 17 patients were enrolled in this study. Demographic details are shown in Table 1. Due to multiple adverse events, the inclusion was terminated at 17 patients.

Three patients did not complete the five treatments and were not included in the (per protocol) analysis (since there was no 6 months follow-up data available for these patients); one patient did not show up again after two treatments, one patient developed mild scarring after one treatment after which participation was terminated, and one patient with a history of diabetes developed a mild wound on her lower extremity (on the Er:YAG treatment site), and withdrew consent. Fourteen patients completed the treatment per protocol and were included in the analysis (Table 1).

Table 1. Baseline Demographic and Clinical Characteristics

Characteristics	Patients (n=17)	Patients (n=14)
Age (years), median (IQR)	29 (23 - 45)	31 (23 - 45)
Female, n (%)	13 (77)	10 (71)
Caucasian, n (%)	17 (100)	14 (100)
Fitzpatrick skin type, n (%)		
II	8 (47)	6 (43)
III	9 (53)	8 (57)
Prior laser treatment, n (%)	3 (18)	2 (14)
Location, n (%)		
Shoulder / upper back	5 (29)	5 (36)
Lower back	2 (12)	2 (14)
Thorax	2 (12)	2 (14)
Upper extremity	3 (18)	2 (14)
Lower extremity	5 (29)	3 (21)

Treatment evaluation

Colorimetric assessment

At six months follow-up, there were no statistically significant differences in percentage clearance between the gold standard PDL treatment and each of the three experimental treatments (Table 2). There was also no statistically significant difference in percentage clearance with or without Er:YAG laser removal of the stratum corneum (mean difference of 3% (95% CI, - 4 to 9; $P = 0.373$).

Table 2. Percentage clearance at six months follow-up, and the difference between PDL and each of the three experimental treatments

Treatment	Percentage clearance; mean (SD)	Mean difference with PDL treatment	*P*-value
PDL	16% (34)	-	
PDL + rapamycin	8% (29)	8% (95% CI, -11 to 27)	$P = 0.21$
PDL + Er:YAG + rapamycin	5% (34)	11% (95% CI, -7 to 28)	$P = 0.381$
Rapamycin monotherapy	-11% (38)[a]	27% (95% CI, 3 to 51)	$P = 0.033$[b]

PDL, Pulsed Dye Laser; Er:YAG, erbium yttrium-aluminum-garnet

[a] In three patients, only the rapamycin monotherapy treatment area still showed erythema at six months follow-up, which could explain the negative percentage. Only in one patient all rapamycin treated sites had negative outcomes because of erythema.

[b] Not statistically significant when corrected for multiple testing.

Photographic evaluation

The agreement between the three independent experts was 0.907 (95% CI, 0.844-0.945). The clearance with PDL treatment was graded with a median score of 1.8 (IQR 1.0-2.9), with PDL + rapamycin with a median of 1.5 (IQR 1.0-2.3), with PDL + Er:YAG + rapamycin with a median of 1.7 (IQR 1.0-2.5), and with rapamycin monotherapy with a median of 1.0 (IQR 1.0-1.3). There was a statistically significant difference between PDL monotherapy and rapamycin monotherapy ($P = 0.007$), but not between the other treatments.

Patient satisfaction

The highest treatment satisfaction was achieved by PDL treatment with a median of 65 (IQR 8-80), followed by PDL + Er:YAG + rapamycin with a median of 60 (IQR 25-75), and PDL + rapamycin with a median of 50 (IQR 13-70), and rapamycin monotherapy with a median of 0 (0-7). There was a statistically significant difference between PDL treatment and rapamycin treatment ($P = 0.007$), but not between the other treatments.

Treatment-related pain

The highest pain scores during treatment were given to PDL + Er:YAG + rapamycin treatment with a median of 24 (IQR 9-41), followed by PDL + rapamycin treatment with a median of 13 (IQR 8-40), and PDL with also a median of 13 (IQR 8-36), and rapamycin monotherapy with a median of 0 (IQR 0-4). Pain scores in the two weeks after treatment were low with a median of 12 (IQR 0-21) for PDL + Er:YAG + rapamycin, a median of 10 (IQR 0-15) for PDL + rapamycin, a median of 5 (IQR 0-9) for PDL, and a median of 1 (IQR 0-10) for rapamycin. However, it should be noted that distinction between the treatment areas was, for some patients, difficult to make.

Safety

Adverse events

A summary of the adverse events is presented in Table 3. The most common adverse event was application-site pruritus (occurred in 12 out of 17 patients). Due to pruritus six patients (in total on 16 occasions) had to remove the bandages with rapamycin before the seven days were completed. One patient developed an itching rash on her entire upper body after the fifth treatment, which was confirmed by patch testing as an allergic contact dermatitis to rapamycin. No serious adverse events occurred.

Table 3. Adverse events

Adverse events	Number
Application-site pruritus	29
Erythema	4
Xerosis cutis	4
Allergic contact dermatitis	1
Allergy to bandages (skin rash)	1
Crustae	1
Pustule	1
Burning sensation	1
Hyperpigmentation	1
Blistering	1
Erosive skin	1
Tinnitus	1
Constipation	1
Eye infection	1
Wound	1
Scarring (mild)	1

Systemic rapamycin exposure

Blood examinations throughout the study did not show any abnormalities. Of the 14 patients who completed the treatment protocol, nine were available 24 hours after the last treatment to determine the serum level of rapamycin. In seven patients, the rapamycin level was below the lower limit of detection, and two patients had detectable levels of 2.1 µg/L, and 2.2 µg/L, respectively.

DISCUSSION

This study shows that the commercially available solution of rapamycin (Rapamune® 0.1% oral solution), used as an adjuvant topical treatment during PDL treatment, does not appear to improve PWS blanching. The addition of ablation of the stratum corneum with the Er:YAG laser to optimize (trans)dermal absorption of rapamycin, did not result in better PWS blanching. Treatment with rapamycin was associated with frequently occurring local skin reactions, which led us to terminate the study before the total number of 20 patients was included.

The greatest difference in efficacy at six months follow-up was observed between PDL and rapamycin monotherapy. This difference is most likely due to the lack of efficacy of rapamycin as monotherapy without PDL pre-treatment and thereby lack of activation of the HIF-1α and VEGF-mediated pathways in PWS. Rapamycin monotherapy could be considered as a negative control in this study. Also, due to erythema on the rapamycin monotherapy treatment area in three patients at six months follow-up (resulting in negative clearance), the difference in clearance with PDL monotherapy was even larger.

The use of topical rapamycin in the treatment of PWS showed promising results in *in vitro* and animal studies [12-14, 22, 23], and also in one clinical trial [16], and one case report [24]. However, the creams used in these clinical studies are not commercially available, and the stability and efficacy of these compounded creams cannot be ensured [25]. The use of the commercially available oral solution of rapamycin (Rapamune® 1mg/ml) used as a topical formulation was thought to be more feasible for everyday clinical practice. However, in our study, we did not find significant improvement with topical rapamycin as an adjuvant to PDL treatment compared to PDL monotherapy. This could be caused by a lack of efficacy of rapamycin in PWS blanching, or by the lack of efficacy of the rapamycin solution used in this study.

Most studies on topical rapamycin for PWS or facial angiofibromas chose to apply topical rapamycin once or twice daily [16, 26-31]. Since the design of our study (multiple adjacent squares with different treatment combinations) made home/self-application impossible, we decided to leave the rapamycin on the test areas for seven days to inhibit neo-angiogenesis, which is most active in the first week after PDL treatment [12]. Theoretically, our study design, where rapamycin is applied only once under occlusion, could have led to a shorter suppression of the mTOR pathway. Consequently, this could be a reason why the rapamycin treatment areas were not positively impacted.

The 0.1% concentration of rapamycin used in this study was lower than the concentration used in other clinical studies (1% [16] and 0.5% [24]). The concentrations used in the *in vitro* and animal studies were also higher, ranging from 0.5 to 2%. We tried to compensate for the lower concentration of the commercially available rapamycin used in our study by removing the stratum corneum, leading to increased absorption of the

compound. Higher concentrations may be more effective, although Jia et al. did not find the reperfusion rate to be linearly proportional to the rapamycin concentration in animals [22]. Also, higher concentrations could potentially induce more side effects.

The solution we used in this study caused frequent local skin reactions (i.e. pruritus, skin irritation), which may have prevented effective treatment since rapamycin often had to be removed before the seven days were completed. These side effects could be caused either by rapamycin itself (confirmed in one of our patients) or by ingredients of the rapamycin solution (e.g. ethanol). Skin irritation after topical use of the commercially available solution of rapamycin twice daily has been described before, but when it was used once daily it was tolerated [26, 28]. However, since we applied rapamycin under occlusion for seven days, this may have decreased skin tolerability. Occlusion by the chamber itself tapped to the skin for seven days could also have caused, or contributed to the pruritus complaints.

In two patients a detectable level of systemically absorbed rapamycin was measured. The levels were low (2.1 µg/L and 2.2 µg/L) and did not result in any adverse effects. Systemic absorption of topical rapamycin has been described before [16].

Faurschou et al. found that PDL treatment resulted in more than 25% reduction in redness in 50-100% of participants [32]. We found an average blanching rate of 16% with PDL monotherapy. This difference may be explained by the fact that we only included extra-facial PWS. Nguyen et al. found anatomic location to be the most important predictor of treatment response [33]. The PWS located over bony areas of the face (e.g., forehead, peripheral face) showed the most successful response, where central facial lesions were more resistant [33, 34]. However, extremities, and especially the lower limb showed the lowest clearance rates [35, 36].

Strengths of this study are the intra-patient comparison, the randomization of treatment location, and the use of a solution of rapamycin that is available for everyday clinical practice. Limitations are the early termination of patient inclusion, the exclusion of facial lesions, and the frequent occurrence of adverse effects preventing optimal treatment evaluation. Also, the potential use of rapamycin under occlusion on larger areas would be challenging for some anatomic locations.

In summary, our results did not demonstrate adjuvant topical rapamycin after PDL treatment to improve PWS blanching. There may be a lack of efficacy of rapamycin in PWS blanching, or the commercially available solution of rapamycin (Rapamune® 0.1%) may not be optimal for topical use, due to frequent local skin reactions, and the relatively low concentration. Future studies should focus on determining the efficacy of rapamycin in PWS blanching. Oral rapamycin has shown promising results in one patient [15], and should be further investigated regarding efficacy, adverse effects (during short-term use), and cost effectiveness. Also, the risk of allergic contact dermatitis to topical rapamycin should be considered.

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Chapter 2.2

Allergic contact dermatitis caused by topical sirolimus used as an adjuvant for laser treatment of port wine stains

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Sirolimus (rapamycin) is a specific inhibitor of mammalian target of rapamycin, and, because of its anti-angiogenic properties, its use as an adjuvant for the laser treatment of port wine stains (PWS) has recently been suggested [1]. We report a case of allergic contact dermatitis caused by sirolimus (Rapamune® 0.1% oral solution) which was used as an adjuvant topical treatment during laser treatment of a PWS.

CASE REPORT

A 32-year-old healthy woman participated in our clinical trial in which a PWS on her left flank was treated with laser therapy, directly followed by topical application of sirolimus on three separate areas of 1cm^2 (small areas were chosen in order to allow comparison of different experimental treatment combinations in one PWS, before treating the whole PWS with the 'best' treatment). Sirolimus was applied topically under occlusion on the treated PWS and left *in situ* for seven days. This treatment was repeated five times, with 2-week intervals. After the second and third treatments, the patient developed mild dermatitis (erythema) at the sirolimus application sites, which forced her to remove the occlusive bandage after five days. The skin irritation cleared spontaneously within two weeks. The treatment was repeated two more times according to the study protocol, but, because of increased itching and dermatitis, the occlusive bandages had to be removed after two days on both occasions. After the last treatment, the patient developed an itching rash on her entire upper body, in addition to the erythematous skin reaction at the application site, which slowly resolved within two weeks after daily applications of desoximetasone cream.

To evaluate the possibility of allergic contact dermatitis, patch tests were performed with the European baseline and additional series supplemented with topical Rapamune® (1mg sirolimus/ml) 'as is', 1:3 in olive oil, and 1:3 aq. Patch tests were conducted with van der Bend Chambers® on the patient's back, and read on day (D)2 and D3, according to *ICDRG* criteria [2]. Readings showed extreme positive reactions to Rapamune®, and some positive reactions to common allergens, which were deemed to be of no relevance to this case. The positive results are summarized in Table 1.

Table 1. Patch test results

Allergens	D2	D3
Rapamune® pure	++	+++
Rapamune® 1:3 olive oil	+	++
Rapamune® 1:3 aq.	+	+
p-Phenylenediamine	++	++
Caine mix III	++	+
Quaternium 15	-	+
Formaldehyde	++	++
Methyldibromo glutaronitrile	+	+
Carba mix	-	+
1,2-benzisothiazolin-3-one	-	+

Sirolimus is a macrolide immunosuppressant, and, to evaluate the possibility of cross-reactivity with, and subsequent allergy to macrolide antibiotics [3], further patch tests were performed five months later with azithromycin, clarithromycin, and erythromycin. To evaluate the possibility of an allergy not to sirolimus itself but to constituents of the solution, propylene glycol (5% pet.) and polysorbate (5% pet.) were also tested. The other ingredients of Rapamune® solution (i.e. phosphatidylcholine, monodiglycerides, ethanol, soya fatty acids, and ascobyl palmitate) were not available for patch testing but were considered unlikely allergens. All additional patch tests, again read D2 and D3, showed no reactions.

We did not perform patch tests with Rapamune® in control patients; however, in our clinical trial, 16 other PWS patients had been treated with topical Rapamune® under occlusion (in van der Bend Chambers®, to match the 1cm^2 treatment areas exactly); five of these developed irritant skin reactions, but no allergic reactions.

DISCUSSION

We conclude that our patient most likely experienced an allergic reaction to topically applied sirolimus. Although there are some reports of skin irritation caused by topical sirolimus [4, 5], to our knowledge this is the first report of a true allergic contact dermatitis.

As there is increasing interest in the use of topical sirolimus in the treatment of PWS, and also of angiofibromas [5], attention should be drawn to the risk of allergic contact dermatitis.

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Chapter 3

Pain management

Chapter 3.1

Non-invasive anaesthetic methods for dermatological laser procedures - a systematic review

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ABSTRACT

Pain is a common side-effect of dermatological laser procedures. Non-invasive anaesthetic drugs and anaesthetic procedures can be used to provide pain relief and increase patient satisfaction and treatment efficacy. However, it remains unclear which method provides the best pain relief. The objective of this systematic review was therefore to assess the efficacy and safety of non-invasive anaesthetic methods during dermatological laser procedures. A systematic literature search was conducted. Randomized and non-randomized controlled clinical trials (RCTs and CCTs) were included. Two authors independently assessed study eligibility, extracted data and assessed the risk of bias. The quality of evidence was rated using Grading of Recommendations Assessment, Development and Evaluation (GRADE). Twenty RCTs and 12 CCTs were included, involving nine different laser indications: hair removal (n = 9), resurfacing/rejuvenation (n = 5), port wine stains (n = 8), leg telangiectasia (n = 3), facial telangiectasia (n = 2), tattoo removal (n = 2), nevus of Ota (n = 1), solar lentigines (n = 1), and human papilloma virus lesions (n = 1). The non-invasive anaesthetic methods (i.e. topical anaesthetic drugs, skin cooling, and pneumatic skin flattening [PSF]), types of lasers, laser settings, application time and types of pain scales varied widely among the included studies. All of the studies had an unclear or high risk of bias and the overall quality of evidence was rated as low. In general, active non-invasive anaesthetic methods seemed to provide favourable results compared to placebo or no anaesthesia, and topical anaesthetic drugs and PSF seemed to result in a better pain reduction than skin cooling. However, the current evidence is insufficient to provide recommendations for daily clinical practice. There is a need for more high quality (head-to-head) RCTs. Future studies should also evaluate sex differences in pain perception, have uniformity with regard to validated pain measurement scales, and address clinically significant differences in pain reduction besides statistically significant differences.

INTRODUCTION

Pain is a bothersome side effect during dermatological laser procedures and can decrease patient satisfaction and also treatment efficacy as pain sometimes makes it necessary to use lower laser settings.

Local anaesthetics can be used to reduce treatment-related discomfort and pain. However, invasive anaesthetics are often painful to administer and are less suitable for needle phobic patients. Non-invasive anaesthetic drugs and anaesthetic procedures on the other hand, are painless to administer, and, in case of non-invasive drugs, decrease the risk of systemic exposure.

Non-invasive methods for pain reduction during dermatological laser procedures include topical anaesthetic drugs, skin cooling, and pneumatic skin flattening (PSF), which all have different modes of action.

Topical anaesthetic drugs block the influx of sodium and prevent nerve cell depolarization and the sensory input to the central nervous system [1]. The first topical anaesthetic that was discovered was cocaine [2]. Later, ester anaesthetics (e.g. tetracaine) were developed, but allergic contact dermatitis was a frequently occurring adverse effect [3]. Subsequently, amide anaesthetics (e.g. lidocaine, prilocaine) were created and became the preferred anaesthetic. Non-steroidal anti-inflammatory drugs (NSAIDs) can also be used as local anaesthetic drugs. Their mode of action is based on the inhibition of the cyclo-oxygenase enzymes (COX-2) that synthesize prostaglandins [4]. Today, various prescription and over-the-counter topical anaesthetic drugs and combinations are available.

Skin cooling can lower the skin temperature during laser procedures, and thereby improve pain and discomfort. Furthermore, skin cooling is often used to protect the epidermis from thermal injury as melanin in the epidermis is an unwanted site for absorption of light, with the exception of epidermal pigmented lesions [5]. Different skin cooling methods are available, including cold gels, contact cooling, dynamic cooling (using cryogen spray), and cold air. Cooling can be delivered pre-, during-, and post-treatment.

Pneumatic skin flattening utilizes a vacuum chamber covered by a transparent sapphire window through which the laser light can be delivered [6]. When the handpiece is placed on the skin, negative pressure is automatically generated. The high vacuum activates enough pressure receptors in the skin to reduce pain through afferent inhibition of pain transmission to the dorsal horn (gate control theory of pain) [7, 8]. The vacuum is released at the end of the light pulse.

Which non-invasive method of anaesthesia provides the best pain reduction during different dermatological laser procedures has never been systematically investigated. This information could be used to facilitate decision making in daily clinical practice.

The aim of this systematic review was therefore to provide an overview of the efficacy and safety of all non-invasive methods for skin anaesthesia during dermatological laser procedures.

METHODS

Types of study

Both randomized controlled trials (RCTs) and non-randomized controlled clinical trials (CCT's) were included. Language was restricted to English or Dutch. No restrictions were made as to publication date.

Types of participants

Patients of all ages undergoing laser or intense pulsed light (IPL) procedures for dermatological indications were included. Patients undergoing endovenous laser treatment or photodynamic therapy were excluded.

Types of interventions

All non-invasive anaesthetic methods were included. Control interventions could be any other non-invasive anaesthetic method, placebo, or no treatment.

Types of outcome measures

The primary outcome was patient self-reported pain, measured on any pain scale. The secondary outcome was adverse events.

Search strategy

We searched the following databases from inception until 15 February 2016: EMBASE, MEDLINE, the Cochrane Central Register of Controlled Trials (CENTRAL), Web of science and PubMed (Supplementary Table 1). References of the included articles were screened to identify additional relevant studies.

Study selection

The preliminary selection was done by two independent investigators (KG and LL) based on titles and abstracts. Subsequently, a second selection was made based on the full text. This selection was done by two independent investigators as well (KG and LL) and based on predefined inclusion and exclusion criteria. Any disagreements were resolved by discussion.

Data extraction and quality assessment

Two authors (KG and LL) independently extracted data from the included studies, using a predefined data extraction form. The following data was extracted: authors, year of publication, study design, sample size, male/female ratio, intervention, comparative control, type of laser, laser settings, type of pain rating scale, outcomes, adverse effects, conflicts of interest and sponsors. In case of unclear information in the included studies, authors were approached for clarification.

Methodological quality of the articles was rated by two independent authors (KG and LL) using the Cochrane Collaboration's tool for assessing risk of bias [9]. Selection bias, performance bias, detection bias, attrition bias, and reporting bias were evaluated. Disagreements were resolved, and consensus was reached. The overall quality of evidence was rated using Grading of Recommendations Assessment, Development and Evaluation (GRADE) [10].

The results of our systematic review were reported according to the PRISMA guidelines for reporting systematic reviews [11].

RESULTS

Study characteristics

The database search yielded 4185 articles, of which 2635 remained after removing duplicates. Titles and abstracts of these studies were screened, and 2535 references were excluded. We obtained full text of the remaining 100 studies and included 32 studies based on our inclusion and exclusion criteria (figure 1). No additional articles were included after the reference search.

Twenty RCTs and 12 CCTs, published between 1990 and 2012 and comprising 983 participants, were included in this review.

The included trials investigated the following dermatological laser indications: hair removal, resurfacing/rejuvenation, port wine stains, leg telangiectasia, facial telangiectasia, tattoo removal, nevus of Ota, solar lentigines, and human papilloma virus (HPV) related lesions.

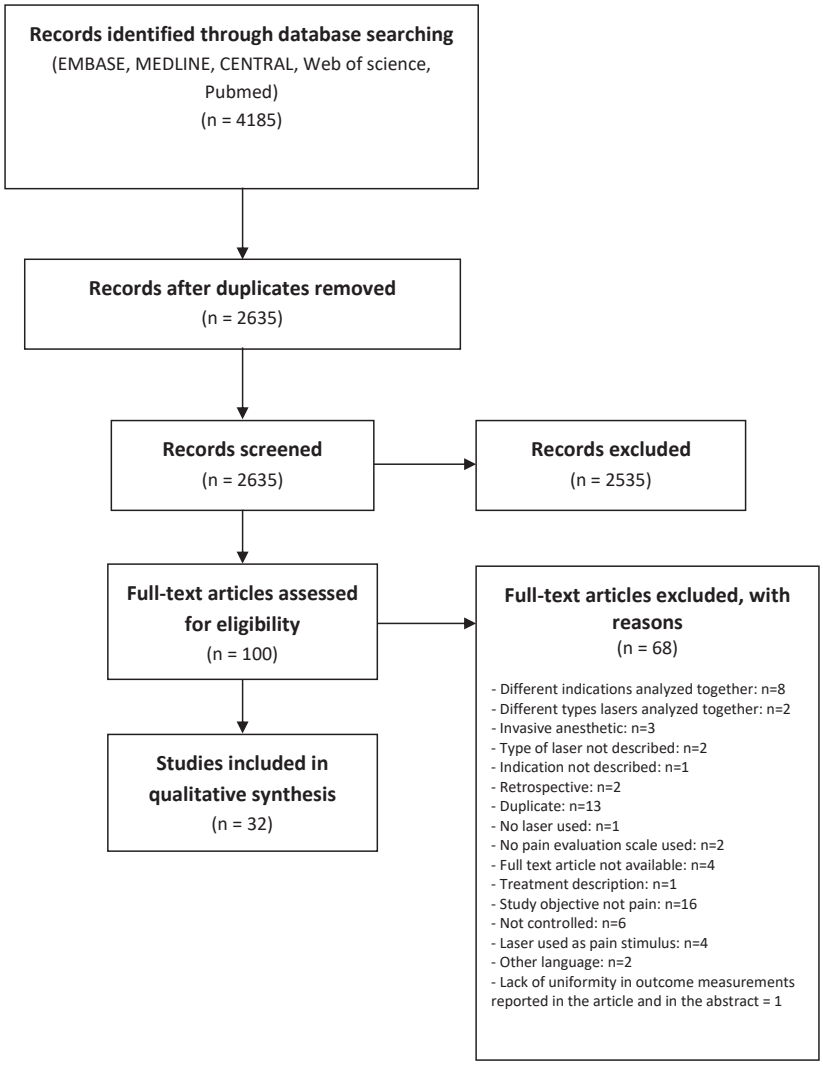

Figure 1. Flow chart of our search strategy and study selection

Hair removal

Nine studies, including a total of 258 patients, reported on non-invasive anaesthetic methods during laser-assisted hair removal. Study characteristics and results are shown in Table 1.

Table 1. Hair removal – characteristics of included studies and summary of results

Author	Study type	N	% male	Intervention	Control	Laser (type)	Fluence (J/cm2)	Pulse duration (ms)	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Akinturk, 2007[12]	RCT	50	0	Piroxicam gel 0.5% *45 minutes under occlusion	Saline (placebo) *45 minutes under occlusion	Nd:YAG 1064 nm	Mean (SD) Piroxicam: 44 (7) Placebo: 43 (8)	Mean (SD) Piroxicam: 56 (8) Placebo: 55 (7)	VAS (0-10)	Piroxicam gel: 2 (2) Saline (placebo): 7 (2) *$P < 0.001$	Erythema, edema, and folliculitis were more frequent in the placebo group ($P < 0.001$)	Not reported
Akinturk, 2009[16]	RCT	50	0	Piroxicam gel 0.5% *60 minutes under occlusion	EMLA cream *60 minutes under occlusion	Nd:YAG 1064 nm	Mean (SD) EMLA: 42 (7) Piroxicam: 43 (7)	Mean (SD) EMLA: 53 (8) Piroxicam: 55 (9)	VAS (0-10)	Piroxicam gel: 2 (2) EMLA cream: 2 (1) *no statistically significant difference	Blanching, erythema, and inflammatory side effects (e.g. edema, folliculitis) occurred more often in the EMLA group ($P < 0.001$)	Not reported
Bernstein, 2007[7]	RCT, split lesion	10	0	Pneumatic Skin Flattening	Dynamic skin cooling (Cryogen)	Alexandrite 755 nm	14 - 26	3	McGill Pain Questionnaire (10-point scale)	PSF: 1.5 (range 0-5) Cryogen spray: 5.7 (range 3-8) *$P < 0.001$	None reported	Dr. Bernstein is chairman of the scientific advisory board for Candela Corporation
Eremia, 2000 [13]	RCT, split lesion	20	0	5% lidocaine cream *60 minutes, + skin cooling	No treatment *+ skin cooling	-Alexandrite 755 nm 8 mm spotsize -Alexandrite 755 nm 12 mm spotsize -Diode 800 nm 9 mm spotsize	Maximum of 40	Alexandrite: 3 Diode: half of the fluence	Not specified	Without cream vs with cream: - Alexandrite 8mm: 4.5 vs. 2.47 - Alexandrite 12 mm: 5.17 vs. 3.42 - Diode: 5.64 vs. 3.95 *No *P*-values reported	None reported	No significant interest with commercial supporters Coherent Inc provided the diode laser system and Candela Inc the cryogen spray
Guardiano, 2005[17]	RCT, split lesion	64	63	EMLA cream * 30 minutes *no occlusion, + skin cooling	Topical lidocaine 5% *30 minutes, + skin cooling	Nd:YAG 1064 nm	30-50	20-30	VAS (0-100)	EMLA: 34.53 (23.264) LMX: 35.73 (23.783) * $P = 0.647$	None reported	No significant interest with commercial supporters

Table 1. Hair removal – characteristics of included studies and summary of results (continued)

Author	Study type	N	% male	Intervention	Control	Laser (type)	Fluence (J/cm2)	Pulse duration (ms)	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Ke, 2007[14]	RCT, split lesion	10	?	Pneumatic Skin Flattening	No treatment	Diode laser 800 nm	25-30	12.5-15	1-10 scale	With PSF: 2.1 Without PSF: 3.7 *No *P*-values were reported	None reported	Not reported
Nahm, 2002[15]	CCT, split lesion	10	50	Dynamic cooling device (0, 20, 40, 60, 80, 100 ms cryogen spurt durations)	No treatment	Alexandrite 755 nm	15-35	3	0-10 scale	Lower mean pain scores with skin cooling, and an overall decrease in pain with increased spurt durations *No mean pain scores or *P*-values reported.	Transient hyperpigmentation 1/10 with skin cooling, and 6/10 without skin cooling	Not reported
Rashidi, 2012[18]	CCT, split lesion	32	0	Cooling device	1.EMLA cream 2.Diclofenac gel * both 60 minutes, under occlusion	Alexandrite 755 nm	unknown	unknown	Numeric Rating Scale (1-10)	Cooling device: 10 Diclofenac gel: 8.09 EMLA: 5.46 *Cooling vs topical anaesthetics $P = 0.01$ * EMLA vs diclofenac $P = 0.01$	None reported	None declared

Overall, topical anaesthetic drugs, PSF and skin cooling resulted in a better pain reduction than placebo or no anaesthesia [12-15]. There was no difference in efficacy found between EMLA (lidocaine 2.5% / prilocaine 2.5%) cream and piroxicam gel [16], or between EMLA cream and lidocaine 5% cream [17]. One CCT showed better results with EMLA cream than diclofenac gel, while both were superior to skin cooling [18]. Pneumatic skin flattening was compared to skin cooling and resulted in a better pain reduction [7, 19].

Resurfacing / rejuvenation

Five studies, including a total of 78 patients reported on non-invasive anaesthetic methods during laser resurfacing or rejuvenation. Study characteristics and results are shown in Table 2. Pain was better reduced with topical anaesthetic drugs or skin cooling, than with placebo or no anaesthesia [20, 21]. S-Caine peel (lidocaine 7% / tetracaine 7%, alternative brand name: Pliaglis®) provided better pain reduction than EMLA [22], and pain was better reduced when the stratum corneum was ablated before lidocaine 5% application [23]. One CCT compared PSF to skin cooling, and, like for hair removal, showed a better pain reduction with PSF [24].

Table 2. Resurfacing/rejuvenation – characteristics of included studies and summary of results

Author	Study type	N	% male	Intervention	Control	Laser (type)	Flucence	Pulse duration	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Alster, 2002[22]	RCT, split lesion	20	15	S-Caine peel	EMLA cream *under occlusion	1 pass CO_2 10600 nm	Pulse energy of 300 mJ, Pulse power of 60 W	950 µsec	VAS (0-10)	S-Caine: 2.66 EMLA: 5.25 *No *P*-values were reported	S-Caine peel: mild erythema (75%), skin blanching (15%); EMLA: skin blanching (90%), erythema and edema (10%)	Authors indicated no significant interest with commercial supporters. Zars Corporation provided the anaesthetic materials
Doshi, 2003[20]	RCT, split lesion	20	5	S-Caine peel *+ skin cooling	Placebo cream + skin cooling	Non-ablative 1450 nm diode laser	10-14 J	250 ms	VAS (0-100)	S-Caine peel: 15 Placebo: 47 *$P < 0.001$	S-Caine peel: mild erythema, transient moderate erythema (n=1) Placebo: -	Zars inc. supported this study
Kono, 2010[24]	CCT, split lesion	11	?	Pneumatic Skin Flattening	Dynamic skin cooling (Cryogen)	Nd:YAG 1064 nm	30 J/cm2	50/cm2 ms	VAS (0-10)	PSF: 2.4 (0.5) CSC: 6.9 (1.2) *$P < 0.01$	PSF: mild ecchymosis (n=2) Cooling: -	No conflicts of interest
Tierney, 2012[21]	CCT, split lesion	15	27	Cold-air anaesthesia *+ bupivacaine / lidocaine/tetracaine topical mix for 30 min	No treatment *+ bupivacaine / lidocaine/tetracaine topical mix for 30 min	Fractionated CO_2 laser 10600 nm	30 W	500 to 1000 µsec	0-10 pain scale	No cold-air: 7.47 Cold-air: 4.27 *$P < 0.01$	The intensity and duration of post treatment erythema, edema and crusting was lower on the side treated with cold-air	Funding sources: None Conflicts of interest: None declared
Yun, 2002[23]	RCT, split lesion	12	0	Er:YAG laser ablation of stratum corneum + lidocaine 5% (under occlusion)	Lidocaine 5% (under occlusion)	Er:YAG 2940 nm	Stratum corneum: 1.3 J/cm2, Full face resurfacing: 20-25 J/cm2	350 µsec	VAS (0-10)	First pass: Laser + lido 5%: 2.86 (0.37) Lidocaine 5%: 3.85 (0.46) *$P < 0.0123$ Second pass: Laser + lido 5%: 4.77 (0.43) Lidocaine 5%: 6.85 (0.67) *$P < 0.0001$	None reported	Funding sources: None. Conflicts of interest: None identified

Port Wine Stains

Eight studies, including a total of 235 patients, reported on non-invasive anaesthetic methods during laser port wine stain treatment. Study characteristics and results are shown in Table 3. Overall, topical anaesthetic drugs and skin cooling provided a better pain reduction than placebo or no anaesthesia [25-31]. There was no difference in efficacy between iontophoresis of lidocaine 4% with or without epinephrine [27]. However, iontophoresis of lidocaine 5% with epinephrine was more effective than iontophoresis of mepivacaine 2% with epinephrine [32], and amethocaine 4% was found to be more effective than EMLA cream [29].

Table 3. Port Wine Stains – characteristics of included studies and summary of results

Author	Study type	N	% male	Intervention	Control	Laser (type)	Fluence (J/cm2)	Pulse duration	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Fiskerstrand, 1996[25]	CCT, split lesion	12	?	Dynamic cooling (cryogen spurts)	No treatment	PDL 585 nm	6-10	45 ms	Numeric pain intensity scale (0-10)	Median (IQR): Uncooled area: 4 (3.3-5) Cooled areas: 2 (1.3-3) *$P = 0.01$	Cooled areas: onset of erythema delayed, duration of purpura shorter; No differences in degree of onset of edema or occurrence of hyper-pigmentation in the cooled and uncooled test sites	Not reported
Greve, 2001[26]	CCT, split lesion	13	31	Cold Air Cooling	No treatment	PDL 585 nm	6	450 µsec	Unchanged / stronger / weaker	9 patients (69%) stated that the analgesic effect was stronger with cooling, and 3 patients (23%) indicated no difference. *No *P*-values reported	Less erythema and edema when patients were treated with cold air	No significant interest with commercial supporters indicated
Kennard, 1992[27]	RCT, split lesion	11	45	- Ionthophoresis of lidocaine HCL 4% - Iontophoresis of lidocaine HCL 4% with epinephrine 1:50.000	-Iontophoresis of 0.9% NaCL (placebo) - No treatment	PDL 585 nm	6-7	450 µsec	VAS (0-10)	No mean VAS scores were reported *Lower pain scores with interventions than controls: $P < 0.001$ *With vs without epinephrine: $P = 0.0659$ * Placebo vs no treatment: $P = 0.5082$	None reported	Neither author has financial interest in ZOMED, Znc., or Perimed, Znc.

Table 3. Port Wine Stains – characteristics of included studies and summary of results (continued)

Author	Study type	N	% male	Intervention	Control	Laser (type)	Fluence (J/cm2)	Pulse duration	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Mallory, 1993[28]	CCT, split lesion	14	14	Topical lidocaine 25% in 70% dimethyl sulfoxide (DMSO)-ethanol	No treatment	PDL 585 nm	6 - 6.75	450 µsec	VAS 0-10	13/14 patients had some degree of anaesthesia with lidocaine, with a mean improvement of 51.4% compared to no anaesthesia. *No mean VAS scores were reported *No *P*-values reported	None reported	Not reported
McCafferty, 1997[29]	RCT, split lesion	29	?	- EMLA - 4% amethocaine gel *Both under occlusion	Placebo *Under occlusion	PDL 585 nm	6.25-6.5	450 µsec	VAS (0-10)	No mean VAS scores reported *Both interventions were superior to placebo ($P < 0.001$) *Amethocaine gel was superior to EMLA ($P < 0.005$)	None reported	Not reported
Núñez, 1997[32]	RCT	36	36	- Iontophoresis of lidocaine 5% with epinephrine 1:50,000	- Mepivacaine 2% with epinephrine 1:50,000 - 0.9% NaCL (placebo)	PDL 585 nm	7	450 µsec	VAS (0-10)	Lidocaine: 3.926 Mepivacaine: 6.224 Placebo: 7.650 *Lidocaine vs mepivacaine $P < 0.0001$	The sites receiving iontophoresis with epinephrine showed minimal degrees of blanching	Not reported
Tan, 1992[30]	RCT, split lesion	73	48	EMLA *Under occlusion	- Placebo - No cream *Under occlusion	PDL 577 nm	unknown	360 µsec	VAS 100 mm	EMLA: 10.9 Placebo: 32.1 Untreated: 38.6 *EMLA vs placebo or untreated: $P < 0.0001$	No adverse events reported	ASTRA pharmaceuticals supported this study
Waldorf, 1997[31]	CCT, split lesion	47	?	Dynamic cooling device (cryogen spurts)	No treatment	PDL 585 nm	unknown	450 µsec	0 (none); 1 (slight); 2 (moderate); 3 (severe).	Mean change in pain rating: -1.02 (0.92) *Lower pain scores with cooling ($P < 0.05$) *Means per group not reported	Post treatment purpura were equal for both sites; If pigmentation changes were more or less likely with cooling could not be indicated	Not reported

Leg telangiectasia

Three studies (total of 178 patients) investigated non-invasive anaesthetic methods during laser treatment of leg telangiectasia. Study characteristics and results are shown in Table 4. Lower mean pain scores were reported for sides treated with cryogen spray than for sides treated with second skin moist gel pad and no cooling [33]. S-caine peel (lidocaine 7% / tetracaine 7%) was found to be superior to placebo after 30, 60, and 90 minutes application [34, 35].

Table 4. Leg telangiectasia – characteristics of included studies and summary of results

Author	Study type	N	% male	Intervention	Control	Laser (type)	Fluence	Pulse duration	Pain rating scale	Results; mean (SD)	Adverse events	Conflict of interest / Sponsor
Buscher, 2000[33]	CCT, split lesion	18	0	Dynamic Cooling Device (cryogen spray)	No cooling *+ Second Skin Moist Gel Pad	PDL 595 nm	20 J/cm2, and 24 J/cm2	1.5 ms	Scale of 1 to 3: 0 (none), 1 (slight), 2 (moderate), and 3 (severe)	With cooling: 1.36 Without cooling: 1.8 *P-values were not reported	- Incidence of blistering: 16.7% for skin cooled sites versus 12.2% for the non-cooled sites - Hyperpigmentation occurred slightly more often on the sites treated with skin cooling	Not reported
Chen, 2003[34]	2 RCTs, split lesion	Study 1: 60 Study 2: 40	?	S-Caine peel *study 1: 30 or 60 minutes *study 2: 60 or 90 minutes	Placebo	Nd:YAG 1064 nm	140 to 400 J/cm2	30 to 100 ms	VAS (0-100)	Study 1: pain scores lower for S-Caine peel sites *$P = 0.046$ * mean VAS scores not reported Study 2: S-Caine peel vs placebo 60 min: 25.2 (20.6) vs 48.0 (28.1) *$P = 0.01$ 90 min: 15.2 (17.7) vs 42.5 (20.2) * $P = 0.001$	No differences between sites in occurrence of erythema, blanching or edema	Zars inc. sponsored this study and supplied materials used in this study
Jih, 2004[35]	RCT, split lesion	60	0	S-Caine peel	Placebo	Nd:YAG 1064 nm	Center 1: 250-370 J/cm2 Center 2: 126 J/cm2	Center 1: 45-60 ms Center 2: 8 ms	VAS (0-100)	S-Caine peel: 26.9 mm (range 0-86) Placebo: 42.7 mm (range 3-97) *$P < 0.001$	No differences between sites in occurrence of erythema, blanching or edema.	Not reported

Facial telangiectasia

Two studies (total of 36 patients) investigated non-invasive anaesthetic methods during laser treatment of facial telangiectasia. Study characteristics and results are shown in Table 5.

Table 5. Facial telangiectasia – characteristics of included studies and summary of results

Author	Hammes, 2005[36]	Kauvar, 2002[37]
Study type	CCT, split lesion	RCT, split lesion
N	17	19
% male	12	?
Intervention	- Cold air cooling to 20 degrees - Cold air cooling to 17 degrees	Water cooled handpiece
Control	No Cooling	Water cooled handpiece + 2mm film of aqueous gel (Surgilube gel)
Laser (type)	PDL 585 nm	KTP 532 nm
Fluence	3.5 J/cm2	9.5 J/cm2
Pulse duration	0.45 milliseconds	10 ms
Pain rating scale	Numerical analog scale (NAS) from 0 (meaning no) to 3 (meaning high)	0=none; 1=mild; 2=moderate; 3=severe
Results; mean (SD)	Without cooling: 2.41 Cooling to 20 degrees: 1.06 Cooling to 17 degrees: 0.76 *No *P*-values reported	Without aqueous gel: 5% mild pain, 68% moderate pain, and 26% severe pain; With aqueous gel 16% no pain, 68% mild pain, 16% moderate pain, and 0% severe pain *No mean pain scores were reported *Statistical analyses were not done
Adverse events	Cooling reduced purpura compared to no cooling	Use of the aqueous gel decreased the incidence and severity of erythema, edema, and crusting.
Conflict of interest / Sponsor	Not reported	Not reported

Anaesthesia with cold air to 20 degrees was compared to 17 degrees and to no cooling [36]. The lowest mean pain scores were measured with cooling to 17 degrees, then cooling to 20 degrees, and the highest mean pain scores without cooling. It was stated that patients preferred cooling to 20 degrees. Skin cooling with a water cooled handpiece chilled to 4 degrees Celsius was compared to skin cooling with the water cooled handpiece combined with a 2 mm film of aqueous gel (Surgilube gel) [37]. Lower pain scores were found when skin cooling was combined with the aqueous gel.

Tattoo removal

Two studies (total of 41 patients) investigated non-invasive anaesthetic methods during laser tattoo removal. Study characteristics and results are shown in Table 6. The topical anaesthetic drug S-Caine peel (lidocaine 7% / tetracaine 7%) and PSF reduced pain better than placebo or no anaesthesia [38, 39].

Table 6. Tattoo removal – characteristics of included studies and summary of results

Author	Chen, 2005[38]	Lapidoth, 2007[39]
Study type	RCT, split lesion	CCT, split lesion
N	30	11
% male	27	18
Intervention	S-Caine peel	Pneumatic Skin Flattening
Control	Placebo	No treatment
Laser (type)	Q-switched Nd:YAG 1064 nm	Q-switched Nd:YAG 1064 nm
Fluence	4-5 J/cm2	*Medlite: 3-5 J/cm2 * Quantum: 4.2 J/cm2,
Pulse duration	Unknown	Unknown
Pain rating scale	VAS (0-100)	Modified McGill pain questionnaire: five level scale
Results; mean (SD)	S-caine peel: 42.5 Placebo: 66.3 *P = 0.001	Without PSF: 4.09 With PSF: 2.36 *Z = 0.003
Adverse events	No difference between treatment sites	None reported
Conflict of interest / Sponsor	Material for this study was provided by Zars inc.	Not reported

Nevus of Ota

One CCT (37 patients, ten male) investigated non-invasive anaesthesia during Q-switched Alexandrite 755 nm laser treatment of nevus of Ota [40]. To measure pain, a 10-cm horizontal line was used. The study was supported by a research grant given by the Hong Kong Research Grant Council. Skin cooling (Cool sapphire plate [DermaCool]) was compared to no cooling in a split-lesion design. On both sides, EMLA cream (under occlusion) was applied to the lesion 2 hours before treatment. Skin cooling was superior to no cooling, with mean pain scores of 2.97, and 3.91 respectively (P = 0.001). No adverse effects were reported.

Solar lentigines

One CCT (20 patients, 0 male) investigated non-invasive anaesthesia during IPL treatment of solar lentigines [41]. To measure pain, a 10-level scale modified McGill Pain

Questionnaire was used. Conflict of interest or sponsors were not reported. Lower pain scores were achieved with PSF than without PSF, with mean pain scores of 0.82 and 3.76 respectively. No *P*-values were reported. Erythema and edema occurred more often without PSF than with PSF. No other adverse events occurred.

HPV lesions

One RCT (80 patients, 0 male) investigated non-invasive anaesthesia during CO_2 10600 nm laser treatment of HPV lesions [42]. A VAS scale (100 mm) was used to measure pain. Conflict of interest or sponsors were not reported. EMLA (lidocaine 2.5% / prilocaine 2.5%) ($n = 60$) was compared to placebo ($n = 20$), after an application time of 1-75 minutes. Pain scores were lower when EMLA cream was used, with median (IQR) pain scores of 12 (0-96) with EMLA and 80 (34-100) with placebo ($P < 0.01$)[42]. Erythema was reported more often in the EMLA group.

Quality of the studies

We assessed the studies for risk of bias. All studies had a high or unclear risk of bias (Supplementary Table 2). Most trials, except two for hair removal [12, 16], one for PWS [32], and one for HPV lesions [42] used intra-patient comparisons.

Effects of interventions

A summary of the primary outcome and the evidence quality assessed using GRADE is presented in Table 7. The overall quality of evidence was rated as low.

Table 7. Summary of findings. Outcome: self-reported pain

Treatment	Outcome	Comparator	No of Participants (studies)	Laser	Minimal important difference[1]	Quality of evidence (GRADE)
HAIR REMOVAL						
Piroxicam	>	Placebo	50 (1 RCT)	Nd:YAG	Yes	++OO Low[a,b]
	=	EMLA	50 (1 RCT)	Nd:YAG	N.A.	
Lidocaine 5%	>	No treatment	20 (1 RCT, split lesion)	Alexandrite / Diode	?	++OO Low[a,b]
	=	EMLA	64 (1 RCT, split lesion)	Nd:YAG	N.A.	
EMLA	=	Piroxicam	50 (1 RCT)	Nd:YAG	N.A.	++OO Low[a,b]
	=	Lidocaine 5%	64 (1 RCT, split lesion)	Nd:YAG	N.A.	
	>	Diclofenac gel	32 (1 CCT, split lesion)	Alexandrite	Yes	
	>	Cooling	32 (1 CCT, split lesion)	Alexandrite	Yes	
Diclofenac gel	>	Cooling	32 (1 CCT, split lesion)	Alexandrite	Yes	++OO Low[a,b]
	<	EMLA	32 (1 CCT, split lesion)	Alexandrite	Yes	
PSF	>	No treatment	10 (1 RCT, split lesion)	Diode	?	++OO Low[a,b]
	>	Cooling	40 (2 RCTs, split lesion)	Alexandrite / Nd:YAG	Yes, ?	
Cooling	>	No treatment	10 (1 CCT, split lesion)	Alexandrite	?	++OO Low[a,b]
	<	EMLA	32 (1 CCT, split lesion)	Alexandrite	Yes	
	<	Diclofenac	32 (1 CCT, split lesion)	Alexandrite	Yes	
	<	PSF	20 (2 RCTs, split lesion)	Alexandrite / Nd:YAG	Yes, ?	

Table 7. Summary of findings. Outcome: self-reported pain (continued)

Treatment	Outcome	Comparator	No of Participants (studies)	Laser	Minimal important difference[1]	Quality of evidence (GRADE)
			REJUVENATION			
S-Caine peel	>	Placebo	20 (1 RCT, split lesion)	Diode	Yes	++OO Low[a,b]
	>	EMLA	20 (1 RCT, split lesion)	CO_2	Yes	
Cooling	>	No treatment	15 (1 CCT, split lesion)	Fractionated CO_2	?	++OO Low[a,b]
	<	PSF	11 (1 CCT, split lesion)	Nd:YAG	Yes	
EMLA	<	S-Caine peel	20 (1 RCT, split lesion)	CO_2	Yes	++OO Low[a,b]
PSF	>	Cooling	11 (1 CCT, split lesion)	Nd:YAG	Yes	++OO Low[a,b]
Er:YAG + lidocaine 5%	>	Lidocaine 5%	12 (1 RCT, split lesion)	Er:YAG	Yes	++OO Low[a,b]
			PWS			
Iontophoresis lidocaine 4%	>	Placebo / no treatment	11 (1 RCT, split lesion)	PDL	?	++OO Low[a,b]
	=	Iontophoresis lidocaine 4% with epinephrine	11 (1 RCT, split lesion)	PDL	N.A.	
Iontophoresis lidocaine 4% + epinephrine	>	Placebo / no treatment	11 (1 RCT, split lesion)	PDL	?	++OO Low[a,b]
	=	Iontophoresis lidocaine 4%	11 (1 RCT, split lesion)	PDL	N.A	
Iontophoresis lidocaine 5% with epinephrine	>	Placebo (iontophoresis 0.9% NaCl)	36 (1 RCT)	PDL	Yes	++OO Low[a,b]
	>	Iontophoresis mepivacaine 2% with epinephrine	36 (1 RCT)	PDL	Yes	

Table 7. Summary of findings. Outcome: self-reported pain (continued)

Treatment	Outcome	Comparator	No of Participants (studies)	Laser	Minimal important difference[1]	Quality of evidence (GRADE)
Iontophoresis mepivacaine 2% with epinephrine	>	Placebo (iont.0.9% NaCl)	36 (1 RCT)	PDL	Yes	++OO Low[a,b]
	<	Iont. lido 5% with epinephrine	36 (1 RCT)	PDL	Yes	
Cooling	>	No treatment	72 (3 CCTs, split lesion)	PDL	Yes, ?, ?	++OO Low[a,b]
Lidocaine 25% in 70% DMSO-ethanol	>	No treatment	14 (1 CCT, split lesion)	PDL	?	++OO Low[a,b]
Amethocaine 4%	>	Placebo	29 (1RCT, split lesion)	PDL	?	++OO Low[a,b]
	>	EMLA	29 (1 RCT, split lesion)	PDL	?	
EMLA	>	Placebo / no treatment	102 (2 RCTs, split lesion)	PDL	?, Yes	++OO Low[a,b]
	<	Amethocaine 4%	29 (1 RCT, split lesion)	PDL	?	
			LEG TELANGIECTASIA			
S-Caine peel	>	Placebo	160 (3 RCTs, split lesion)	Nd:YAG	?, Yes, Yes	++OO Low[a,b]
Cooling	>	Second skin moist gel pad	18 (1 CCT, split lesion)	PDL	?	++OO Low[a,b]
			FACIAL TELANGIECTASIA			
Cooling 17 or 20 decrees	>	No treatment	17 (1 CCT, split lesion)	PDL	?	++OO Low[a,b]
Cooling 17 degrees	>	Cooling 20 degrees	17 (1 CCT, split lesion)	PDL	?	++OO Low[a,b]

Table 7. Summary of findings. Outcome: self-reported pain (continued)

Treatment	Outcome	Comparator	No of Participants (studies)	Laser	Minimal important difference[1]	Quality of evidence (GRADE)
Cooling + 2 mm film of aqueous gel	>	Cooling	19 (1 RCT, split lesion)	KTP	?	++OO Low[a,b]
			TATTOO REMOVAL			
S-Caine peel	>	Placebo	30 (1 RCT, split lesion)	Q-switched Nd:YAG	Yes	++OO Low[a,b]
PSF	>	No treatment	11 (1 CCT, split lesion)	Q-switched Nd:YAG	?	++OO Low[a,b]
			NEVUS OF OTA			
Cooling	>	No treatment	37 (1 CCT, split lesion)	Q-switched Alexandrite	Yes	++OO Low[a,b]
			SOLAR LENTIGINES			
PSF	>	No treatment	20 (1 CCT, split lesions)	IPL	?	++OO Low[a,b]
			HPV LESIONS			
EMLA	>	Placebo	80 (1 RCT)	CO2	Yes	++OO Low[a,b]

>; better than, =; equal to, <; worse than , N.A.; not applicable

1.The smallest change in instrument score that patients perceive is known as the minimal important difference (MID), and facilitates the interpretation of results. The MID for the VAS is 9-18 mm on a 100 mm visual analog scale (VAS) [51-53], and for numeric rating scale (NRS) a reduction of 18% [54].

a. Downgraded for risk of bias

b. Downgraded for imprecision, because the evidence is based on the results of one or few studies.

DISCUSSION

This systematic review provides an overview of the efficacy and safety of non-invasive anaesthetic methods that are used during dermatological laser procedures. The types of non-invasive anaesthetic methods varied widely. Also, the types of lasers, laser settings, application time and pain scales were often dissimilar. This precluded pooling of the data and subsequent meta-analysis. Additionally, all included studies had an unclear-to-high risk of bias, and the overall quality of evidence was low. Therefore, any recommendations should be made with caution.

Most of the reviewed studies compared active anaesthetic methods to placebo or no treatment. For all dermatological laser indications investigated in this review, active anaesthetic methods of any kind (topical drugs, skin cooling, PSF) showed favourable results with regard to pain reduction compared to placebo or no treatment. To facilitate recommendations for daily clinical practice, active treatments should preferably (also) be compared to other active treatments in head-to-head clinical trials. In general, topical anaesthetic drugs and PSF seemed to provide a better pain reduction than skin cooling during the laser treatment of hairs, PWS, and resurfacing/rejuvenation. For the indications leg telangiectasia, facial telangiectasia, tattoos, nevus of Ota, solar lentigines, and HPV lesions no head-to-head studies were included in this systematic review. All anaesthetic methods were well-tolerated, and no serious adverse events occurred.

Different topical anaesthetic drugs were explored in the studies included in this review. The efficacy of topical anaesthetic drugs can be enhanced in several ways, of which some were used in the above-mentioned studies. One way is to optimize the rate of (trans)dermal absorption, which is normally limited by the stratum corneum barrier. Different methods can be used to overcome this barrier. Yun et al. used laser ablation to remove the stratum corneum [23], but also tape stripping, or degreasing with acetone have been described [43]. Occlusion and heat can enhance the penetration through the skin by increasing temperature and hydration of the stratum corneum. Iontophoresis is a method of delivering a topical anaesthetic using a mild electric current, which facilitates the passage of ionized local anaesthetics into and across the skin barrier [43]. Another option is adding epinephrine to topical anaesthetic drugs, which causes vasoconstriction and thereby prolongs the anaesthetic effect and decreases systemic absorption.

When choosing a non-invasive anaesthetic, not only the efficacy should be taken into account, but also the ease of use, side effects, and costs are important factors. The need to apply some topical anaesthetic drugs under occlusion can be bothersome, especially for certain anatomical locations. Also, the duration of the application time may be a disadvantage for both patients and physicians, and there may be a risk of excessive systemic absorption when applied to larger areas or in case of disruption of the stratum corneum [44]. No serious adverse events were described in the included studies. However,

allergic reactions are often reported with the ester type of anaesthetics, and methemoglobinemia has been described for other local anaesthetics, particularly prilocaine [45]. Skin cooling and PSF are both easy to use, and adverse events are mild and transient. Cost-effectiveness was beyond the scope of this article, but should be included in the decision-making process.

Pain is a very subjective outcome measurement and therefore difficult to investigate. Additionally, there are differences in pain perception and analgesic response between men and woman. These sex differences may be explained by biological, psychological, and sociocultural factors, including sex hormones, endogenous opioid function, genetic factors, pain-related catastrophizing, and gender roles [46, 47]. Since the majority of patients in the included studies in this review were female, this may complicate extrapolating results to the general population.

Strengths of this review are the extensive search, the fact that pain reduction was the primary objective in all the included studies, and that all non-invasive methods of anaesthesia were evaluated. Limitations of this review are the small sample sizes in most of the studies, the unclear to high risk of bias, and the fact that the quality of the included studies was low. Remarkably, several studies did not perform or report their statistical analysis and did not provide *P*-values or confidence intervals. Some studies did not use randomisation, however, this might be less important in case of a split-lesion design. Also, blinding was not always possible, which may be especially important since pain is a subjective outcome measurement. Furthermore, there is a possibility of publication bias as only published studies were considered.

In summary, we conclude that the current evidence is insufficient to compare the efficacy and safety of the different non-invasive anaesthetic methods. Therefore recommendations for daily clinical practice should be made with caution. In general, active non-invasive anaesthetic methods seemed to provide favourable results compared to no anaesthesia. Therefore, when patients experience pain during laser therapy, a certain method of anaesthesia may be used to improve patient satisfaction and treatment efficacy. Topical anaesthetic drugs and PSF seemed to result in a better pain reduction than skin cooling. As the number of laser procedures is increasing worldwide, more high-quality head-to-head RCTs are needed in order to facilitate recommendations for daily clinical laser practice. Future studies should also evaluate cost-effectiveness and sex differences, and uniformity in validated pain measurement scales is recommended. For example, the VAS or NRS, since they are equally sensitive and appropriate for a patient's subjective feeling of the intensity of pain [48]. Furthermore, not only statistically significant differences, but also minimal important differences should be addressed since only this translates to real life situations in clinical practice [49, 50].

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SUPPLEMENTARY TABLES

Supplementary Table 1. Digital search strategy

Database	Search string
EMBASE **n = 1986**	('local anesthetic agent'/de OR EMLA/de OR 'local anesthesia'/exp OR cryoanesthesia/de OR 'transdermal patch'/de OR 'low temperature procedures'/de OR cooling/de OR ((gel/de OR 'transdermal drug delivery system'/de OR 'topical treatment'/de OR 'topical drug administration'/de OR 'transdermal drug administration'/de OR 'cutaneous drug administration'/de) AND (anesthesia/de OR analgesia/de)) OR cryogel/de OR hydrogel/de OR (cryoanalgesi* OR cryoanesth* OR cryoanaesth* OR ((analgesi* OR anesth* OR anaesth* OR lidocaine OR tetracain* OR prilocain*) NEAR/3 (local OR topical OR cutaneous OR wipe OR wipes OR cold OR plaster* OR patch* OR peel OR gel OR gels OR transdermal OR cutaneous OR cream*)) OR cooling OR emla OR pliaglis OR ((local OR topical) NEAR/3 (pain OR discomfort) NEAR/3 (manage* OR relief OR reduc*)) OR ((medicat* OR drug* OR cain) NEAR/3 (plaster* OR patch* OR peel)) OR hydrogel* OR cryogel*):ab,ti) AND ('laser'/exp OR 'low level laser therapy'/de OR 'laser surgery'/de OR 'IPL device'/de OR 'intense pulsed light therapy'/de OR (laser* OR 'intense pulsed light' OR ipl):ab,ti) AND (dermatology/de OR 'skin disease'/exp OR (dermatolog* OR skin OR cutaneous)) NOT ([animals]/lim NOT [humans]/lim)
MEDLINE (OVID) **n = 1002**	("Anesthesia, Local"/ OR (Lidocaine/ AND Prilocaine/) OR Cryoanesthesia/ OR "Transdermal Patch"/ OR ((Gels/ OR "Administration, Topical"/ OR "Administration, Cutaneous"/) AND (anesthesia/ OR analgesia/)) OR Cryogels/ OR Hydrogels/ OR (cryoanalgesi* OR cryoanesth* OR cryoanaesth* OR ((analgesi* OR anesth* OR anaesth* OR lidocaine OR tetracain* OR prilocain*) ADJ3 (local OR topical OR cutaneous OR wipe OR wipes OR cold OR plaster* OR patch* OR peel OR gel OR gels OR transdermal OR cutaneous OR cream*)) OR cooling OR emla OR pliaglis OR ((local OR topical) ADJ3 (pain OR discomfort) ADJ3 (manage* OR relief OR reduc*)) OR ((medicat* OR drug* OR cain) ADJ3 (plaster* OR patch* OR peel)) OR hydrogel* OR cryogel*).ab,ti.) AND (exp "lasers"/ OR "Laser Therapy"/ OR "Intense Pulsed Light Therapy"/ OR (laser* OR "intense pulsed light" OR ipl).ab,ti.) AND (dermatology/ OR exp "skin diseases"/ OR (dermatolog* OR skin OR cutaneous)) NOT (exp animals/ NOT humans/)
CENTRAL **n = 176**	((cryoanalgesi* OR cryoanesth* OR cryoanaesth* OR ((analgesi* OR anesth* OR anaesth* OR lidocaine OR tetracain* OR prilocain*) NEAR/3 (local OR topical OR cutaneous OR wipe OR wipes OR cold OR plaster* OR patch* OR peel OR gel OR gels OR transdermal OR cutaneous OR cream*)) OR cooling OR emla OR pliaglis OR ((local OR topical) NEAR/3 (pain OR discomfort) NEAR/3 (manage* OR relief OR reduc*)) OR ((medicat* OR drug* OR cain) NEAR/3 (plaster* OR patch* OR peel)) OR hydrogel* OR cryogel*):ab,ti) AND ((laser* OR 'intense pulsed light' OR ipl):ab,ti) AND ((dermatolog* OR skin OR cutaneous))
Web of science **n = 1000**	TS=(((cryoanalgesi* OR cryoanesth* OR cryoanaesth* OR ((analgesi* OR anesth* OR anaesth* OR lidocaine OR tetracain* OR prilocain*) NEAR/2 (local OR topical OR cutaneous OR wipe OR wipes OR cold OR plaster* OR patch* OR peel OR gel OR gels OR transdermal OR cutaneous OR cream*)) OR cooling OR emla OR pliaglis OR ((local OR topical) NEAR/2 (pain OR discomfort) NEAR/2 (manage* OR relief OR reduc*)) OR ((medicat* OR drug* OR cain) NEAR/2 (plaster* OR patch* OR peel)) OR hydrogel* OR cryogel*)) AND ((laser* OR "intense pulsed light" OR ipl)) AND ((dermatolog* OR skin OR cutaneous)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR rabbit OR rodent* OR swine OR pig OR pigs OR porcine) NOT (human* OR patient*)))

Supplementary Table 1. Digital search strategy (continued)

Database	Search string
PubMed **n = 21**	("Anesthesia, Local"[mh] OR (Lidocaine[mh] AND Prilocaine[mh]) OR Cryoanesthesia[mh] OR "Transdermal Patch"[mh] OR ((Gels[mh] OR "Administration, Topical"[mh] OR "Administration, Cutaneous"[mh]) AND (anesthesia[mh] OR analgesia[mh])) OR Cryogels[mh] OR Hydrogels[mh] OR (cryoanalgesi*[tiab] OR cryoanesth*[tiab] OR cryoanaesth*[tiab] OR ((analgesi*[tiab] OR anesth*[tiab] OR anaesth*[tiab] OR lidocaine OR tetracain*[tiab] OR prilocain*[tiab]) AND (local OR topical OR cutaneous OR wipe OR wipes OR cold OR plaster*[tiab] OR patch*[tiab] OR peel OR gel OR gels OR transdermal OR cutaneous OR cream*[tiab])) OR cooling OR emla OR pliaglis OR ((local OR topical) AND (pain OR discomfort) AND (manage*[tiab] OR relief OR reduc*[tiab])) OR ((medicat*[tiab] OR drug*[tiab] OR cain) AND (plaster*[tiab] OR patch*[tiab] OR peel)) OR hydrogel*[tiab] OR cryogel*[tiab])) AND ("lasers"[mh] OR "Laser Therapy"[mh] OR "Intense Pulsed Light Therapy"[mh] OR (laser*[tiab] OR "intense pulsed light" OR ipl)) AND (dermatology[mh] OR "skin diseases"[mh] OR (dermatolog*[tiab] OR skin OR cutaneous)) NOT (animals[mh] NOT humans[mh]) AND publisher[sb]

Supplementary Table 2. Risk of bias

	Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
	Adequate Sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data addressed	Free of selective outcome reporting	Other bias
			HAIR REMOVAL				
Akinturk 2007	Y	Y	U	Y	Y	U	-
Akinturk 2009	Y	Y	U	Y	Y	U	-
Bernstein 2007	U	U	U	U	U	U	-
Eremia 2000	U	U	U	U	U	U	-
Guardiano 2005	Y	Y	Y	Y	U	U	-
Ke 2007	U	U	U	U	U	U	-
Nahm 2002	N	N	U	U	Y	U	-
Rashidi 2012	N	N	Y	U	U	U	-
Yeung 2010	U	U	U	U	Y	U	-
			RESURFACING / REJUVENTATION				
Alster 2002	U	U	Y	Y	Y	U	-
Doshi 2003	U	U	U	U	U	U	-
Kono 2010	N	N	U	U	U	U	-
Tierney 2012	N	N	U	U	U	U	-
Yun 2002	Y	Y	U	U	Y	U	-
			PORT WINE STAINS				
Fiskerstrand 1996	N	N	U	U	U	U	-
Greve 2001	N	N	U	U	U	U	-
Kennard 1992	U	U	Y	Y	Y	U	-
Mallory 1993	N	N	U	U	U	U	-

Supplementary Table 2. Risk of bias (continued)

	Selection bias		Performance bias	Detection bias	Attrition bias	Reporting bias	Other bias
McCafferty 1997	U	U	U	U	U	U	-
Núñez 1997	U	U	U	U	Y	U	-
Tan 1992	U	U	U	U	Y	U	-
Waldorf 1997	N	N	U	U	U	U	-
			LEG TELANGIECTASIA				
Buscher 2000	N	N	U	U	Y	U	-
Chen 2003 /1	U	U	U	U	Y	U	-
Chen 2003 / 2	U	U	U	U	Y	U	-
Jih 2004	U	U	Y	Y	Y	U	-
			FACIAL TELANGIECTASIA				
Hammes 2005	N	N	U	U	Y	U	-
Kauvar 2002	U	U	U	Y	Y	U	-
			TATTOO REMOVAL				
Chen 2005	U	U	U	U	Y	U	-
Lapidoth 2007	N	N	U	U	U	U	-
			NEVUS OF OTA				
Chan 2003	N	N	U	U	U	U	-
			SOLAR LENTIGINES				
Fournier 2007	N	N	U	U	U	U	-
			HPV LESIONS				
Rylander 1990	Y	U	U	U	Y	U	-

Y, yes; N, no; U, unclear

Chapter 3.2

Comparison of lidocaine/tetracaine cream and lidocaine/prilocaine cream for local anaesthesia during laser treatment of acne keloidalis nuchae and tattoo removal: results of two randomized controlled trials.

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ABSTRACT

Background

Pain is a common adverse effect of dermatological laser procedures. Currently, no standard topical anaesthetic cream exists for deeper dermal laser procedures.

Objective

To compare the efficacy of lidocaine/tetracaine cream and lidocaine/prilocaine cream in reducing self-reported pain during deeper dermal laser treatments of acne keloidalis nuchae (AKN) and tattoos.

Methods

We conducted two randomized, double-blind, controlled clinical trials with intra-patient, split-lesion designs: study A included patients with AKN (n = 15); study B included patients with black tattoos (n = 15). The primary endpoint was the patients' self-reported pain on a 10-cm visual analog scale (VAS). Secondary objectives were the percentage of patients with adequate pain relief, willingness to pay €25 for the cream that provided the best pain relief, and safety of the creams.

Results

In both studies, VAS scores were lower for lidocaine/prilocaine cream, with a mean VAS difference in study A of 1.9 (95% confidence interval [CI], 1.0-2.8) and in study B of 0.6 (95% CI, –0.7-1.9). In study A, adequate pain relief was achieved in 13% (2/15) with lidocaine/tetracaine cream vs. 73% (11/15) with lidocaine/prilocaine cream (P = 0.004), and in study B in 53% (8/15) versus 80% (12/15) respectively (P = 0.289). In study A, 47% (7/15) was willing to pay an additional €25 vs. 73% (11/15) in study B. No serious adverse events occurred.

Conclusion

Lidocaine/prilocaine cream under plastic occlusion is the preferred topical anaesthetic during painful laser procedures targeting dermal chromophores.

INTRODUCTION

Pain is a common and bothersome adverse effect during dermatological laser procedures. To prevent thermal damage to the epidermis and to alleviate laser-induced pain, most laser devices use simultaneous local skin cooling. However, local cooling often does not provide adequate pain relief. In such cases, local anaesthetics can be used to reduce procedure-related pain and increase patient satisfaction. Anaesthetics inhibit the sensory input to the central nervous system by blocking voltage-gated sodium channels [1]. The blockade of sodium influx prevents action potentials and inhibits nerve cell depolarization. B fibres (myelinated autonomic preganglionic fibres) are blocked first, followed by C fibres (non-myelinated fibres) and, finally, A fibres (myelinated somatic fibres), which regulate pain and temperature [2, 3].

Injectable anaesthetics can be painful to administer and cannot be used in patients with needle phobia. Topical anaesthetics may be an alternative option. One of the most widely used products is an eutectic mixture of 2.5% lidocaine and 2.5% prilocaine cream (EMLA®, AstraZeneca, London, U.K.; henceforth referred to as 'LP cream'). A practical disadvantage of LP cream is the need for it to be applied under plastic occlusion. A recently (re-)introduced topical anaesthetic, which contains the highest concentration of active anaesthetic ingredients available in a Food and Drug Administration-approved topical cream, is an eutectic mixture of 7% lidocaine and 7% tetracaine cream (Pliaglis®, Galderma Laboratories, Fort Worth, TX, U.S.A.; henceforth referred to as 'LT cream'). As LT cream forms a self-occlusive film when exposed to air, it is said to not require plastic occlusion. LT cream has shown superiority over placebo in pain reduction during several dermatological laser procedures [4-7], and lower pain scores compared to LP cream during (superficial) single-pass CO_2 laser skin resurfacing [8]. However, apart from this single study, no head-to-head studies have been performed for other dermatological indications or laser systems. It is, therefore, unknown whether LT cream is also superior to LP cream in providing anaesthesia when targeting dermal chromophores.

Two examples of common indications for laser treatment in dermatology which require deeper dermal anaesthesia are hair removal, such as in acne keloidalis nuchae (AKN), and tattoo removal. AKN is a chronic, scarring folliculitis in which hair follicles are the principle contributors to inflammation [9]. Laser-assisted hair removal causes coagulation necrosis of the viable hair follicles and hair shafts in the deep dermis, and has shown positive results for AKN [10, 11]. In tattoos, using nanosecond Q-switched laser pulses, the ink particles are fragmented into smaller pieces, which can then be phagocytosed by macrophages and cleared from the skin via the lymph vessels to the draining lymph nodes [12].

The aims of these studies were to evaluate the efficacy of LT cream compared with LP cream in reducing self-reported pain during laser treatments of AKN (study A) and tattoos (study B).

MATERIALS AND METHODS

Study population

Eligible for inclusion in study A were patients aged ≥18 years with AKN, and eligible for inclusion in study B were those aged ≥18 years with a black, professionally placed tattoo. Key exclusion criteria for both studies included known hypersensitivity or contact allergy to any components of the test materials, use of any other pain medication 24 h prior to the study visit, pregnant or breast-feeding women, and damaged skin at the designated treatment area.

Study design and randomization

These double-blind, randomized, controlled trials with intra-patient, split-lesion design were approved by the medical ethical review board of the Erasmus Medical Centre (MEC-2014-517; 14-11-2014) and registered at ClinicalTrials.gov (NCT02372786). All patients provided written informed consent before study participation. Both studies were of single-centre design and all patients were treated at the Erasmus University Medical Centre in Rotterdam, The Netherlands.

Study design and treatment protocols were similar for both studies. The treatment areas (i.e. necks in study A, and tattoos in study B) were divided into two equal, anatomically similar treatment sides (left and right). Both sides were separated by an area of 1 cm, which was left untreated to avoid possible spill-over effects of the two anaesthetic creams. Both creams were applied to each patient, and the order of cream application was randomized. A computer-generated randomization list was provided by the department of Biostatistics of the Erasmus MC, and was only available to the unblinded study nurse. The creams were applied by our unblinded study nurse according to the manufacturer's instructions and according to randomization. LT cream dried when exposed to air (self-occlusive), and LP cream was applied under occlusion using an occlusive plastic film (Fig. 1). The creams were removed by the unblinded study nurse after 60 minutes, after which the patient was transferred to the laser treatment room. The skin was then examined by the blinded investigator and any local reactions were recorded. All laser procedures were started within five minutes after removal of the anaesthetic creams. In all cases, the laser treatment began on the left treatment side followed by the right treatment side, to minimalize any potential order effect. Immediately after laser treat-

Figure 1. Application of lidocaine/tetracaine cream (self-occluding) on the left treatment side and lidocaine/prilocaine cream under plastic occlusion on the right treatment side in a patient with acne keloidalis nuchae.

ment of each side, pain scores were assessed using a standard 10-cm visual analog scale (VAS), with lower scores indicating less pain.

One week after the visit all patients were contacted by telephone, to assess potential adverse events after treatment.

Laser treatment

Laser settings were determined based on the safe clinical responses to previously performed test spots. Test spots are part of the normal routine procedure in our daily clinical practice, and were executed before study participation.

Study A (Acne keloidalis nuchae)

A neodymium-doped yttrium aluminum garnet (Nd:YAG) laser (Cynosure Cynergy, Westford, MA, U.S.A.) emitting a wavelength of 1,064 nm was used for hair removal in patients with AKN. A spot size of 7 or 10 mm, fluences between 35 to 60 J/cm^2, pulse duration of 25 ms, and frequency of 1 Hertz (Hz) were used. Two passes were applied. Standardized air-cooling (Zimmer air cooler; Zimmer, Irvine CA, U.S.A.) was administered during treatment for thermal protection of the epidermis, and additional pain relief, whereby both sides were (pre)cooled in an identical manner.

Study B (tattoos)

A Q-switched Nd:YAG laser (Quanta Systems, Solbiate Olona, Italy), emitting a wavelength of 1,064 nm was used for tattoo removal. A spot size of 3 mm, fluences between 6 to 10 J/cm^2, and frequency of 1 Hz were used. A single pass was applied.

Study objectives

The primary objective of both studies was to compare the efficacy of LT cream and LP cream in reducing self-reported pain, using a 10-cm VAS, during a single laser procedure. Secondary objectives of both studies were to evaluate the percentage of patients who reported adequate pain relief (yes/no), to monitor the nature and frequency of adverse events, and to evaluate if patients were willing to pay approximately €25 for the treatment that provided superior pain reduction. This last question was included as LP cream is, in The Netherlands, reimbursed by health insurance and LT cream has to be purchased for approximately €25 (per 15 grams).

Statistical analysis

For both studies, a sample size of 15 patients was calculated to provide 80% power to detect a difference of 2 (SD 2.5) in VAS, which is determined to be (approximately) the minimal clinically important difference that a patient can detect [13], with a two-sided type I error level of 5%. A t-test for paired samples was used.

The results of the two studies were analysed separately. The differences in VAS scores between the LT and LP creams were analysed using a t-test for paired samples. Normality of the variables was tested using a Shapiro-Wilk test. The differences between LT cream and LP cream regarding adequate pain relief were analysed using a McNemar test. The side on which the treatment was least painful and willingness to spend approximately €25 for the cream used on this side are shown as percentages and absolute numbers of cases. *P*-values < 0.05 were considered statistically significant. SPSS Statistics 21 (IBM, Armonk, NY, U.S.A.) was used for all analyses.

RESULTS

Between February and September 2015, a total of 15 patients were enrolled in study A (AKN), and between February and July 2015 a total of 15 patients were enrolled in study B (tattoos). Demographic details of both studies are shown in Table 1. There were no dropouts or exclusions after randomization in both studies.

Table 1. Patient demographics

	Study A (AKN)	Study B (tattoos)
Patients (*n*)	15	15
Gender (*n*)		
Male	15	5
Female	0	10
Mean (SD) age (years)	41 (12)	39 (10)
Fitzpatrick skin type (*n*)		
I	-	1
II	-	7
III	1	3
IV	2	1
V	9	3
VI	3	0
Previous laser treatment (*n*, %)		
No	15 (100)	7 (47)
Yes	-	8 (53)
Mean number of previous treatments	-	5
Localization (*n*)		
Face	-	1
Neck	15	1
Chest	-	1
Upper extremities	-	10
Lower extremities	-	2

AKN; acne keloidalis nuchae, n; number, SD; standard deviation.

Study A: acne keloidalis nuchae

The mean (SD) VAS scores were 5.5 (1.7) for LT cream and 3.7 (2.6) for LP cream (Fig. 2), with a mean VAS difference of 1.9 (95% confidence interval [CI], 1.0-2.8; $P = 0.001$).

Adequate pain relief during the laser procedure was achieved in 13% (2/15) of the patients receiving LT cream and 73% (11/15) of the patients receiving LP cream ($P = 0.004$) (Fig. 3).

Seven percent (1/15) rated the side treated with LT cream as least painful vs. 80% (12/15) of the patients receiving LP cream. Thirteen percent (2/15) of the patients did not have a preference for either side. Of all patients, 47% (7/15) were willing to pay €25 for future treatment with the cream that provided the best pain relief.

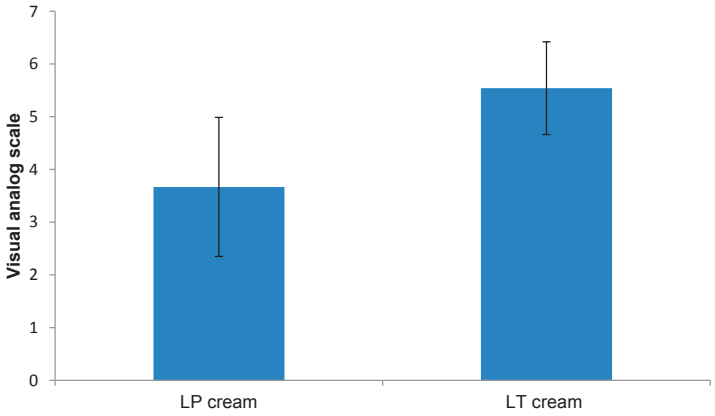

Figure 2. Study A: patients with acne keloidalis nuchae (n=15). Visual Analog Scale scores after application of lidocaine/tetracaine (LT) cream (self-occluding) on one treatment side and lidocaine/prilocaine (LP) cream under plastic occlusion on the other treatment side ($P = 0.001$).

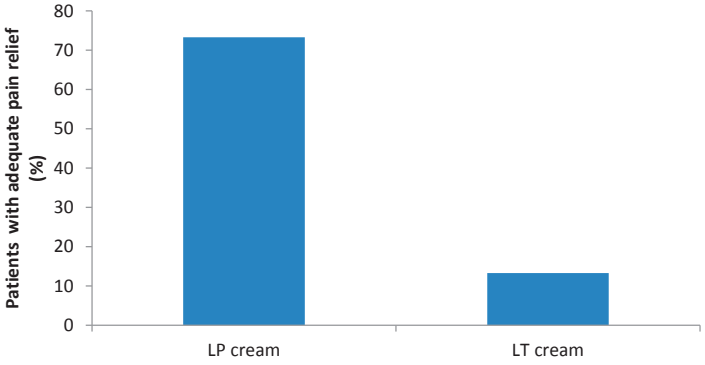

Figure 3. Study A: patients with acne keloidalis nuchae (n=15). Percentage of patients who perceived adequate pain relief after application of lidocaine/tetracaine (LT) cream (self-occluding) on one treatment side and lidocaine/prilocaine (LP) cream under plastic occlusion on the other treatment side ($P < 0.004$).

Study B: tattoos

The mean (SD) VAS scores were 4.3 (1.8) for LT cream and 3.7 (2.3) for LP cream (Fig. 4). The mean VAS difference of 0.6 (95% CI, -0.7 to 1.9; $P = 0.335$) was not statistically significant.

Adequate pain relief during the laser procedure was achieved in 53% (8/15) of the patients with LT cream and 80% (12/15) of the patients with LP cream ($P = 0.289$) (Fig.5).

Of all patients, 33% (5/15) rated the side treated with LT cream as least painful vs. 60% (9/15) of the patients for LP cream. Seven percent (1/15) of the patients did not have a preference for either side. Of all patients, 73% (11/15) were willing to pay €25 for future treatment with the cream that provided the best pain relief.

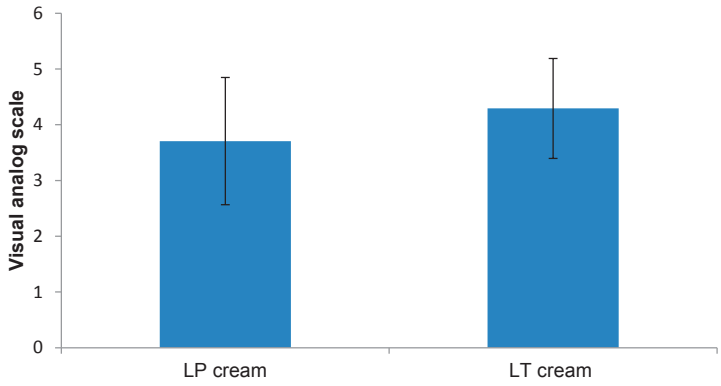

Figure 4. Study B: patients with tattoos (n=15). Visual Analog Scale scores after application of lidocaine/tetracaine (LT) cream (self-occluding) on one treatment side and lidocaine/prilocaine (LP) cream under plastic occlusion on the other treatment side ($P = 0.335$).

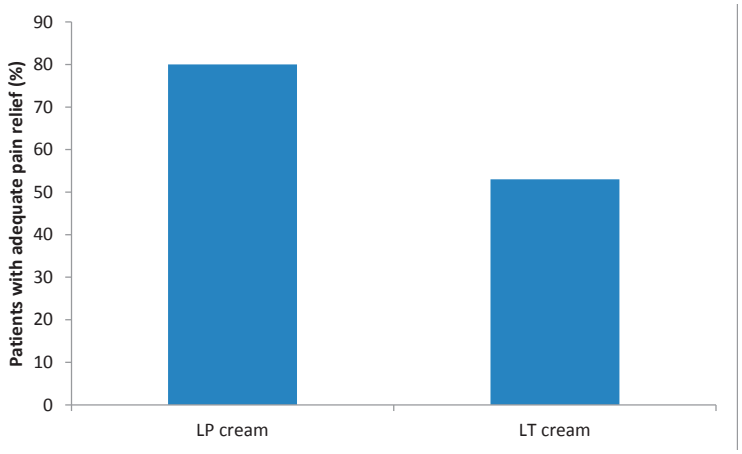

Figure 5. Study B: patients with tattoos (n=15). Percentage of patients who perceived adequate pain relief after application of lidocaine/tetracaine (LT) cream (self-occluding) on one treatment side and lidocaine/prilocaine (LP) cream under plastic occlusion on the other treatment side ($P = 0.289$).

Local reactions and adverse events of both studies

During examination of the skin immediately after cream removal, the side treated with LT cream showed blanching in 0% (0/15), edema in 20% (3/15) and erythema in 27% (4/15) in study A, and blanching in 0% (0/15), edema in 13% (2/15) and erythema in 53% (8/15) in study B. On the side treated with LP cream no local reactions were seen after cream removal in study A, and in study B blanching was seen in 47% (7/15), oedema in 7% (1/15) and erythema in 0% (0/15) of the patients. These local reactions were all mild and transient.

Thirteen patients of study A and 13 patients of study B were available for telephone consultation one week after treatment. In study A, two patients reported adverse events: burning sensation (n=1), and itching and crusting (n=1). In study B, five patients reported adverse events: swelling (n=1), itching (n=2) and mild blistering (n=2). All adverse events were transient and most likely related to the laser treatments. No serious adverse events occurred.

DISCUSSION

The main finding of study A was that LP cream provided statistically significant lower pain scores than LT cream during the Nd:YAG laser treatment of AKN. Previous studies defined the minimal clinically important difference (i.e. the smallest clinically relevant change that a patient can detect) on a 100-mm VAS to be between 9 to 18 mm [13, 14]. Thus, the statistically significant 19-mm VAS difference we found between the two creams during the treatment of AKN (favouring LP cream) is also clinically relevant.

The results of study B showed no significant differences between the two creams in reducing self-reported pain during the Q-switched Nd:YAG laser treatment of tattoos, although the numerical difference appears to be in favour of LP cream. Possibly, the lack of statistical significance in this study, compared with study A, is caused by the fact that laser-assisted hair removal in AKN is more painful than tattoo removal. Breivik et al. showed that the power to detect a difference in pain intensity is higher when there is a large difference and a higher baseline pain [15, 16]. Terminal hair follicles are located deeper in the dermis than tattoo ink particles and, together with the larger spot size and higher fluence (causing more volumetric bulk heating of the dermis), treatment with a long-pulsed Nd:YAG laser for hair removal is associated with more (painful) thermal injury to the skin than the ultrashort-pulsed Nd:YAG laser used for tattoo removal. Also, in laser tattoo removal there is less photothermal and more of a photoacoustic effect, which is less painful. Additionally, the AKN group was very homogeneous group, whereas the tattoo group was more heterogeneous, including various anatomic locations, sexes, skin types and number of previous laser treatments. These are all factors that could have increased the variability and therefore have influenced the statistical significance of the results.

The results appear to be in contrast with the only published head-to-head study which reported self-occluding LT cream to be superior to LP cream under occlusion, after a 30 minutes application time, during single-pass CO_2-laser skin resurfacing [8]. However, it might not be possible to extrapolate the results found during this superficial laser treatment to treatments with longer wavelength lasers used for both AKN and tattoo removal. Longer wavelength lasers penetrate much deeper into the dermis, triggering

far more nerves than a single-pass CO_2 laser skin ablation. Furthermore, as the label-recommended application time for LP cream is a minimum of 60 minutes (https://www.medicines.org.uk), the results of Alster et al. may have been influenced by the shorter application time of only 30 minutes.

The effectiveness of an anaesthetic cream mainly depends on the rate of (trans)dermal absorption. This absorption rate is determined by the thickness of the stratum corneum, and the pKa (acid dissociation constant) of the anaesthetic [2]. Penetration through the skin can be enhanced by occlusion, which increases temperature and hydration of the stratum corneum. The thinner the stratum corneum and the closer the pKa matches the pH of the skin (~5.5 [17]), the better the anaesthetic penetrates through the skin. As every patient in both of our studies was his or her own control, the thickness of the stratum corneum was equal for both treatment sides. Both creams contained lidocaine (pKa 7.7), and the pKa of prilocaine and tetracaine did not differ substantially (7.9 and 8.6, respectively [18]). Therefore, the main factor that could have made a difference in (trans)dermal absorption between the two creams is the different method of occlusion. The flexible film formed by LT cream might not have facilitated optimal drug absorption, and despite its higher concentrations of anaesthetics, provided less (deeper) dermal pain reduction.

Besides pain reduction, potential side effects, costs, and ease of use should be taken into account when choosing an anaesthetic cream. Mild local reactions were observed after cream removal, but no clinically important differences between the two creams were evident. In The Netherlands, the retail price of LT cream is approximately 2.5 times higher than that of LP cream, and LT cream is not reimbursed by the health insurance. In our experience, LT cream was easier to apply, but sometimes difficult to remove. On the contrary, LP cream was more difficult to apply using plastic occlusion but easier to remove.

Limitations of both studies were the relatively small sample sizes, and that patients were not completely blinded as the methods of occlusion were different. However, it was assumed patients did not know which method belonged to which cream.

In summary, we conclude that a 60-minute application of LP cream under plastic occlusion is the preferred topical anaesthetic during painful laser procedures targeting dermal chromophores.

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We wish to thank Ingrid Boesten for her assistance during these studies and drs. Tim van Meurs and dr. Ewout Baerveldt for their help with patient recruitment. Funding sources: lidocaine/tetracaine cream (Pliaglis®) was donated by the manufacturer (Galderma).

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Chapter 4

Lentigo maligna

Chapter 4.1

Epidemiology of lentigo maligna and lentigo maligna melanoma in the Netherlands, 1989 – 2013

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ABSTRACT

Lentigo maligna (LM) is considered a precursor to LM melanoma (LMM). We assessed trends in LM and LMM incidence rates between 1989 and 2013 in The Netherlands, and estimated the risk of an LMM after LM. Data on newly diagnosed LM and LMM were obtained from the Netherlands Cancer Registry and PALGA (Dutch Pathology Registry). Age-standardized incidence rates (European standardized rate), estimated annual percentage changes (EAPC), and the cumulative incidence of LMM after LM were calculated. Between 1989 and 2013, 10,545 patients were diagnosed with a primary LM and 2,898 with a primary LMM in The Netherlands. The age-standardized incidence rate for LM increased from 0.72 to 3.84 per 100,000 person-years, and for LMM from 0.24 to 1.19 between 1989 and 2013. LM incidence increased from 2002 to 2013 with 6.8% annually, before an even steeper rise in LMM incidence from 2007 to 2013 (EAPC: 12.4%). The cumulative incidence of LMM after a primary LM after 25-years follow-up was 2.0% for males and 2.6% for females. The increased incidence of LM and LMM in The Netherlands seems, besides increased awareness and increased histological confirmation of LM, to reflect a true increase. The absolute risk of an LMM (at any location) after a histologically confirmed LM was low (2.0 – 2.6%).

INTRODUCTION

Lentigo maligna (LM) is the most common subtype of melanoma *in situ*, accounting for three-quarters of all cases [1, 2]. It is considered a precursor of lentigo maligna melanoma (LMM), which represents 4% - 15% of all invasive melanomas [3]. Both LM and LMM are related to cumulative sun exposure and occur mostly in the head and neck region of elderly individuals [4].

Incidence rates of LM and LMM have increased in the recent decades [1, 5]. There is controversy about whether this represents a truly increased incidence (caused by increased ultraviolet exposure), or that it is caused by a growing awareness of skin cancer among both patients and physicians (i.e. increased detection) [6]. The stronger increase of melanoma *in situ* incidence than invasive melanoma incidence suggests at least a role for earlier detection [7, 8].

Surgical excision is considered the gold standard treatment for LM [9]. However, non-surgical treatment options gain more interest, especially for large facial lesions or elderly patients, and even a wait-and-see policy may be considered [9]. Choosing less invasive, non-surgical treatments is supported by data showing that the estimated lifetime risk of LMM in patients with a (clinically defined) LM appears to be low (2.2% to 4.7%) [10]. However, this risk is based on a single epidemiological study from 1987 [10], and has not been investigated since.

The aims of this study were to investigate and compare the incidence trends of LM and LMM in the Netherlands over a period of 25 years (1989-2013), and to estimate the risk of an LMM after a histologically confirmed LM. In addition, the 5-year relative survival of both LM and LMM is presented.

METHODS

Data were obtained from the Netherlands Cancer Registry (NCR) on all cases of LM and LMM recorded between 1989 and 2013 in the Netherlands. The NCR is based on all histologically confirmed malignancies in the Netherlands since 1989 by the automated pathological archive (PALGA), a nationwide network and registry of histopathology and cytopathology [11]. The completeness on skin cancers (excluding basal cell carcinoma) has been estimated in 1994 to be 93% [12].

All patients with a first primary diagnosed LM or LMM (International Classification of Diseases for Oncology (ICD-O) morphology codes 8742/2 and 8742/3) between 1989 and 2013 were included. ICD-O topography codes were used to categorize anatomical sites: skin of the lip (C44.0), eyelid (C44.1), external ear (C44.2), skin of other and unspecified parts of the face (C44.3), skin of the scalp and neck (C44.4), skin of the trunk (C44.5),

skin of the upper limb and shoulder (C44.6), skin of the lower limb and hip (C44.7), and other sites (C44.8-C44.9). The study period was divided into five time periods to study trends: 1989-1993, 1994-1998, 1999-2003, 2004-2008, and 2009-2013. Age-specific incidence rates were computed for 5-year age groups (0-4, ….,80-84, and 85+). Data were stratified by gender.

If a patient with a primary LM had a subsequent diagnosis of a primary LMM, the LM and LMM ICD-O topography codes were compared. NCR data were additionally linked to PALGA to obtain more specific information on the exact anatomic location, and determine if both locations were identical.

Statistical analysis

Incidence rates were calculated per 100,000 person-years, using population size (determined on 1 January each year) derived from Statistics Netherlands, and age-standardized to the European standard population (European Standardized Rate [ESR]), World standard population 1968 (World Standardized Rate [WSR]), World (WHO 2000-2025) standard population (World Standardized Rate [WSR2000-2025]) and US 2000 standard population (United States Standardized Rate [USSR]).

Estimated annual percentage changes (EAPC) with corresponding 95% confidence intervals (CI) were calculated to evaluate incidence trends for LM and LMM over time. Joinpoint regression analyses were used to identify the years in which a significant change in trends occurred.

To estimate the risk of an LMM after a histologically confirmed LM, the cumulative risk up to 25 years after diagnosis of the first LM was calculated using a cumulative incidence curve taking the competing risk of death into account [13].

Five-year relative survival using traditional cohort analysis was calculated for LM and LMM as a proxy for disease-specific survival.

All analyses were carried out using SPSS statistics 21, SAS software (version 4.3, SAS Institute, Cary, North Carolina, U.S.A.) and Joinpoint regression program version 4.2.0.1 [14]. *P*-values were two-sided and considered significant if $P < 0.05$.

RESULTS

Between 1989 and 2013, 10,545 patients were diagnosed with a primary histologically confirmed LM in The Netherlands and 2,898 with a primary LMM. Of all patients with LM, 58% were female ($n = 6,114$), which was comparable to the 57% ($n = 1,649$) females among patients with LMM. Most LM (74%, $n = 7,845$) and LMM (69%, $n = 2,002$) were located in the head and neck region.

The absolute annual number of patients with a histologically confirmed first primary LM increased from 110 in 1989 to 903 in 2013 and for LMM from 38 in 1989 to 292 in 2013. During the same period the median age at diagnosis of LM increased for men (from 68 to 71 years) and women (from 67 to 71 years). For LMM the median age at diagnosis increased as well for both men (from 68 to 73 years) and women (from 71 to 72 years).

Time trends for LM

The age-adjusted incidence rates (ESR) of LM increased between 1989 and 2013 from 0.68 to 3.57 per 100,000 person-years for males and from 0.76 to 4.16 per 100,000 person-years for females (Fig. 1). For both sexes combined, the incidence rates increased from 0.72 to 3.84 per 100,000 person-years. Incidence rates of LM age-standardized to other standard populations can be found in supplementary Table 1.

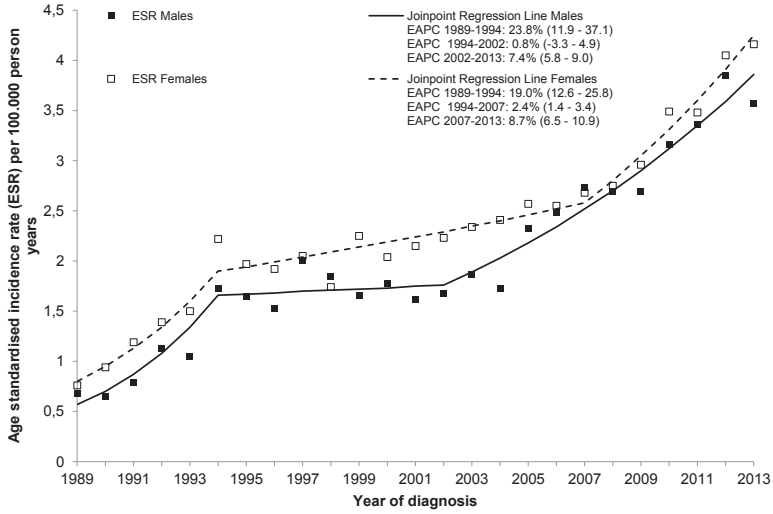

Figure 1. Lentigo maligna. Age-standardized incidence rates and trends. Age-standardized incidence rates per 100,000 person years (ESR) and Joinpoint analyses with Estimated Annual Percentage Changes (EAPC) of lentigo maligna in The Netherlands from 1989-2013 in males and females. *ESR; European Standardized Rate.*

Incidence rates of LM increased for males between 1989 and 1994 (EAPC 23.8% [95% CI, 11.9 - 37.1]) and between 2002 and 2013 (EAPC: 7.4% [95% CI, 5.8 - 9.0]), but remained stable between 1994 and 2002 (EAPC: 0.8% [95% CI, -3.3 - 4.9]) (Fig. 1.). The same pattern with the largest annual increases in incidence rates at the beginning and the end of the study period was observed for females (EAPC 1989-1994: 19.0% [95% CI, 12.6 - 25.8], EAPC 2007-2013: 8.7% [95% CI, 6.5 - 10.9]). The increase in incidence rates among females

was also statistically significant between 1994 and 2007 (EAPC: 2.4% [95% CI,1.4 - 3.4]) (Fig. 1). For both sexes combined, the incidence rates increased each year with 22.1% (95% CI, 15.2 - 29.4) between 1989 and 1994, 0.5% (95% CI, -1.8 - 2.9) between 1994 and 2002, and 6.8% (95% CI, 5.9 - 7.7) between 2002 and 2013.

Time trends for LMM

The age-adjusted incidence rates (ESR) of LMM increased between 1989 and 2013 from 0.26 to 1.25 per 100,000 person-years for males and from 0.22 to 1.18 per 100,000 person-years for females (Fig. 2). For both sexes combined, the incidence rates increased from 0.24 to 1.19 per 100,000 person-years. Incidence rates of LMM age-standardized to other standard populations can be found in supplementary Table 2.

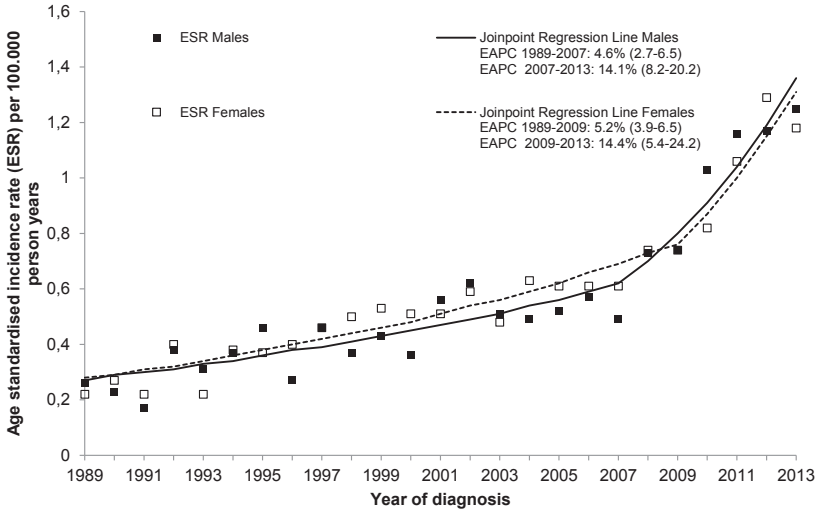

Figure 2. Lentigo maligna melanoma. Age standardized incidence rates and trends. Age standardized incidence rates per 100,000 person years (ESR) and Joinpoint analyses with Estimated Annual Percentage Changes (EAPC) of lentigo maligna melanoma in The Netherlands from 1989-2013 in males and females. *ESR; European Standardized Rate.*

Joinpoint regression analyses showed statistically significant increased incidence rates among males between 1989 and 2007 (EAPC 4.6% [95% CI, 2.7 - 6.5]) and between 2007 and 2013 (EAPC: 14.1% [95% CI, 8.2 - 20.2]) (Fig. 2). Among females, the incidence rates increased in a similar pattern, with 5.2% (95% CI, 3.9 - 6.5) between 1989 and 2009 and 14.4% (95% CI, 5.4 - 24.2) between 2009 and 2013 (Fig. 2). For both sexes combined, the incidence rates increased between 1989-2007 with 4.8% (95% CI, 3.4 - 6.2) annually, and between 2007-2013 with 12.4% (95% CI, 8.0 - 16.9%).

The age-specific incidence rates of both LM and LMM increased with each study period, with the largest increase, for both sexes, during the most recent period (2009-2013), and among elderly (data not shown).

Risk of LMM after LM

Of the 10,545 patients diagnosed with LM, 124 were subsequently diagnosed with an LMM (at any anatomic location). The 5-year cumulative risk of getting an LMM after a histologically confirmed primary LM was 0.7% (95% CI, 0.5 – 1.0) for males and 0.8% (95% CI, 0.6 – 1.1) for females. The 10-year cumulative risk was 1.0% (95% CI, 0.6 – 1.3) and 1.6% (95% CI, 1.2 – 2.0), the 15-year cumulative risk was 1.4% (95% CI, 0.9 – 1.9) and 1.9% (95% CI, 1.4 – 2.3), the 20-year cumulative risk was 2.0% (95% CI, 1.2 – 2.8) and 2.2% (95% CI, 1.7 – 2.8), and the 25-year cumulative risk 2.0% (95% CI, 1.2 – 2.8) and 2.6% (95% CI, 1.9 – 3.3), for males and females, respectively (Fig. 3.).

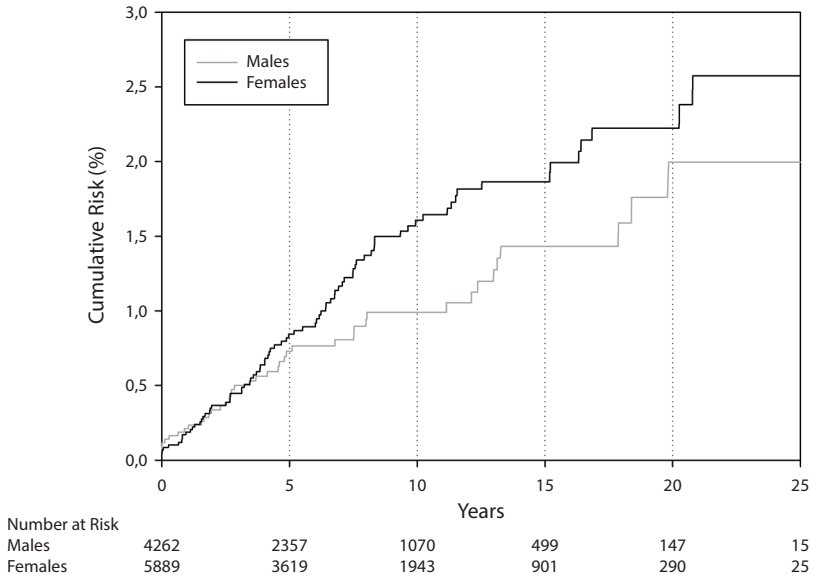

Figure 3. Cumulative incidence curve. Cumulative risk of a lentigo maligna melanoma (at any anatomical location) after a first histologically confirmed lentigo maligna.

Of the 124 patients with an LMM after LM, 101 LMMs had the same ICD-O topography and laterality codes as the LMs: skin of the lip (n = 1), skin of the external ear (n = 3), skin of other and unspecified parts of the face (n = 88), skin of the scalp and neck (n = 4), skin of the trunk (n = 3), skin of the upper limb and shoulder (n = 1), and skin of the lower limb and hip (n = 1).

To determine if the LMM occurred at the exact same anatomical location as the LM, these 101 cases were linked to PALGA. The exact locations could be determined in 71 cases. Of these 71 cases, the LM and subsequent LMM occurred at the same location in 64 cases. In 7 cases, this location was different. In the remaining 30 cases, the localizations remained unclear.

Of these 64 cases with similar anatomic locations, the LM and subsequent LMM occurred on the cheek (n = 39), nose (n = 5), forehead (n = 5), temporal area (n = 5), scalp (n = 3), ear (n = 3), eyelid (n = 1), upper limb (n = 1), neck (n = 1), and upper lip (n = 1).

Of the 64 LMs, 62 were treated, 51 (80%) with surgery, 7 (11%) with cryosurgery, and 4 (7%) with other or unknown therapies. The distribution of treatments was different compared to LM without LMM at the same location (n = 10,481), of which 10,265 LMs were treated, 9,369 (90%) with surgery, 262 (2%) with cryosurgery, and 634 (6%) with other or unknown therapies ($P = 0.004$).

Of all LMMs (n=2,898), 662 (23%) were > 1mm deep. There was no significant difference between cases with prior LM (28%, 18 of 64) and cases without prior LM (23%, 644 of 2,834) ($P = 0.309$).

Relative survival

The 5-year relative survival for LM was 104% (95% CI, 104 - 104), and for LMM 99% (95% CI, 97 - 101).

Of the 64 LMs with subsequent LMMs, three patients died in the first 5-year follow-up. The group was too small to calculate a 5-year relative survival.

DISCUSSION

The results of this large, population-based study showed that the age-standardized incidence rates for both LM and LMM in the Netherlands increased between 1989 and 2013. During the most recent period, incidence rates increased with 7% for LM and 12% for LMM. The absolute risk of an LMM (at any anatomical location) after a histologically confirmed LM was low (2-3% after 25 years). The 5-year relative survival was high for both LM (104%), and LMM (99%).

When examining the rise in LM and LMM incidence over time, there are two interesting periods. The first is the steep increase of LM incidence between 1989 and 1994. Most likely, this steep increase could be explained by under-reporting, because completeness of the database in the first years is unknown. Also, the inception of higher awareness for skin cancer among the population and improved screening [15, 16], leading to an increased histological confirmation of LM, may have contributed to this steep rise as well. The second interesting period is between 2002 and 2013, where the second increase of LM

incidence is seen. This most recent acceleration of LM incidence coincided with an even steeper increase in LMM incidence rates; therefore, we think that this reflects, at least in part, a true increase. Another explanation could be the changed market forces in The Netherlands in 2006, which stimulated clinicians to treat skin cancer more rigorously than before [17]. Diagnostic drift has also been suggested as an explanation for more recent increases in melanoma incidence rates [18], as dermatopathologists are now more likely to diagnose melanoma in biopsies than they were 20 years ago [19]. Also, because immunohistochemistry is nowadays more commonly used in the histopathological diagnosis of LM and LMM than before, this has improved identification, and may have contributed to an increased detection as well.

Studies on incidence rates of *in situ* melanoma often do not report on histological subtypes specifically [20-22], or the LM subtype is excluded because of concerns about consistency of identification over time [7, 23, 24]. Two population-based studies that did report on LM subtype both observed an average annual increase in incidence: 3.9% (95% CI, 1.7 – 6.2) in age-group 45-64 years and 6.8% (95% CI, 5 – 8.7) in age-group ≥ 65 years for both sexes in Northern California 1990-2000 [1], and 1.7% (95% CI, 0.5 – 3.0) for men and 2.7% (95% CI, 1.7 – 3.8) for woman in Denmark 1997-2011 [5]. For LMM, other population based studies reported increased incidence rates in the order of 4-6% [1, 25-27], similar to our earlier study period. However, as steep as the 12% increase that we found in our most recent study period has not been reported previously.

Age-adjusted incidence rates of LM and LMM were almost similar among men and women during the study period. This is different compared with cutaneous melanoma in which there is a predominance found in women in European countries, and the opposite is found in Australia and North America [7, 28-30]. Cutaneous melanoma predominates on the lower limb in women and the trunk in men, whereas the upper limb and head and neck were similar for both sexes [30, 31]. LM and LMM occur mostly in the head and neck region, which may explain why we found no differences between sexes.

The risk of progression of LM to LMM is an important criterion that clinicians consider when deciding on a treatment strategy (e.g. surgical or local treatment). It is, however, very difficult to study the risk of progression, because the ideal study would comprise a prospective cohort in which LM lesions would be subjected to an intensive wait-and-see policy with a very long follow-up. In our study, we could not assess the progression of an individual lesion, and also the anatomic locations of LM and their subsequent LMM were different in 30 (of 124) cases and remained unknown in another 30 (of 124) cases. Therefore, we estimated the risk of a subsequent LMM at any anatomical location after a histologically confirmed LM. The cumulative risk of developing an LMM after a primary LM was estimated to be between 2% and 3% after 25 years. Whether this is a true risk or an overestimation or underestimation is debatable. The number of LM included only histologically confirmed LM and is therefore most likely an underestimation of the

true number of LM in The Netherlands, caused by elderly patients left undiagnosed, or treated (with non-surgical treatments or conservatively) without histopathological confirmation of the diagnosis. On the contrary, the registration of LMM is assumed to be virtually complete. The under-registration of LM combined with a complete registration of LMM may have resulted in an overestimation of the cumulative risk of LMM after LM.

On the other hand, it is also possible that there may be an underestimation of the cumulative risk of LMM after LM, because LM is normally treated in The Netherlands, which would decrease the risk of progression. Also, some of the LM diagnosed as an *in situ* lesion by initial biopsy or conventional excision may have already had an unrecognized component of dermal invasive melanoma and were actually representing LMM [32-34]. Thirdly, LMM registered without a previous diagnosis of LM would not have been included in our analysis and may also have resulted in an underestimation.

Strengths of this study are the use of the national population-based cancer registry in the Netherlands that covers the whole Dutch population with high quality of the data over a long follow-up time [12]. Also, the link with the pathology database to retrieve if LMM occurred at the exact same anatomical location as LM provided important additional information. A limitation is the lack of information on non-histologically confirmed LM, and on LMM without a previous diagnosis of LM, which could have led to an respectively overestimation or underestimation of the risk of a subsequent LMM. Finally, the histopathological diagnosis of LM and LMM can be difficult because of sun-damaged skin, making these diagnoses challenging [35].

In summary, our results demonstrate an increasing incidence of LM and LMM in The Netherlands between 1989 and 2013. Besides factors like increased awareness, increased histological examination, diagnostic drift and changed market forces, this increased incidence also seems to reflect a true increase as the most recent accelerated increase of LM was followed by an even steeper accelerated increase of LMM. The absolute risk of an LMM (at any anatomical location) after a histologically confirmed LM was 2-3% after 25 years. The relative survival of both patients with LM and those with LMM was excellent 5 years after diagnosis. Our data may help physicians and patients to weigh the advantages and disadvantages of the different treatments for LM.

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Supplementary Table 1. Incidence rates (per 100,000 person years) of LM in The Netherlands in males and females from 1989-2013, age-standardized to the European standard population (European Standardized Rate [ESR]), World standard population 1968 (World Standardized Rate [WSR]), World (WHO 2000-2025) standard population (World Standardized Rate [WSR2000-2025]) and US 2000 standard population (United States Standardized Rate [USSR])).

Incidence Year	Gender	ESR	WSR	WSR2000	USSR
1989	Males	0.68	0.46	0.52	0.71
1990	Males	0.65	0.44	0.49	0.71
1991	Males	0.79	0.56	0.62	0.81
1992	Males	1.13	0.77	0.88	1.17
1993	Males	1.05	0.69	0.81	1.15
1994	Males	1.73	1.17	1.34	1.87
1995	Males	1.65	1.09	1.29	1.83
1996	Males	1.53	1.04	1.20	1.66
1997	Males	2.00	1.34	1.53	2.18
1998	Males	1.85	1.26	1.44	2.00
1999	Males	1.66	1.09	1.26	1.81
2000	Males	1.78	1.18	1.35	1.96
2001	Males	1.62	1.05	1.23	1.81
2002	Males	1.68	1.10	1.30	1.87
2003	Males	1.87	1.23	1.44	2.10
2004	Males	1.73	1.14	1.32	1.92
2005	Males	2.32	1.51	1.76	2.61
2006	Males	2.48	1.64	1.88	2.72
2007	Males	2.73	1.76	2.05	3.09
2008	Males	2.69	1.74	2.04	3.07
2009	Males	2.69	1.82	2.07	2.91
2010	Males	3.16	2.12	2.44	3.46
2011	Males	3.36	2.19	2.54	3.73
2012	Males	3.85	2.54	2.95	4.29
2013	Males	3.57	2.34	2.72	4.00
1989	Females	0.76	0.54	0.60	0.76
1990	Females	0.94	0.69	0.76	0.98
1991	Females	1.19	0.84	0.94	1.21
1992	Females	1.39	0.99	1.09	1.39
1993	Females	1.50	1.05	1.15	1.51
1994	Females	2.22	1.53	1.73	2.33
1995	Females	1.97	1.36	1.54	2.05
1996	Females	1.92	1.33	1.51	2.00
1997	Females	2.05	1.40	1.59	2.19
1998	Females	1.74	1.21	1.37	1.83

Supplementary Table 1. Incidence rates (per 100,000 person years) of LM in The Netherlands in males and females from 1989-2013, age-standardized to the European standard population (European Standardized Rate [ESR]), World standard population 1968 (World Standardized Rate [WSR]), World (WHO 2000-2025) standard population (World Standardized Rate [WSR2000-2025]) and US 2000 standard population (United States Standardized Rate [USSR])). (continued)

Incidence Year	Gender	ESR	WSR	WSR2000	USSR
1999	Females	2.25	1.55	1.73	2.27
2000	Females	2.04	1.38	1.56	2.16
2001	Females	2.15	1.47	1.67	2.27
2002	Females	2.23	1.57	1.73	2.25
2003	Females	2.34	1.60	1.80	2.43
2004	Females	2.41	1.67	1.86	2.48
2005	Females	2.57	1.72	1.97	2.70
2006	Females	2.55	1.74	1.96	2.71
2007	Females	2.68	1.82	2.04	2.82
2008	Females	2.75	1.94	2.12	2.80
2009	Females	2.96	2.00	2.25	3.14
2010	Females	3.49	2.38	2.67	3.66
2011	Females	3.48	2.39	2.68	3.66
2012	Females	4.05	2.77	3.11	4.27
2013	Females	4.16	2.82	3.19	4.42

Supplementary table 2. Incidence rates (per 100,000 person years) of LMM in The Netherlands in males and females from 1989-2013, age-standardized to the European standard population (European Standardized Rate [ESR]), World standard population 1968 (World Standardized Rate [WSR]), World (WHO 2000-2025) standard population (World Standardized Rate [WSR2000]) and US 2000 standard population (United States Standardized Rate [USSR])).

Incidence Year	Gender	ESR	WSR	WSR2000	USSR
1989	Males	0.26	0.18	0.20	0.26
1990	Males	0.23	0.16	0.17	0.22
1991	Males	0.17	0.13	0.14	0.18
1992	Males	0.38	0.25	0.30	0.43
1993	Males	0.31	0.21	0.24	0.34
1994	Males	0.37	0.25	0.28	0.42
1995	Males	0.46	0.30	0.35	0.52
1996	Males	0.27	0.17	0.21	0.33
1997	Males	0.46	0.32	0.35	0.48
1998	Males	0.37	0.23	0.27	0.40
1999	Males	0.43	0.28	0.33	0.50
2000	Males	0.36	0.23	0.27	0.40
2001	Males	0.56	0.37	0.43	0.62
2002	Males	0.62	0.41	0.47	0.70

Supplementary table 2. Incidence rates (per 100,000 person years) of LMM in The Netherlands in males and females from 1989-2013, age-standardized to the European standard population (European Standardized Rate [ESR]), World standard population 1968 (World Standardized Rate [WSR]), World (WHO 2000-2025) standard population (World Standardized Rate [WSR2000]) and US 2000 standard population (United States Standardized Rate [USSR])). (continued)

Incidence Year	Gender	ESR	WSR	WSR2000	USSR
2003	Males	0.51	0.31	0.39	0.61
2004	Males	0.49	0.31	0.37	0.57
2005	Males	0.52	0.33	0.40	0.60
2006	Males	0.57	0.38	0.45	0.64
2007	Males	0.49	0.31	0.37	0.58
2008	Males	0.73	0.48	0.56	0.83
2009	Males	0.74	0.48	0.57	0.87
2010	Males	1.03	0.65	0.78	1.20
2011	Males	1.16	0.73	0.87	1.38
2012	Males	1.17	0.75	0.89	1.37
2013	Males	1.25	0.79	0.93	1.46
1989	Females	0.22	0.15	0.17	0.24
1990	Females	0.27	0.19	0.22	0.28
1991	Females	0.22	0.15	0.17	0.22
1992	Females	0.40	0.27	0.31	0.43
1993	Females	0.22	0.15	0.17	0.24
1994	Females	0.38	0.25	0.30	0.42
1995	Females	0.37	0.25	0.29	0.42
1996	Females	0.40	0.27	0.31	0.44
1997	Females	0.46	0.31	0.35	0.49
1998	Females	0.50	0.33	0.39	0.58
1999	Females	0.53	0.36	0.41	0.55
2000	Females	0.51	0.34	0.40	0.60
2001	Females	0.51	0.34	0.39	0.57
2002	Females	0.59	0.41	0.46	0.65
2003	Females	0.48	0.32	0.38	0.54
2004	Females	0.63	0.41	0.48	0.71
2005	Females	0.61	0.42	0.47	0.64
2006	Females	0.61	0.40	0.46	0.65
2007	Females	0.61	0.40	0.46	0.69
2008	Females	0.74	0.50	0.57	0.80
2009	Females	0.74	0.49	0.56	0.84
2010	Females	0.82	0.55	0.62	0.90
2011	Females	1.06	0.70	0.81	1.18
2012	Females	1.29	0.85	1.00	1.45
2013	Females	1.18	0.78	0.89	1.29

Chapter 4.2

A two-stage treatment of lentigo maligna using ablative laser therapy followed by imiquimod: excellent cosmesis, but frequent recurrences on the nose

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ABSTRACT

Background

Although surgery is the recommended treatment for lentigo maligna (LM), it may be less desirable for large facial lesions or elderly patients.

Objective

To determine recurrence rates, cosmetic results and patient satisfaction after treatment of LM with ablative laser therapy followed by topical imiquimod 5% cream.

Methods

Data were collected from patients with histologically proven LM who had been treated with ablative laser therapy followed by imiquimod cream between 2008 and 2014. Case records and pathology reports were used to determine histologically proven recurrences, cosmetic results (0-10), treatment-related discomfort (0-10) and if patients would recommend this treatment to others (yes/no).

Results

Thirty-five patients were identified and evaluated, with a median follow-up time of 19 (interquartile range [IQR] 13-38) months. Local recurrences were found in six patients, two after eight months, one after 12 months, one after 15 months and two after 18 months follow-up. Five recurrences occurred on the nose. Patients rated the cosmetic outcome with a mean score of 8.5 (95% CI, 8.2 to 8.9) and the treatment-related discomfort with a mean score of 4.9 (95% CI, 3.9 to 5.9). Ninety percent would recommend this treatment to others. None of the patients developed lentigo maligna melanoma.

Conclusions

Ablative laser therapy followed by imiquimod cream is a feasible alternative to surgery in patients who are unsuitable for surgical treatment of LM, and on other body sites than the nose. Future work should include an analysis of possible differences between LM on the nose and other anatomical locations. Until more data are available it is important to inform patients about the potential advantages and disadvantages, and it remains crucial to follow-up these patients closely.

INTRODUCTION

Lentigo maligna (LM) is the most common subtype of melanoma *in situ*, with a rapidly growing incidence [1, 2]. It typically arises in chronically sun-exposed skin of elderly individuals. Estimates of the lifetime risk of LM progressing to LM melanoma (LMM) vary from 2.2 to 4.7% [3]. The primary treatment goal is to completely eradicate the lesion with prevention of recurrences. Since LM occurs mainly in the head and neck region [4], important secondary goals are to minimize functional and cosmetic deformities.

Surgical and non-surgical treatments are available for LM. Although surgical excision is the recommended treatment with the lowest recurrence rates [5], it may be less desirable in large facial lesions or elderly patients with extensive comorbidities [6, 7]. In these cases, alternative non-surgical treatments, or even a wait-and-see policy, may be considered [6, 8-10].

Aiming to find a non-surgical treatment with low recurrence rates and excellent cosmetic and functional outcomes, our department has introduced a novel treatment combination of ablative laser therapy followed by six weeks of topical imiquimod 5% application. In our previous publication we reported the absence of recurrences in 12 patients after a mean follow-up of 22 months, with good-to-excellent cosmetic results [11].

We have now reviewed 35 patients who were treated in a similar fashion and present the recurrence rates and patient satisfaction of this cohort, including the long-term follow-up results of the original cohort.

MATERIALS AND METHODS

The current investigation involved a retrospective evaluation of the recurrence rates, cosmetic results and patient satisfaction after treatment with ablative laser therapy followed by topical imiquimod 5% application.

During this treatment, LM lesions were first treated with ablative laser therapy with margins of 2-3 cm of adjacent skin to remove the large bulk of atypical melanocytes. The epidermis and superficial papillary dermis were ablated with either 2,940 nm erbium-doped yttrium aluminium garnet (Er:YAG) laser or 10,600 nm CO_2 laser. End points of the laser treatment were complete pigment clearance and visible punctuate bleeding. After laser treatment, patients started daily application of imiquimod 5% cream on the erosive skin for five days a week for six weeks. This topical Toll-like receptor 7 and 8 agonist induced an inflammatory immune response to clear any residual atypical melanocytes. The degree of inflammation was assessed at regular intervals, and depending on the local inflammatory reaction the frequency of application was increased to twice daily, or reduced to three days a week. Potential flu-like symptoms caused by imiquimod were

counteracted by 1 gram of paracetamol 1-2 hours before and 6-8 hours after application of imiquimod cream.

All patients with histologically proven LM, who had undergone ablative laser therapy followed by imiquimod cream at either the Erasmus University Medical Centre in Rotterdam or at DermaTeam Clinic in Middelburg or Vlissingen, between 2008 and 2014, were retrospectively identified from our prospectively updated database. All patients had declined surgical treatment and were extensively informed about the potential advantages and disadvantages of this experimental combination treatment.

Data collected included age, sex, anatomical site of the lesion, histopathological results, treatment details (laser device, duration and dosing regimen of imiquimod cream), follow-up period, biopsies taken after treatment, progression to invasive or metastatic melanoma, and disease-related mortality data. Patients who had returned to the care of their referring dermatologists were contacted and data were collected.

Patient satisfaction was determined by the cosmetic outcome (0-10, with 10 being the best possible cosmetic outcome), treatment-related discomfort (0-10, with 10 being the highest degree of discomfort), and whether the patients would recommend this treatment to others (yes/no). If this information was not available in the case records, patients were contacted by telephone to collect this information.

Statistical analysis

The continuous variables were presented as mean and standard deviation (SD) or median and interquartile range (IQR). The categorical variables were presented as numbers and their corresponding percentages. The risk of recurrence was calculated for different time points using a cumulative incidence curve from the cmprsk package from R (http://cran.r.r-project.org) and the CumIncidence function [12].

RESULTS

Patients

Between 2008 and 2014, 36 patients were identified with histologically proven LM, who consented to treatment with ablative laser therapy followed by six weeks treatment with imiquimod. One patient was excluded because remaining pigmentation (residue) was visible immediately after the (too superficial) laser treatment. He was subsequently treated with micrographically controlled staged surgical excision.

Most patients were treated at the Erasmus University Medical Centre in Rotterdam (27 of 35) and a smaller cohort at the DermaTeam Clinic in Vlissingen (6 of 35) and Middelburg (2 of 35). Twelve of the 35 patients were part of the original cohort and described in our previous publication [11].

The demographic details of the study population and treatment details are listed in Table 1. Of the 28 primary cases of LM, eight had persistent disease following previously attempted treatments (two after surgical excision, four after cryotherapy, one after monotherapy with topical imiquimod cream and one after electrosurgery and curettage). Of the seven patients with recurrent LM, six were previously treated with surgical excision and one with cryotherapy.

Table 1. Patient characteristics and treatment details

	35 patients
Sex, *n*	
Male	12
Female	23
Age (years), mean (SD)	71 (12)
Body site, *n*	
Nose	15
Cheek	12
Frontal area	4
Temporal area	1
Preauricular area	1
Upper lip	1
Leg	1
Cases, *n*	
Primary	28
Recurrent	7
Laser, *n*	
CO_2	11
Er:YAG	20
Both	4
Imiquimod	
Duration (week), mean	5.7
Intensity (times per week), mean	4.5
Follow-up (months), median (IQR)	
Original cohort (*n* = 12)	42 (37-60)
Additional cohort (*n* = 23)	14 (12-19)
Complete cohort (*n* = 35)	19 (13-38)
Inflammatory response, *n*	
Yes	35
No	0
Patient satisfaction, mean (95% CI)	
Cosmetic result	8.5 (8.2 to 8.9)

Table 1. Patient characteristics and treatment details (continued)

	35 patients
Treatment discomfort	4.9 (3.9 to 5.9)
Recommendation to others, % (*n* = 31)	
Yes	90
No	10
Cumulative incidence of recurrence, % (95% CI)	
After one year	9.4 (2.3 to 22.6)
After two years	23.5 (8.9 to 42.0)
After three years	23.5 (8.9 to 42.0)

Er:YAG, erbium yttrium aluminium garnet; IQR, interquartile range; CI, confidence interval.

Treatment

All patients responded to imiquimod by developing clear inflammatory reactions. The most common adverse reactions to the imiquimod treatment were application site reactions, flu-like symptoms, and general malaise.

Follow-up

Twenty-eight patients had their follow-up at either the Erasmus MC or DermaTeam Clinics and their case records could be evaluated. Five patients were returned to the care of their referring dermatologists and follow-up information was collected by telephone. There was no additional follow-up information available of two patients from the original cohort since last publication. One patient confirmed by telephone that there was no new visible pigmentation since treatment, but he declined a visit to the outpatient clinic. The other patient had died at the age of 89 years of brain metastases from an unknown primary tumour.

The median (IQR) follow-up time of the original cohort of 12 patients was 42 (37-60) months. The median (IQR) follow-up time of the 23 additional patients was 14 (12-19) months. When we combined all patients (n=35) we calculated a median (IQR) follow-up time of 19 (13-38) months.

Response to treatment

We found local, histologically confirmed recurrences in six patients. After one year of follow-up (number at risk = 28) there had been three recurrences (two after eight months, one after 12 months), and the cumulative incidence of recurrence was 9.4% (95% confidence interval [CI], 2.3 - 22.6%). After two years of follow-up (number at risk = 13) there had been an additional three recurrences (one after 15 months, two after 18 months) and the cumulative incidence of recurrence was 23.5% (95% CI, 8.9 - 42.0%).

After three years follow-up (number at risk = 11) there had been no additional recurrences and the cumulative incidence of recurrence remained 23.5% (95% CI, 8.9 - 42.0%).

Five of the recurrences were located on the nose (out of the 15 patients with LM on the nose) and one of the recurrences was located on the cheek (out of the 12 patients with LM on the cheek). No recurrences occurred on other anatomical locations. The recurrences were treated as follows; one patient was treated with ablative laser therapy and imiquimod for a second time, two patients chose micrographically controlled staged surgical excision, one patient received wide local excision and two patients were treated with an excisional biopsy, of which one followed by topical imiquimod application for six weeks. None of the patients in our cohort developed LMM.

Because of visible pigmentation, biopsies were taken from an additional two patients of the extended cohort during follow-up. Histopathological analysis showed a cyst and scar tissue. No atypical melanocytes were seen.

Patient satisfaction

Information on patient satisfaction was available for 89% of patients (31 of 35). Patients rated the cosmetic outcome with a mean score of 8.5 (95% CI, 8.2 to 8.9) (Fig. 1) and treatment-related discomfort with a mean score of 4.9 (95% CI, 3.9 to 5.9). Ninety percent would recommend this treatment to other patients with LM. One patient developed an ectropion of the left lower eyelid after treatment of LM on the left cheek including

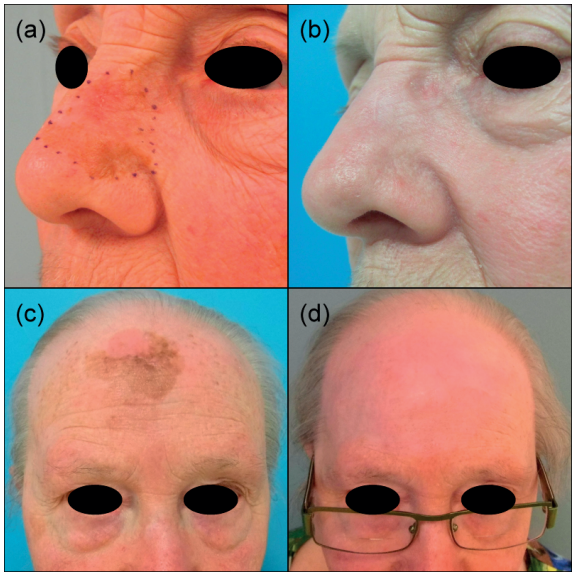

Figure 1. Examples of the cosmetic outcome after ablative laser therapy followed by imiquimod for LM unsuitable for surgical excision; (a) before treatment and (b) six months after treatment, (c) before treatment and (d) 13 months after treatment.

the lower eyelid. The ectropion did not occur until one year after the superficial Er:YAG laser treatment. Therefore we do not suspect any causality. None of the other patients developed functional deformities.

DISCUSSION

Since our previous publication on 12 LM patients who were treated with ablative laser therapy followed by imiquimod cream, we have extended our cohort with an additional 23 patients. With this larger patient number, in combination with the long-term follow-up time of the original cohort, we now present a more accurate representation of the recurrence rate, cosmetic results, and patient satisfaction.

A possible explanation of recurrences after non-surgical treatments may be incomplete clearance of atypical melanocytes extending down into the pilosebaceous units (PSU) [13]. Because of the substantial possibility of sampling error and possible compromise of the cosmetic result, we decided not to obtain biopsies for microscopic assessment after treatment. Therefore, we do not have histopathological evidence that all atypical melanocytes are in fact removed, and any residual atypical melanocytes may have led to recurrences.

The development of a clinical inflammatory response during imiquimod treatment is described as a strong prognostic feature for effective treatment [14]. Because the epidermis and reticular dermis are removed by the ablative laser, deeper penetration of imiquimod is enabled and it is delivered closer to its target cell (the dermal dendritic cell). We observed a strong inflammatory response in 100% of our patients, in contrast to imiquimod monotherapy where there are also non-responders (14-31%) [14,15]. The inflammatory reaction and adverse effects of imiquimod accounted for much of the perceived treatment-related discomfort in our cohort. Imiquimod cream applications caused stinging and were experienced as painful by most patients.

A recent review on other non-surgical treatments for LM reports mean recurrence rates of 11.5% for radiotherapy (up to 31%), 24.5% for topical imiquimod (up to 50%) and 34.4% for laser therapy (up to 100%), after variable follow-up durations [8]. Combining ablative laser therapy with imiquimod seems to result in lower recurrence rates than both treatments alone. Future work should focus on a randomized controlled trial comparing these different second line, non-surgical treatments for LM.

A potential disadvantage of non-surgical treatments is the inherent lack of histopathological control. Without histopathological control, possible misdiagnosed LMM cannot be assessed. Hazan et al. [15] found that after excision, 16% of the LM diagnosed initially on biopsy had an unrecognized component of dermal invasive melanoma. Lower percentages have been reported by Abdelmalek et al. [16] (11.7%) and Iorizzo et al.

[17] (8.1%). Among our surgical patients we recently found LMM in 4% (4/100) of the LM previously diagnosed on biopsy [18]. This lower percentage may be due to the fact that our pathologists section and examine the complete excision biopsies to rule out LMM. We recommend that non-surgical treatment for LM is only considered when the lesions are clinically unsuspicious for LMM, that (multiple) biopsies are taken from the darkest (or palpable) parts of the lesion for diagnostic purposes and that the excision biopsies are sectioned and examined completely.

Since LM mainly occurs in the head and neck region, cosmetic and functional outcomes play an important role in patients' satisfaction. The patients in our cohort rated the cosmetic outcome with an 8.5 of 10, while surgical treatment at our department has an average score of 7.8 of 10 [18]. The fact that this difference is lower than expected may be explained by the patients' expectations before treatment and our subjective assessment method. For example, patients who refuse surgical treatment are often more concerned about undesirable cosmetic outcomes and may weigh any imperfect outcome more severely. On the other hand, patients who underwent surgical excision may be expecting a number of negative effects including scarring. If the cosmetic impact is less than expected, they might be inclined to inflate a positive evaluation.

The treatment-related discomfort was scored as moderate. The inflammatory reaction and side effects of imiquimod cream account for much of the perceived treatment-related discomfort. Because of pre-treatment with the ablative laser we have chosen a shorter treatment duration of imiquimod (six weeks) than described in other literature [19], which may reduce the discomfort and burden for patients.

A limitation of this study is its retrospective design. A prospective randomized controlled trial (RCT) comparing our combination treatment to surgical excision or to other non-surgical treatments could provide better evidence and optimize treatment choice. However, in this extended cohort, we have collected relevant information about our experimental combination treatment. This has raised new questions about possible differences in recurrence risk for different anatomic locations. These questions should first be addressed. We are aware that availability of ablative laser therapy may be a limiting factor and that the costs of this treatment may be relatively high. Therefore, in addition to the recurrence rate and cosmetic outcome, future studies should also include a cost-effectiveness analysis.

In summary, we conclude that combined treatment of LM with ablative laser therapy followed by topical imiquimod cream is a feasible alternative to surgery in patients who decline or are unsuitable for surgical treatment, and on other body sites than the nose. The major advantages are the cosmetic and functional outcomes. Until more data are available it is important to inform patients about the potential advantages and disadvantages, and it remains crucial to follow-up these patients closely.

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Chapter 4.3

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

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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 hematoxylin and eosin 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.

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 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 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 hematoxylin 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

Blanc slides were cut from the selected corresponding FFPE blocks at 4µm, 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 minutes, followed by primary antibody incubation at 36 °C for 16 minutes. 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 hematoxylin. 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. 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 (IQR). 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 an 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 (14) years and in the cheek group 73 (13) years. Gender distribution in the nose group was 14 female, eight male; and in the cheek group 15 female and seven 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 to 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 to 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).

Table 1. Differences 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

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

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.

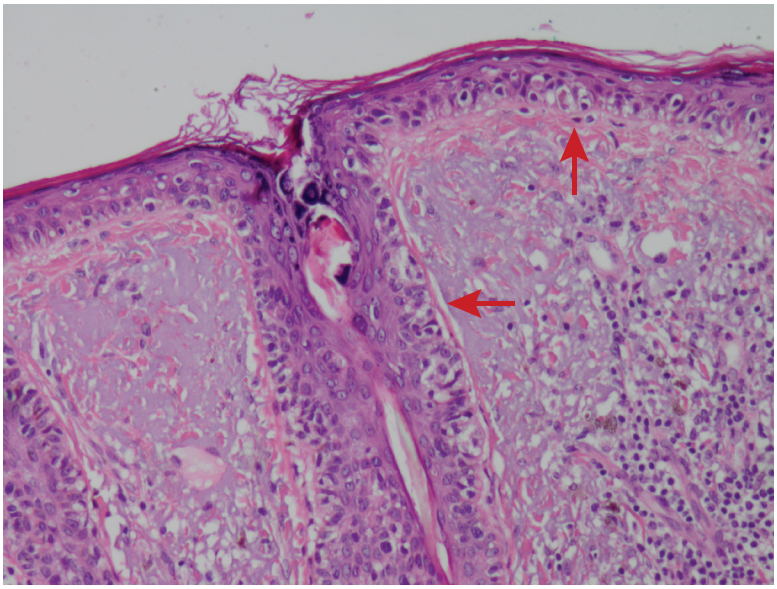

Figure 1. Lentigo maligna; continuous proliferation of atypical melanocytes along the dermo-epidermal junction. Clear extension downward, along the hair follicle is shown. In the background, severe solar elastosis. Arrows point out atypical melanocytes (HE 100x).

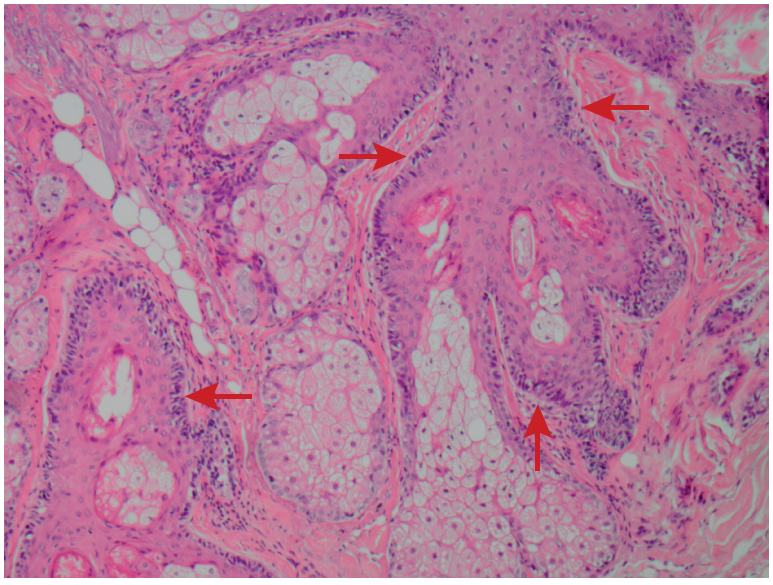

Figure 2. Very deep extension along pilosebaceous units. Arrows point out atypical melanocytes (HE 50x).

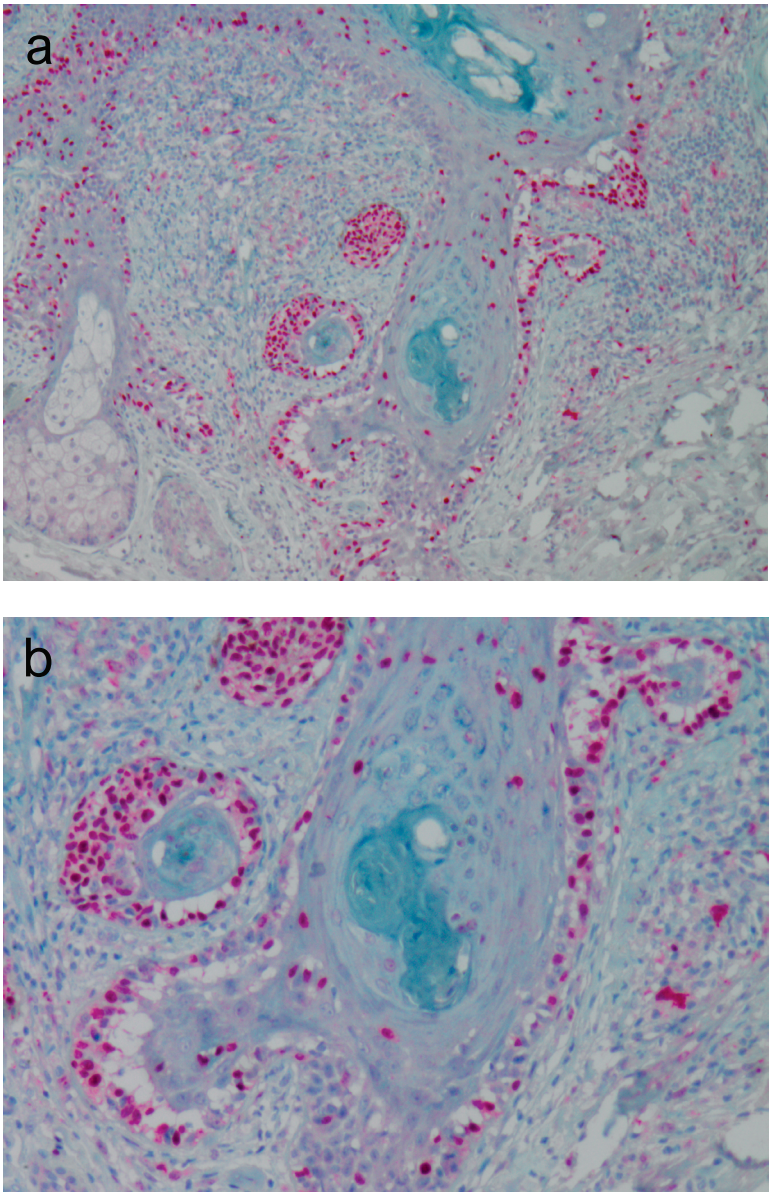

Figure 3 (a,b). Microphthalmia transcription factor (MITF) immunohistochemistry, emphasizing the number of continuous atypical melanocytes extending downward. The positive staining cells in the stroma are macrophages. (a) MITF 50x, (b) MITF 100x.

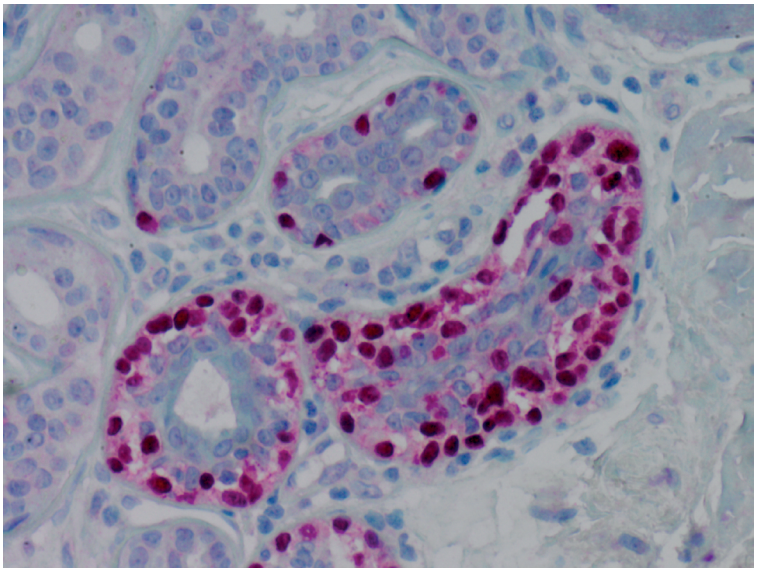

Figure 4. Microphthalmia transcription factor (MITF) immunohistochemistry. In one of the samples the downward growth along the sweat glands was even deeper than along the pilosebaceous units (MITF 100x).

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 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 of 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 (2,940 nm) and the carbon dioxide *(*CO_2) laser (10,600 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 CO2 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 ten passes of the CO_2 laser are needed, and many more using the Er:YAG laser, which in addition would be hampered by bleeding because 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 tumor 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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Chapter 4.4

Recurrence rate of lentigo maligna after micrographically controlled staged surgical excision.

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ABSTRACT

Background

Lentigo maligna (LM) is a slowly growing melanoma *in situ*. Current guidelines advise wide local excision with a margin of 5 mm as the treatment of first choice, which has recurrence rates ranging from 6% to 20%.

Objective

To determine retrospectively the recurrence rate of LM after staged surgical excision.

Methods

Records of all patients with LM treated with our method of staged surgical excision between 2002 and 2011 were retrieved. To identify recurrences, we used the computer program Sympathy®, which is linked to PALGA, a nationwide network and registry of histopathology and cytopathology in the Netherlands.

Results

We identified 100 patients, who were treated with staged surgical excision with 100% immunohistopathological control of lateral margins. Digital pictures were used to facilitate orientation during the several stages of surgery. After a mean follow-up of 60 months, four patients had a recurrence, after 37, 58, 74 and 77 months of follow-up.

Conclusion

Staged surgical excision is superior in clearance and recurrence rates to wide local excision for LM and should be considered as treatment of first choice in national and international guidelines.

INTRODUCTION

Lentigo maligna (LM) is a melanoma *in situ* clinically characterized by a slowly growing pigmented macular lesion on sun-exposed skin, most commonly in the face. The incidence increases with age, reaching 5 per 100,000 in the 45-64-year-old age group [1, 2]. For individuals aged > 65 years, the incidence is about 23 per 100,000 [1, 2]. Lentigo maligna is an epidermal lesion. When atypical melanocytes have invaded the dermis, the lesion has progressed to LM melanoma (LMM). As LM is regarded as melanoma *in situ*, treatment is usually recommended.

The treatment of first choice, according to the current guidelines, is wide local excision [3, 4]. However, clinical margins are often difficult to delineate, and wide local excision with a margin of 5 mm is often inadequate, with clearance rates ranging from 24% to 70% of cases and recurrence rates ranging from 6 to 20% [5, 6]. Therefore, several methods for staged surgical excision (SSE) have been used. Also, some surgeons have used Mohs micrographic surgery (MMS) to treat LM, or have modified this technique and used permanent sections, a technique referred to as 'slow Mohs'.

We analysed our results after ten years' experience with a modified method of SSE with paraffin-embedded histological sections, allowing for standard haematoxylin and eosin and immunohistological examinations, and compared our results with previously published data on SSE methods and MMS.

MATERIALS AND METHODS

Inclusion of patients

Records of all patients diagnosed with LM between 2002 and 2011 were retrieved from our Electronic Health Record system. The correspondence to general practitioners was searched for the terms 'lentigo maligna', 'Hutchinson's melanotic freckle', 'Dubreuilh' and 'melanosis precancerosa'. The files were reviewed and all patients treated with SSE were included.

Surgical procedure

In all patients the diagnosis of LM was made by microscopic examination of histological sections from at least one diagnostic biopsy by a dermatopathologist. After confirmation of the diagnosis, SSE was performed. Immediately prior to excision, the lesion was marked with a margin of 3 mm (Fig. 1). This relatively small margin was chosen to conserve as much tissue as possible.

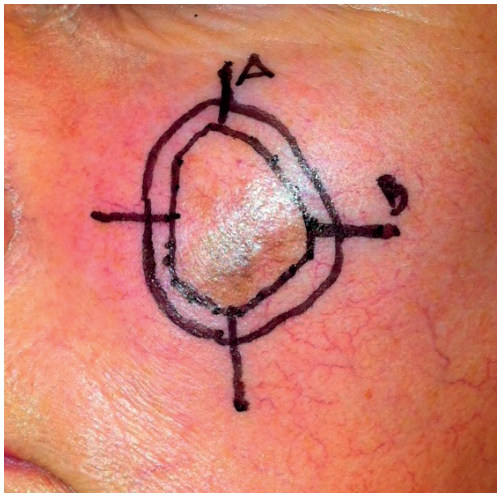

Figure 1: Markings on the skin immediately prior to the first stage of excision.

In general, no Wood's lamp was used to delineate subclinical margins prior to the first excision. Subsequently, the marked area was excised and pinned on a piece of cardboard, marked and oriented according to the *in vivo* orientation (Fig. 2).

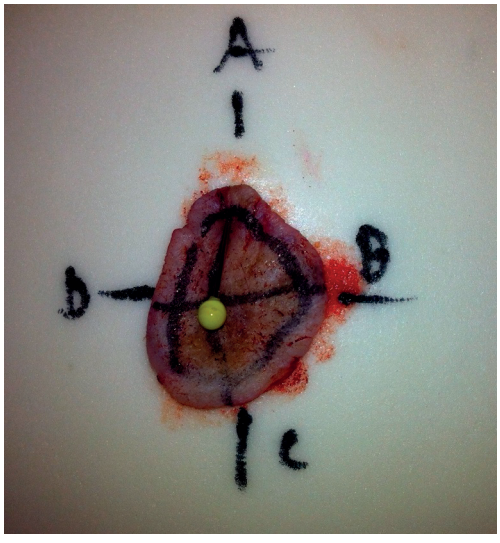

Figure 2: The excised material is pinned on a piece of cardboard, marked and oriented according to the *in vivo* orientation.

Stitches were used to maintain the *in vivo* orientation. Also, pictures were taken from the wound and the excised skin on the cardboard to help maintain orientation. The resulting skin defects were not closed, but covered instead with non-adherent bandages (Fig. 3).

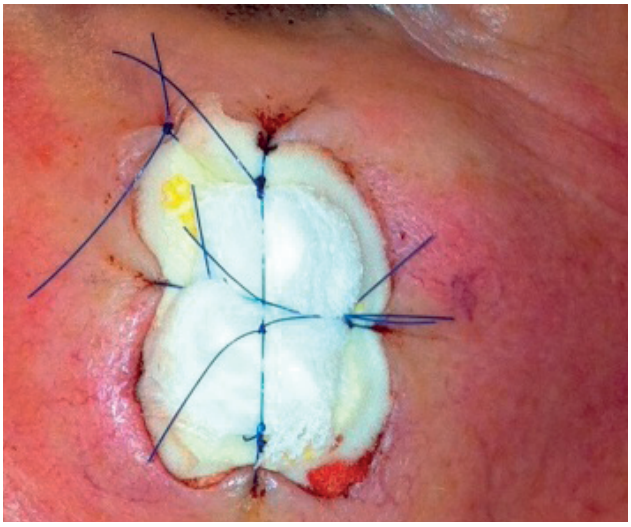

Figure 3: The resulting skin defect is covered with non-adherent bandages. Stitches are used both to keep bandages in place and to maintain orientation during the week the patient is sent home. If any residual LM is found during histopathological examination, an additional 5 mm of tissue is excised at the specific location where the LM was seen.

The margins were examined histologically by one of our three dermatopathologists within one week. Instead of examination by conventional vertical 'bread-loaf' sectioning through the material, strips of skin along the surgical margins of the resected specimen were separated from the central portion, fixed in formalin and embedded in paraffin. Sections of tissue were cut from each of the peripheral margins in a direction parallel to the margin, and stained with hematoxylin and eosin, for *en face* margin assessment of lateral margins. Slides were also stained using S100 and MelanA stains. In this way 100% of the lateral margins could be examined (Fig 4).

Although LM is an epidermal lesion, the central portion of the specimen, including the deep margin, was examined using conventional serial vertical sections ('bread-loaf' sectioning) in order to exclude invasive melanoma (i.e. LMM).

After a week, patients returned to our department. If any residual LM was found upon histological examination of the margins, an additional 5 mm of tissue was excised at that specific location where the LM was seen. The pictures taken during the first excision facilitated orientation. After excision of the additional tissue, the wound was photographed

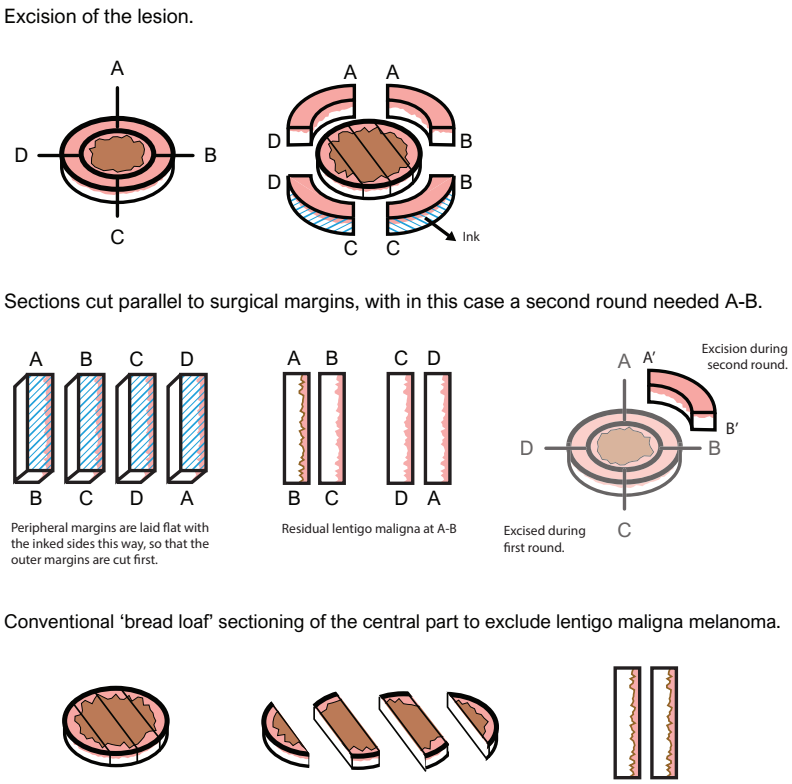

Figure 4: Sectioning and visualization of peripheral margins and central portion of the lesion.

again and then covered with non-adherent bandages for another week. This procedure was repeated until all margins were free of LM and subsequently closed. Generally, no prophylactic antibiotics were prescribed during or in between surgery sessions.

Most patients were seen for follow-up after three months, and if there were no problems the patients were discharged and were followed up by their referring physicians/dermatologists.

Evaluation of recurrences

Most patients returned to their referring dermatologist for follow-up after treatment. Therefore, we used Sympathy 2.8, a program of our pathology department that is linked to PALGA, a nationwide network and registry of histopathology and cytopathology in the Netherlands, to search for any biopsies taken from the same patient because of suspicion of a LM in the time period after the procedure, in order to assess recurrence rates. If a patient had died in the period between surgical treatment and the moment

we searched in the Sympathy database, the period between surgical treatment and date of death was used as the follow-up period. In these cases, the cause of death was noted.

Patient satisfaction

During evaluation of the results of our procedure, we contacted a random sample of one in three patients who had undergone SSE at our department. We questioned them about their satisfaction with the procedure and the cosmetic results, and asked them to rate this with a score from 0 to 10.

RESULTS

Patient archives from our electronic health record system were searched for patients with a histopathologically proven LM treated with our method for SSE. Using these criteria we found 100 patients. In addition, we found 17 patients with clinically diagnosed LM that proved to be LMM on biopsy, who were also treated using our method for SSE described above (Table 1). These patients were not included in our results.
Of the 100 patients with LM, 42 patients were male and 58 patients were female. All

Table 1. Staged surgical excision for lentigo maligna melanoma

Total number of patients	17
Male/female ratio	4/13
Primary disease/recurrent disease	3/14
Breslow thickness on biopsy, range	0.46-2.53 mm
Size of lesion at baseline (largest diameter), mean (range)	23.75 mm (6-50 mm)*
Breslow thickness after excision, range	0.4-2.5 mm
Rounds needed to achieve free margins, mean (range)	1.9 (1-5 rounds)
Number of recurrences	0
Complications	Infraorbital lymphedema (n = 1) Revision of scar after healing (n = 3)
Mean follow-up	58 months (4.8 years)

*Data on one patient missing.

but three lesions were located on the face or scalp. The mean size of the lesion (largest diameter) was 20.1 mm (range 4 - 50 mm, eight data missing). Twelve patients were referred to our department with a recurrent LM, while the other 88 patients had primary disease. Five patients had LM in the diagnostic biopsy, but LMM was found in the excision material (with Breslow thicknesses of 0.35 mm, 0.5 mm, 0.7 mm, 0.9 mm and 1.4 mm). As decision for surgical treatment was based on the biopsy, we included

these patients in our study. After the first round, 49 lesions (49%) had free margins. This relatively low clearance rate after the first round may be due to the fact we chose 3 mm as the first margin, especially in areas were the cosmetic and functional outcome is very important. A second round was needed to achieve free margins in 39 patients (39%) and a third round in nine patients (9%). Three patients (3%) had free margins after a fourth round (Table 2).

Several patients had complications after surgery. Nine had a wound infection which needed treatment with oral antibiotics. Two patients had post-surgical bleeding. In one patient, the excised material was erroneously sectioned using the conventional bread-loafing technique without *en face* margin assessment of the lateral margins. Consequently, another round was needed to ensure complete removal of LM. In one patient, the temporal branch of the facial nerve was damaged leading to impairment of the frontalis muscle. Two patients developed mild lymphoedema of the cheek for which lymph drainage was started. Three patients developed ectropion. In addition, in 18 patients the scar was considered suboptimal due to thickening, trapdoor deformity, colour mismatch between graft and surrounding skin or functional impairment. Of these, 12 underwent scar revision or were referred to a plastic surgeon. Four patients had visible terminal hairs in their grafts or reconstruction flap, which were eliminated using laser hair removal.

Table 2. Number of stages and cumulative margin needed to achieve free margins.

Number of stages	Cumulative margin (mm)	Percentage of free margins
1	3 mm	49
2	8 mm	88
3	13 mm	97
4	18 mm	100

The mean follow-up was 60 months (5 years). In this period, four patients had a recurrence, after 37, 58, 74 and 77 months. In these patients, LM was located in the temporal area (one patient) or on the cheek (three patients). One patient had recurrent disease on presentation at our department, and the other three had primary disease. All four patients with a recurrence had LM on biopsy and did not have an invasive melanoma in the excision specimen. Six patients had died from other causes in the period between surgical treatment and the time we searched in the Sympathy database.

The interviewed patients rated their satisfaction with the procedure with a mean score of 7.8 out of 10 and the cosmetic end result also with a mean score of 7.8 out of 10.

DISCUSSION

In our series, the recurrence rate of LM after SSE with a 100% immunohistopathological control of the peripheral margins was four out of 100. Using our technique, the lateral margins were examined for a full 100% control. Deep, central margins were examined using conventional vertical sections. As LM is an epidermal disorder we did not expect the deep margin to contain tumour. However, examination of the central portion of the lesion remains important, as several authors found that some lesions had to be upstaged to invasive melanoma based on the excision specimen. Abdelmalek et al. reported that 11.7% of lesions that were diagnosed as LM had an invasive component that was discovered after the excisionn[7]. Iorizzo et al. found that 8.1% of LM and melanoma *in situ*, diagnosed on biopsy, were invasive melanoma after examination of excision specimen [8]. In our series, five out of 100 lesions (5%) diagnosed as LM were diagnosed as LMM after excision. This number is lower than reported in literature. One explanation for this finding may be that, in the diagnostic work-up of LM, our pathologists usually section the whole biopsy to search for an invasive component.

By using the Sympathy program to identify recurrences, we could find only recurrences that were proven histologically. Patients who develop LM are typically elderly. Therefore, some patients with recurrences may not have visited their dermatologist or could have been treated by any physician using alternative treatments such as cryotherapy or local treatment with imiquimod. Also, if patients moved abroad, histopathological examination of a recurrence is not listed in the database we used, which is linked to a Dutch national database. Therefore, our recurrence rate may be somewhat underestimated.

We compared our results to previously published data on other surgical techniques. Wide local excision showed relatively low clearance and high recurrence rates that may be explained by the presence of subclinical (for the naked eye invisible) areas with atypical melanocytes, and lack of 100% histopathological margin control [5, 6]. Several methods have been described to achieve more thorough histological examination, including MMS with frozen sections, MMS with paraffin sections ('slow Mohs') and several types of SSE. Staged surgical excision uses paraffin-embedded, permanent sections for histological evaluation of the margins. Final closure is delayed until free margins are achieved.

Several different methods of SSE are available. In the square method, a square is drawn around the lesion and the presumed adequate margin. Two parallel blades are used to excise the square perimeter, and the excised tissue is sectioned *en face*. After achieving free margins the central portion is excised and subsequently examined conventionally. Some tissue has to be sacrificed with this method, especially for large lesions. Therefore, some surgeons excise the entire lesion in a geometric (for example triangle, rectangle, hexagon) fashion, or use the spaghetti technique to match the contour of the lesion. Cut-off strips from the margins are then oriented and processed for *en face* margin

examination. Permanent sections or rush permanent sections can be used. The central portion can be left in place until free margins are achieved, or, during the first step the tumour and margins can be excised en bloc. Leaving the central portion in place until free margins are achieved has the advantage that the wound can be closed during the subsequent rounds of the procedure. However, staging of the tumour is only done after the last round. The deep portion can be sectioned using the conventional 'bread-loaf' technique, or by horizontal sections [6].

For SSE where *en face* sections were used, recurrence rates ranged from 0% to 9.7% after mean follow-up periods of 4.7 - 96 months [6]. Recently, Abdelmalek et al. reported a 1.7% (four of 239) recurrence rate after a mean follow up period of 32.3 months after treatment using geometric staged excision [7]. To our knowledge, only Walling et al. have reported a series of patients treated with some form of SSE with a follow-up longer than that in our study [9]. In their study, 41 patients were treated with SSE, and after a mean follow-up of 95 months, three recurrences were found (7.3%).

Instead of permanent sections, MMS uses frozen sections, with the advantage that the definitive excision and closure can be performed on the same day. For MMS, recurrence rates of 0% - 33% are reported with mean follow up periods of 22 - 117 months. If one study with 18 patients is excluded, recurrence rates for MMS are 0% - 6.3% after a follow-up period of 22 - 108 months [6]. Kunishige et al. reported a 0.3% recurrence rate after MMS for 1,120 *in situ* melanomas, with a mean follow up of 4.7 years [10]. The major disadvantage of MMS is the difficulty in accurate diagnosis of melanocytic lesions on haematoxylin and eosin frozen sections, which could explain the discrepancy in recurrence rates. MMS can be combined with direct immunohistochemical stains [11, 12], which showed promising results in a recent study [13], with recurrence rates of 0.34% (two of 597) with a mean follow-up of 2.8 years. However, as MMS facilities and experienced and skilled staff are not widely available, SSE provides a good alternative in the treatment of LM, with favourable recurrence rates compared with wide local excision.

The National Comprehensive Cancer Network mentions wide local excision with a margin of 5 mm as the treatment of first choice for LM in their guideline on melanoma, published in 2008. However, for large melanoma *in situ*, LM type, it is advised to consider wider margins and more thorough histological examination [4]. The European guideline on melanoma treatment also advises wide local excision with a margin of ≥ 5 mm as the treatment of first choice for LM [3]. In this guideline the micrographic control of margins is suggested to achieve free margins. Compared to wide local excision with vertical sectioning of the excised material, techniques whereby the peripheral margins are examined for a full 100% have lower recurrence rates. However, the costs for SSE are higher than for wide local excision. At our department, the costs for SSE are comparable with those of MMS. The costs for MMS are about four times higher than costs for wide local excision with primary closure, and about 2.5 times higher than for wide local excision

followed by closure with flaps or grafts. Although these procedures are more expensive, they may lead to a higher quality of life for treated patients, as less anxiety is expected for (unplanned) resections of residual or recurrent LM.

Importantly, in the European guideline on melanoma treatment, the need to respect the anatomy of the face, including functional and cosmetic results in patients with LM, is stressed, and topical treatment with imiquimod is mentioned as an alternative to surgical treatment in selected cases. Clearance rates of 53 - 82% are reported after treatment with imiquimod, which is lower than after SSE [14]. However, when compared with surgery, a better cosmetic result may be achieved, as no scar will be formed. Most alternative options, including topical treatment with imiquimod, cryotherapy, radiation therapy or ablative laser therapy, have the possibility to treat wide margins around the lesion. A major disadvantage of topical treatments is the lack of histologic control of margins. Also, cryotherapy and radiation therapy may still lead to scar formation. Ablative laser therapy as monotherapy may fail to treat atypical melanocytes residing along skin appendages. A promising treatment modality for patients in whom surgery would be disfiguring or who are unwilling to undergo surgery may be ablative laser therapy followed by topical treatment with imiquimod,[15] but the efficacy of this treatment modality needs to be demonstrated in a larger cohort. However, as LM is an *in situ* malignancy with a low estimated chance of progression to invasive malignancy, in some patients the functional and cosmetic risks of surgery may outweigh the advantage of achieving 100% free margins. This argument may even lead to the decision not to offer treatment to patients with limited life expectancy from other causes, for whom the treatment may be worse than the risks associated with the disease.

In conclusion, compared with conventional excision, our technique has the advantage of 100% immunohistopathological control of the peripheral margins, leading to a high clearance and low recurrence rate. The conventional sections of the central parts of the lesion enable the detection of an invasive component. The main disadvantages are the need for patients to visit our department repeatedly and discomfort of the open wound in the time period between visits. As MMS with direct immunohistochemical stains is not widely available, we feel that our technique is very useful and superior to conventional wide excision, with a low recurrence rate (four of 100) after a mean follow-up period of 5 years. Our data support the recommendation of SSE as primary treatment method for LM, and it should be incorporated into national and international guidelines.

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Chapter 5

General discussion

Lasers have gained an important role in dermatology and are increasingly used for both medical and cosmetic purposes. However, it is still a developing field, and many treatments can often be improved with regard to treatment efficacy and procedure-related discomfort.

Different approaches have been undertaken to optimize laser treatments for dermatological indications. First, different laser devices can be combined. For example, in the treatment of port wine stains (PWS) a dual-wavelength approach has been developed combining a 585-597 nm Pulsed Dye Laser (PDL) and a 1,064 nm neodymium-doped yttrium aluminium garnet (Nd:YAG) laser [1]. The theory behind this approach is that when the PDL pulse is fired first, a chromatic shift in blood takes place converting oxyhemoglobin to methemoglobin. The methemoglobin absorption coefficient aligns with the Nd:YAG laser wavelength, which is fired as a second pulse. However, no RCTs comparing standard of care PDL treatment to this dual-wavelength approach have been published thus far. Other examples of dual wavelength approaches are combinations of the 532 nm potassium titanyl phosphate (KTP) laser and 1,064 nm Nd:YAG laser for the treatment of hyperpigmentation/rejuvenation [2], the fractional Q-switched ruby laser and intense pulsed light for melasma [3], and the long-pulsed 755 nm alexandrite laser and 1,064 nm Nd:YAG laser for rosacea [4].

Second, laser treatments can be optimized by combining lasers with various topical drugs. This has been investigated for multiple indications, including melasma [5], hypopigmented scars [6], actinic keratosis [7], hair removal [8, 9], psoriasis [10, 11], vitiligo [12, 13], rosacea [14], tattoos [15], and onychomycosis [16]. Lasers may also be combined with oral drugs, for example in the treatment of onychomycosis [17].

Third, enhancements in laser technology can optimize treatments, for example the development of ultra-short pulse duration (picosecond) lasers for the treatment of tattoos. Although the evidence for the claimed higher efficacy of this treatment is sparse [18].

A fourth optimization strategy for laser treatments in general is minimizing general discomforts like pain, downtime, adverse events, and treatment duration. An example is the introduction of epidermal cooling, which minimized both adverse events and pain. Also, the development of non-ablative lasers and fractional ablative lasers for resurfacing resulted in treatments with less downtime, pain, adverse events and a shorter treatment duration than with ablative lasers [19, 20].

Fifth, lasers can be used to assist in drug delivery. Ablative lasers and fractional ablative lasers can remove the stratum corneum and enhance dermal drug delivery [21]. For example, laser-assisted corticosteroid delivery for hypertrophic scars [22], fractional laser-assisted topical imiquimod treatment for recalcitrant warts [23], and fractional CO_2 laser-assisted delivery of topical anaesthetics [24].

In this thesis different optimization strategies were evaluated: optimizing the efficacy of laser treatments through the addition of topical drugs, optimizing the efficacy of a

topical drug through optimizing its penetration through the stratum corneum by laser ablation, and optimizing treatment-related discomfort by minimizing pain during laser treatments.

PORT WINE STAINS

Port wine stains are among the oldest and most common indications for laser therapy. Throughout the years, laser treatment for PWS has significantly improved. However, with 0-22% complete clearance rates [25-29], and 14-40% treatment-resistant PWS after gold standard PDL treatment [30], there is clearly room for further improvement. Adjuvant treatment with the anti-angiogenic agent sirolimus (rapamycin) was considered to be one of the most promising treatment options for PWS. Therefore, we conducted a prospective, intra-patient, randomized controlled trial to evaluate the efficacy of PDL treatment followed by application of topical sirolimus (Rapamune® 0.1%), with and without laser ablation of the stratum corneum (**Chapter 2.1**). Topical sirolimus was applied under occlusion and left *in situ* for seven days. Patients received a total of five treatments with two week intervals. Our results showed no improvement of PWS blanching by using adjuvant topical sirolimus after PDL treatment. Moreover, treatment with topical sirolimus was associated with frequent local skin reactions that hampered its use.

One other clinical trial (randomized, double-blind, intra-individual placebo-controlled) has evaluated the efficacy of PDL combined with topical sirolimus in 23 patients with PWS in Sturge-Weber syndrome [31]. In this study, a formulation of 1% sirolimus cream was fabricated and applied daily for 12 weeks. In contrast to our study results, this did appear to be an effective treatment.

There may be a true lack of efficacy of sirolimus in PWS blanching, or the commercially available (oral) solution of sirolimus (Rapamune® 0.1%) that we used may not have been optimal for topical use due to its relatively low concentration and frequent local skin reactions (that interfered with our efficacy measurements as well). Another reason why the sirolimus treatment areas were not positively impacted in our study may be that sirolimus was applied only once under occlusion, and this could have led to a shorter and thus insufficient suppression of the mammalian target of rapamycin pathway.

Furthermore, one of our patients developed an allergic contact dermatitis to topically applied sirolimus (**Chapter 2.2**). Although there are some reports of skin irritation to topical sirolimus [32, 33], to our knowledge this is the first report of a true allergic contact dermatitis. Since topical sirolimus is gaining more interest in the treatment of PWS, and also of angiofibromas [32], the risk of allergic contact dermatitis should also be taken into account.

Future research

Future studies should focus on further determining the efficacy of sirolimus in PWS blanching. Oral sirolimus has shown promising results in one patient [34], and should be further investigated regarding efficacy, adverse effects, and cost effectiveness. Determining the efficacy of different concentrations of topically applied sirolimus and the appropriate excipient for topical application may provide important additional information.

Study protocols of two RCTs, comparing PDL monotherapy with PDL followed by topical sirolimus application, and PDL monotherapy with PDL followed by oral sirolimus, are published in an American clinical trial registry (www.clinicaltrials.gov). These studies are completed, but have not been published at present. However, after contacting the principal investigator of the studies, we were informed that there was a clear lack of efficacy of sirolimus, either applied topically or given orally, and that both studies were closed due to lack of efficacy. Publication of these studies needs to be awaited to obtain more information. A first careful conclusion may be that from these unpublished studies it appears there is a true lack of efficacy of sirolimus in the treatment of PWS, which is in agreement with the findings of our study.

Interestingly, other studies have recently been published investigating the efficacy of different treatments for PWS, including the 532 nm Nd:YAG for superficial bright red facial PWS [35], the 755 nm Alexandrite laser for hypertrophic, dark and/or resistant PWS [36, 37], photodynamic therapy (PDT) [38, 39], hemoporfin-mediated PDT [40], PDL combined with imiquimod [41, 42], and fractional ablative lasers [43]. Some of these treatments have been compared to gold standard PDL treatment and are intriguing, however, clinical validation in larger patient samples is required. Furthermore, protocols have been published (clinicaltrials.gov) of studies investigating new treatment options for PWS, including Bosentan (oral inhibitor of endothelin), electroporation in combination with bleomycin sclerotherapy ('electrosclerotherapy'), brimonidine gel, and combined PDL and PDT treatment. An improved understanding of the genetic, molecular, and cellular changes causing the localized capillary and venular dilatation in PWS may lead to other innovative solutions [44].

PAIN MANAGEMENT

Pain is a common side-effect of virtually all dermatological laser procedures. In order to optimize patient satisfaction and efficacy of laser treatments, procedure-related pain should be minimized. Different non-invasive anaesthetic drugs and procedures are available, which are painless to administer compared to the more invasive injectable anaesthetics. We performed a systematic review summarizing the available data on the

efficacy and safety of non-invasive anaesthetic methods that were used to reduce pain during dermatological laser procedures (**Chapter 3.1**). In general, active non-invasive anaesthetic methods (i.e. topical drugs, skin cooling, pneumatic skin flattening) appeared to provide favourable results compared to placebo or no anaesthesia. Therefore, when patients experience pain during laser therapy, non-invasive anaesthesia may be considered to improve patient satisfaction and treatment efficacy. In addition, topical anaesthetic drugs and pneumatic skin flattening appeared to result in a better pain reduction than skin cooling. However, all of the studies we assessed had an unclear or high risk of bias and the overall quality of evidence was low. Therefore, no recommendations for daily clinical practice could be provided. No serious adverse events occurred in any of the included studies.

In **Chapter 3.2** we compared the efficacy and safety of two commonly used local anaesthetics in daily clinical practice: 2.5% lidocaine / 2.5% prilocaine cream under occlusion (EMLA®, henceforth referred to as 'LP cream'), and self-occlusive 7% lidocaine / 7% tetracaine cream (Pliaglis®, henceforth referred to as 'LT cream'), in two RCTs. The efficacy of the creams was evaluated during the treatment of two painful laser indications: acne keloidalis nuchae (AKN) and tattoo removal. We found that 60 minutes of LP cream under plastic occlusion resulted in lower pain scores than 60 minutes of self-occlusive LT cream during these painful laser procedures targeting dermal chromophores. These (unexpected) results may be explained by the fact that the effectiveness of an anaesthetic cream also depends on the rate of (trans)dermal absorption. We believe that the main contributing factor in the difference in (trans)dermal absorption between the two creams may be related to the different methods of occlusion. The flexible film formed by LT cream might not have facilitated optimal drug absorption, and despite its higher concentrations of anaesthetic drugs provided less (deeper) dermal pain reduction.

Most other studies have compared LT cream with placebo (showing superiority) [45-48], and in only one other study LT cream was compared with LP cream after 30 minutes application during (superficial) single-pass CO_2-laser skin resurfacing [49]. In this study, LT cream resulted in lower pain scores than LP cream. However, it may not be possible to extrapolate these results to treatments with longer wavelength lasers used for both AKN and tattoo removal. Longer wavelength lasers penetrate much deeper into the dermis, triggering far more nerves than a single pass CO_2 laser skin ablation. Furthermore, since the per label recommended application time for LP cream is a minimum of 60 minutes, their results may have been influenced by the shorter application time of only 30 minutes.

Future research

We established that self-occlusive LT cream was not superior to LP cream under plastic occlusion in reducing self-reported pain during painful laser procedures targeting

dermal chromophores. It would be interesting to evaluate if LT cream applied under occlusion would optimize drug absorption and result in deeper dermal pain reduction. However, the risk of excessive systemic absorption and ease of use in daily clinical practice should be considered.

More high quality head-to-head RCTs are needed to provide recommendations about non-invasive anaesthesia for daily clinical laser practice. In contrast to our study, (published) industry-sponsored studies appear to systematically yield favourable results for the sponsors [50]. Therefore, future studies should preferably not be industry-sponsored.

A study protocol has been published of a trial currently also investigating LP cream vs. LT cream and placebo during Q-switched 532 nm laser treatment of lentigines and/or photorejuvenation. These (future) results may add additional knowledge to this topic. Future studies should also evaluate sex differences in pain perception, and not only report and address statistically significant differences, but also clinically important differences since only this translates to real life situations in clinical practice.

To improve general discomforts during laser treatments the focus for future research should be, besides pain, on minimizing side effects, treatment duration, and downtime as well. For example, a recent study has been published investigating risk factors for known intense pulsed light (IPL)-induced side effects, and found that skin pigmentation and IPL fluence were major determinants of side effects after IPL exposure, while exposure to ultraviolet radiation at 30 minutes or 24 hours after treatment did not amplify side effects [51].

LENTIGO MALIGNA

Lentigo maligna (LM) is the most common subtype of melanoma *in situ* and considered a precursor to LM melanoma (LMM). In **Chapter 4.1**, we investigated and compared trends in LM and LMM incidence rates in The Netherlands over a period of 25 years (1989-2013). In this period, the age-adjusted incidence rates (European standardized rate [ESR]) of LM increased from 0.72 to 3.84 per 100,000 person-years, and for LMM from 0.24 to 1.19 per 100,000 person-years. The LM incidence increased from 2002 to 2013 with 6.8% (95% CI, 5.9 – 7.7) annually, prior to the 12.4% (95% CI, 8.0 – 16.9%) rise in LMM incidence from 2007 to 2013. Besides factors like increased awareness, increased histological examination, diagnostic drift and changed market forces, this increased incidence also seemed to reflect a true increase as the most recent accelerated increase of LM was followed by an even steeper accelerated increase of LMM. Other population-based studies that reported on LM also observed an average annual increase in incidence: 3.9% (95% CI, 1.7 – 6.2) in age-group 45-64 years and 6.8% (95% CI, 5 – 8.7) in age-group ≥ 65 years for both sexes in Northern California between 1990 and 2000 [52], and 1.7% (95% CI, 0.5 – 3.0)

for men and 2.7% (95% CI, 1.7 – 3.8) for woman in Denmark between 1997 and 2011 [53]. For LMM, other population-based studies reported increased incidence rates in the order of 4-6% [52, 54-56].

Surgical excision is considered the gold standard treatment for LM. However, alternative non-surgical treatment options are gaining more interest, especially for large lesions in cosmetically sensitive areas. The main concern in the treatment of LM is the risk of developing an LMM. We estimated the absolute risk of an LMM (at any anatomical location) after an LM to be 2-3% after 25 years (**Chapter 4.1**). This low percentage is in accordance with the 2.2% to 4.7% lifetime risk estimated by Weinstock et al. in 1987 [57]. This data may help physicians and patients to weigh the advantages and disadvantages of the different treatments for LM, especially when choosing an alternative non-surgical treatment.

An example of an alternative non-surgical treatment for LM is ablative laser therapy followed by topical imiquimod 5% application [58]. In order to further investigate this two-stage treatment, we increased the size and the follow-up time of our cohort (**Chapter 4.2**). A total of 35 patients were treated with a median (interquartile range [IQR]) follow-up time of 19 (13-38) months. Recurrences were found in 6 patients, two after eight months, one after 12 months, one after 15 months and two after 18 months follow-up. Five of the six recurrences occurred on the nose (out of the 15 patients with LM on the nose). Patients rated the cosmetic outcome with a mean score of 8.5 of 10 (95% CI, 8.2 to 8.9). We concluded that combined treatment of ablative laser therapy followed by topical imiquimod cream for six weeks is a feasible alternative to surgery, in patients that decline or are unsuitable for surgical treatment of LM with special caution for LM on the nose. The major advantages are the cosmetic and functional outcomes.

Since we found that recurrences of LM after our non-surgical combination treatment occurred almost exclusively on the nose, we investigated histological parameters that might be related to a higher recurrence risk on this anatomic location. In **Chapter 4.3**, we compared specimens of LM on the nose with specimens of LM on the cheek and found that the nose had a significantly higher density of pilosebaceous units (PSU) than the cheek. Also, 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 itself was greater on the nose than on the cheek, with a mean (SD) depth of 2.28 mm (0.41) vs. 1.65 mm (0.82), respectively ($P = 0.003$). Therefore, we concluded 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. These results indicate that anatomic location is relevant for the risk of recurrence of LM after non-surgical treatment. This should be taken into account when deciding on the treatment for the nose specifically, and highlighted the need for adaptation of our current treatment protocol. The adapted protocol now

recommends the use of the CO_2 laser instead of the Erbium YAG laser. The CO_2 laser is more efficacious in removing a higher load of atypical melanocytes and has good coagulation properties, enabling deeper ablation without bleeding complications [59, 60].

In order to put the recurrence rates of our non-surgical combination treatment for LM in perspective to the surgical treatment performed at our department, we determined the recurrence rate of LM after staged surgical excision (**Chapter 4.4**). Compared to conventional excision, this technique has the advantage of a 100% immuno-histopathological control of the peripheral margins, and the conventional sections of the central parts of the lesion enable the detection of an invasive component. We found recurrences in four out of 100 patients, after a mean follow-up period of five years.

Future research

Recently, Marsden et al. published a study that reported on the efficacy of imiquimod as primary treatment for LM [61]. Patients were treated with 60 imiquimod applications over 12 weeks followed by a clinical assessment of response, post-treatment biopsies, and subsequent radical resection. They found that the apparent clinical complete response rate and negative targeted biopsies were an unreliable predictor of the pathological complete response (pCR) rate, as only 7 of 11 cases of complete clearance were confirmed as pCR. They also found a pCR rate of 37% (10 of 27), which was considered insufficient to justify a future study investigating imiquimod vs. surgery.

However, with our combination treatment the large bulk of atypical melanocytes is first removed by ablative laser therapy. Because the epidermis and reticular dermis are removed by laser ablation, deeper penetration of imiquimod is enabled and it is delivered closer to its target cell (dermal dendritic cell). Also, removal of Langerhans cells in the epidermis may prevent suppression of an inflammatory immune response [62]. Therefore, we found 0% non-responders to imiquimod in our study, in contrast to the 27% (8 of 29) with an absent or mild local site reaction reported by Marsden et al. Other studies reporting on imiquimod monotherapy reported non-responder rates of 14-31% [63, 64]. Furthermore, Marsden et al. reported 47% (9 of 19) of their patients that had a moderate or severe local site reaction to imiquimod, to have a pCR, whereas 13% (1 of 8) of the patients with mild or no reaction had a pCR. Therefore, we expect to find higher clearance rates. However, this has to be further investigated in a clearance rate assessment study for ablative laser therapy followed by topical imiquimod application.

The next step in the development of our combination treatment of ablative laser therapy and imiquimod for LM would then be a randomized controlled trial comparing this two-stage treatment to surgical excision. Future research should also determine if the adaption of our treatment protocol has led to fewer LM recurrences on the nose. It is debatable whether non-surgical approaches need to be as effective as surgery, provided

treatment failure could be recognised (especially progression to invasive melanoma), and surgery could be performed subsequently.

Imiquimod has also been used as an adjuvant to surgical excision, which may decrease the surgical defect size [65, 66]. However, these patients would still have the disadvantages of imiquimod treatment (i.e. side-effects, treatment duration), as well as the risks (although possibly smaller) of functional impairments and cosmetic deformities after surgery.

Since LM is a pre-malignant lesion, the most significant clinical outcome is the prevention of progression to invasive melanoma. However, since non-surgical treatments have an inherent lack of histopathological control, possible misdiagnosed LMM cannot be assessed. Hazan et al. found that after excision, 16% of the LM diagnosed initially on biopsy had an unrecognized component of dermal invasive melanoma [67]. Lower percentages (11.7%) have been reported by Abdelmalek et al. [68], and Iorizzo et al. (8.1%) [69]. Among our surgical patients, we found LMM in 4% (4 of 100) of the LM previously diagnosed on biopsy [70]. This lower percentage may be due to the fact that our pathologists section and examine the complete punch biopsies to rule out LMM, which is not standard of care in other institutions. We recommend that non-surgical treatment for LM is only considered when the lesions are clinically unsuspicious for LMM, that (multiple) biopsies are taken from the darkest (or palpable) parts of the lesion for diagnostic purposes, and that the punch biopsies are sectioned and examined completely. Until more data are available it is important to inform patients about the potential advantages and disadvantages of non-surgical treatments for LM and it remains crucial to follow-up these patients closely.

LASERS IN DERMATOLOGY

Future research

Lasers can be used for numerous dermatological indications, and new opportunities keep being developed either for laser as the primary treatment or as an adjuvant to other treatments. However, decision-making in laser dermatology is often still based on expert opinions only. In order to yield strong recommendations for daily clinical practice, a more 'evidence-based medicine' approach is needed in the field of laser dermatology. The future use of evidence from well-designed and conducted research, including RCTs, systematic reviews and meta-analyses, should be emphasized.

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Chapter 6

Summary / Samenvatting

Chapter 1 contains the general introduction and general outline of this thesis. Today, lasers are increasingly used for different medical and cosmetic purposes and have gained an important role in dermatology. Although laser treatments have improved in the last few decades, and more safe and effective lasers were created after the development of selective photothermolysis, treatment results are not always optimal. In this thesis we aimed to develop optimization strategies for laser therapy in dermatology. Three different strategies were followed: optimizing an established laser treatment (for port wine stains), optimizing laser treatment-related pain, and optimizing innovative laser treatments as alternative for a surgical treatment indication (lentigo maligna).

In **Chapter 2.1** we evaluated the efficacy of adjuvant use of (commercially available) topical sirolimus (rapamycin) after Pulsed Dye Laser (PDL) treatment in patients with port wine stains (PWS). We conducted a prospective, intra-patient, randomized study, in which four treatment areas of 1 cm^2 were created in each PWS. PDL monotherapy was compared to the following three treatments: PDL + rapamycin, PDL + Erbium YAG laser ablation of the stratum corneum + rapamycin, and rapamycin monotherapy. We also compared PDL + Erbium YAG + rapamycin with PDL + rapamycin. The primary endpoint was the percentage clearance assessed colorimetrically at six months follow-up. Fourteen patients completed the six months follow-up. The highest percentage clearance was achieved with PDL monotherapy (mean [SD] 16% [34]), but there were no statistically significant differences between treatments. Application-site pruritus was a frequent occurring adverse event. There were no serious adverse events. We concluded that topical application of the commercially available solution of rapamycin (Rapamune® 0.1%) as an adjuvant to PDL treatment did not appear to improve PWS blanching.

In **Chapter 2.2** we described a case of allergic contact dermatitis caused by sirolimus (Rapamune® 0.1% oral solution) which was used as an adjuvant topical treatment of a PWS during the above-mentioned study. Although there are some reports of skin irritation caused by topical sirolimus, to our knowledge this is the first report of a true allergic contact dermatitis.

In **Chapter 3.1** we summarized the available data on the efficacy and safety of non-invasive anaesthetic methods during dermatological laser procedures by means of a systematic review. Randomized and non-randomized controlled clinical trials (RCTs and CCTs) were included. Twenty RCTs and 12 CCTs were included, involving nine different laser indications: hair removal (n = 9), resurfacing/rejuvenation (n = 5), port wine stains (n = 8), leg telangiectasia (n = 3), facial telangiectasia (n = 2), tattoo removal (n = 2), nevus of Ota (n = 1), solar lentigines (n = 1), and human papilloma virus lesions (n = 1). The non-invasive anaesthetic methods (i.e. topical anaesthetic drugs, skin cooling, and pneumatic skin flattening [PSF]), types of lasers, laser settings, application time, and types of pain scales varied widely among the included studies. In general, active non-invasive anaesthetic methods seemed to provide favourable results compared to

placebo or no anaesthesia, and topical anaesthetic drugs and PSF seemed to result in a better pain reduction than skin cooling. However, all of the studies had an unclear or high risk of bias and the overall quality of evidence was rated as low. We concluded that the current evidence is insufficient to provide recommendations for daily clinical practice, and that there is a need for more high quality (head-to-head) RCTs.

In **Chapter 3.2** we compared the efficacy of lidocaine/tetracaine cream (Pliaglis®) and lidocaine/prilocaine cream (EMLA®) in reducing self-reported pain during dermal laser treatments. We conducted two randomized, double-blind, controlled clinical trials with intra-patient, split-lesion design: study A in patients with acne keloidalis nuchae (AKN) (n = 15), and study B in patients with black tattoos (n = 15). The primary endpoint was the patients' self-reported pain on a 10 cm visual analog scale (VAS). Secondary objectives were the percentage of patients with adequate pain relief, and safety of the creams. In both studies, VAS scores were lower for lidocaine/prilocaine cream, with a mean VAS difference in study A of 1.9 (95% confidence interval [CI], 1.0 to 2.8) and in study B of 0.6 (95% CI, -0.7 to 1.9). In study A, adequate pain relief was achieved in 13% (2/15) with lidocaine/tetracaine cream versus 73% (11/15) with lidocaine/prilocaine cream (P = 0.004), and in study B in 53% (8/15) versus 80% (12/15) (P = 0.289), respectively. No serious adverse events occurred. We concluded that lidocaine/prilocaine cream under plastic occlusion is the preferred topical anaesthetic during painful laser procedures targeting dermal chromophores.

In **Chapter 4.1** we assessed trends in lentigo maligna (LM) and lentigo maligna melanoma (LMM) incidence rates between 1989 and 2013 in The Netherlands, and we estimated the risk of an LMM after LM. Data on newly diagnosed LM and LMM were obtained from the Netherlands Cancer Registry and PALGA (Dutch Pathology Database). Age-standardized incidence rates adjusted to the European standard population (European standardized rate [ESR]), estimated annual percentage changes (EAPC), and the cumulative incidence of LMM after LM were calculated. In addition, the 5-year relative survival of both LM and LMM was presented. Between 1989 and 2013, 10,545 patients were diagnosed with a primary LM and 2,898 with a primary LMM in the Netherlands. The ESR for LM increased from 0.72 to 3.84 per 100,000 person-years, and for LMM from 0.24 to 1.19 per 100,000 person-years between 1989 and 2013. LM incidence increased from 2002 to 2013 with 6.8% annually, prior to the – even steeper – rise in LMM incidence from 2007 to 2013 (EAPC: 12.4%). The cumulative incidence of LMM after a primary LM after 25 years follow-up was 2.0% for males and 2.6% for females. The 5-year relative survival was high for both LM (104%) and LMM (99%). We concluded that the increased incidence of LM and LMM in The Netherlands seemed, besides increased awareness and increased histological confirmation of LM, to reflect a true increase. The absolute risk of an LMM (at any location) after a histologically confirmed LM was low. Our data may help

physicians and patients to weigh the advantages and disadvantages of the different treatments for LM.

In **Chapter 4.2** we determined recurrence rates, cosmetic results and patient satisfaction after treatment of LM with ablative laser therapy followed by topical imiquimod 5% cream. Data were collected on patients with histologically proven LM who had been treated with ablative laser therapy followed by six weeks imiquimod cream between 2008 and 2014. Case records and pathology reports were used to determine histologically proven recurrences, cosmetic results (0-10), treatment related discomfort (0-10) and if patients would recommend this treatment to others (yes/no). Thirty-five patients were identified and evaluated, with a median (interquartile range [IQR]) follow-up of 19 (13-38) months. Local recurrences were found in six patients. Five of the six recurrences were located on the nose (out of the 15 patients with LM on the nose). Patients rated the cosmetic outcome with a mean score of 8.5 (95% CI, 8.2 to 8.9) and the treatment related discomfort with a mean score of 4.9 (95% CI, 3.9 to 5.9). Ninety percent of the patients would recommend this treatment to others. None of the patients developed LMM. We concluded that ablative laser therapy followed by imiquimod cream is a feasible alternative to surgery in patients that decline or are unsuitable for surgical treatment, and on other body sites than the nose. The major advantages are the cosmetic and functional outcomes. However, until more data are available it is important to inform patients about the potential advantages and disadvantages, and it remains crucial to follow-up these patients closely.

In **Chapter 4.3** we investigated histological parameters that might be related to the previously observed higher incidence of LM recurrences on the nose after ablative laser therapy followed by topical imiquimod cream. We randomly selected 22 surgical specimens of LM on the nose and 22 on the cheek. Histopathological analysis was performed on hematoxylin and eosin 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. We found that 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) versus 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, with a mean (SD) depth of 2.28 mm (0.41) versus 1.65 mm (0.82) ($P = 0.003$). We concluded that the higher recurrence risk of LM on the nose after non-surgical treatment, which 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 have shed more light on our previous findings and learned that anatomical location is relevant for the risk of recurrence of LM after non-surgical treatment and have led to an adaptation of our treatment protocol.

In **Chapter 4.4** we retrospectively determined the recurrence rate of LM after staged surgical excision. Records of all patients with LM, treated with our method of staged surgical excision between 2002 and 2011, were retrieved. To identify recurrences, we used the computer programme Sympathy®, which is linked to PALGA, a nationwide network and registry of histopathology and cytopathology in The Netherlands. We identified 100 patients who were treated with staged surgical excision with 100% immuno-histopathological control of lateral margins. After a mean follow-up of 60 months, four patients had a recurrence; after 37, 58, 74 and 77 months follow-up. We concluded that staged surgical excision is superior in clearance and recurrence rate to wide local excision for LM and should be considered as treatment of first choice in national and international guidelines.

In **Chapter 5** we discussed the main findings of the studies presented in this thesis and placed them into perspective. Also, we argued on future considerations for the treatment of PWS, pain and other discomforts during laser treatment, the treatment of LM, and optimizing laser treatments and laser research in general. Lasers can be used for numerous dermatological indications and new opportunities keep being developed, either for laser as the primary treatment or as an adjuvant to other treatments. However, decision-making in laser dermatology is often based on expert opinions only. In order to yield strong recommendations for daily clinical practice, a more 'evidence based medicine' approach is needed in the field of laser dermatology. The use of evidence from well-designed and conducted research, including RCTs, systematic reviews and meta-analyses, should be emphasized.

SAMENVATTING

Hoofdstuk 1 geeft een algemene introductie van dit proefschrift. Lasers worden tegenwoordig in toenemende mate gebruikt voor verschillende medische en cosmetische indicaties en hebben een belangrijke rol verkregen binnen de dermatologie. Hoewel lasers de afgelopen decennia zijn verbeterd en er veiligere en effectievere lasers zijn gecreëerd na de ontwikkeling van selectieve fotothermolyse, zijn de behandelresultaten niet altijd optimaal. In dit proefschrift hebben we ernaar gestreefd om verbeterstrategieën te ontwikkelen voor laserbehandelingen in de dermatologie. Drie verschillende strategieën werden gevolgd: verbetering van een reeds gevestigde laserbehandeling (voor wijnvlekken), verbetering van pijn tijdens laserbehandelingen, en het optimaliseren van innovatieve laserbehandelingen als alternatieve therapie voor een chirurgische behandeling (voor lentigo maligna).

In **hoofdstuk 2.1** evalueren we de effectiviteit van het adjuvant gebruik van (commercieel beschikbaar) topicaal sirolimus (rapamycine) na de Pulsed Dye Laser (PDL) behandeling van patiënten met wijnvlekken. We hebben een prospectieve, intra-patiënt, gerandomiseerde studie uitgevoerd, waarbij vier behandelgebieden van 1 cm^2 in elke wijnvlek werden gecreëerd. PDL monotherapie werd vergeleken met de volgende drie behandelingen: PDL + rapamycine, PDL + Erbium YAG laser ablatie van het stratum corneum + rapamycine, en rapamycine monotherapie. PDL + Erbium YAG + rapamycine werd ook vergeleken met PDL + rapamycine. Het primaire eindpunt was het percentage verbleking dat zes maanden na de behandeling werd vastgesteld door middel van een kleurmeter. Veertien patiënten voltooiden de zes maanden follow-up. Het hoogste percentage verbetering werd bereikt met de PDL monotherapie (gemiddeld [SD] 16% [34]), maar er was geen statistisch significant verschil tussen de verschillende behandelingen. Jeuk in het applicatie gebied was een vaak voorkomende bijwerking. Er waren geen serieuze bijwerkingen. We concludeerden dat het topicaal aanbrengen van de commercieel beschikbare oplossing van rapamycine (Rapamune® 0.1%), als aanvulling op de PDL behandeling, het oplichten van de wijnvlek niet lijkt te verbeteren.

In **hoofdstuk 2.2** beschrijven we een casus van een allergische contact dermatitis veroorzaakt door sirolimus (Rapamune® 0.1% drank), welke was gebruikt als aanvullende lokale behandeling van een wijnvlek tijdens de bovengenoemde studie. Hoewel er een aantal meldingen zijn van huidirritatie veroorzaakt door lokaal sirolimus is dit, voor zover ons bekend, de eerste melding van een daadwerkelijke allergische contract dermatitis.

In **hoofdstuk 3.1** vatten we de beschikbare gegevens met betrekking tot de effectiviteit en veiligheid van niet-invasieve methoden van anesthesie tijdens dermatologische laser behandelingen samen door middel van een systematische review. Gerandomiseerd en niet-gerandomiseerd gecontroleerde klinische trials (RCTs en CCTs) werden geïnclu-

deerd. Twintig RCTs en 12 CCTs werden geïncludeerd betreffende negen verschillende laser indicaties: ontharing (*n* = 9), resurfacing/rejuvenation (*n* = 5), wijnvlekken (*n* = 8), teleangiëctasieën op het been (*n* = 3), teleangiëctasieën in het gelaat (*n* = 2), tatoeages (*n* = 2), nevus van Ota (*n* = 1), lentigo solaris (*n* = 1), en humaan papillomavirus laesies (*n* = 1). De niet-invasieve methoden van anesthesie (d.w.z. lokale verdovende middelen, huid koeling, en pneumatic skin flattening [PSF]), soorten lasers, laser instellingen, applicatie tijd en soorten pijnschalen varieerden tussen de geïncludeerde studies. In het algemeen leken actieve methoden van anesthesie betere resultaten op te leveren dan placebo of geen anesthesie en leken lokale verdovende middelen en PSF te resulteren in een betere pijnreductie dan koeling van de huid. Alle studies hadden een onduidelijk tot hoog risico op bias en de algehele kwaliteit van de studies was laag. We concludeerden dat het huidige bewijs onvoldoende is om aanbevelingen te doen voor de dagelijkse klinische praktijk en dat er meer vergelijkende studies van hoge kwaliteit nodig zijn.

In **hoofdstuk 3.2** vergeleken we de effectiviteit van lidocaine/tetracaine crème (Pliaglis®) en lidocaine/prilocaine crème (EMLA®) in het verminderen van zelf-gerapporteerde pijn tijdens dermale laserbehandelingen. We voerden twee gerandomiseerde, dubbelblind, gecontroleerde klinische studies uit met een intra-patiënt, split-laesie design: studie A in patiënten met acne keloidalis nuchae (AKN) (*n* = 15), en studie B in patiënten met zwarte tatoeages (*n* = 15). Het primaire eindpunt was de zelf-gerapporteerde pijn van de patiënt op een 10 cm visual analog scale (VAS). De secundaire eindpunten waren het percentage patiënten met adequate pijnbestrijding en de veiligheid van de crèmes. In beide studies waren de VAS scores lager voor lidocaine/prilocaine crème, met een gemiddeld verschil in studie A van 1,9 (95% betrouwbaarheid interval [BI], 1,0 – 2,8) en in studie B van 0,6 (95% BI, -0,7 – 1,9). In studie A werd de pijnbestrijding adequaat bevonden in 13% (2/15) van de patiënten met lidocaine/tetracaine crème versus 73% (11/15) met lidocaine/prilocaine crème (*P* = 0,004) en in studie B in 53% (8/15) versus 80% (12/15) (*P* = 0.289), respectievelijk. Er waren geen serieuze bijwerkingen. We concludeerden dat lidocaine/prilocaine crème, aangebracht onder plastic occlusie, het voorkeursmiddel is voor lokale verdoving tijdens pijnlijke laserbehandelingen van dermale chromoforen.

In **hoofdstuk 4.1** onderzochten we de incidentietrends van lentigo maligna (LM) en lentigo maligna melanoom (LMM) tussen 1989 en 2013 in Nederland en schatten we het risico op een LMM na een LM. Gegevens over nieuw gediagnosticeerde LM en LMM werden verkregen via de Nederlandse Kanker Registratie en PALGA (Nederlandse pathologie database). Leeftijd-gestandaardiseerde incidentiecijfers aangepast voor de Europese standaardbevolking (ESR), geschatte jaarlijkse procentuele veranderingen (EAPC), en de cumulatieve incidentie van LMM na LM werden berekend. Daarnaast presenteerden we de 5-jaarsoverleving van zowel LM als LMM. Tussen 1989 en 2013 werden er in Nederland 10.545 patiënten gediagnostiseerd met een primair LM en 2.898

met een primair LMM. Tussen 1989 en 2013 steeg de ESR voor LM van 0,72 naar 3,84 per 100.000 persoonsjaren, en voor LMM van 0,24 naar 1,19 per 100.000 persoonsjaren. De incidentie van LM steeg van 2002 tot 2013 met 6,8% per jaar, voorafgaand aan de nog steilere stijging van de incidentie van LMM tussen 2007 en 2013 (EAPC: 12,4%). De cumulatieve incidentie van LMM na een primair LM na 25 jaar follow-up was 2,0% voor mannen en 2,6% voor vrouwen. De 5-jaarsoverleving was hoog voor zowel LM (104%) als LMM (99%). We concludeerden dat de gestegen incidentie van LM en LMM in Nederland, naast een toegenomen bewustzijn en toegenomen histologische bevestiging van LM, een werkelijke stijging lijkt weer te geven. Het absolute risico op een LMM (op elke locatie) na een histologisch bevestigd LM was laag. De resultaten van deze studie kunnen artsen en patiënten helpen om de voor- en nadelen van de verschillende behandelingen voor LM tegen elkaar af te wegen.

In **hoofdstuk 4.2** bepaalden we de recidiefkans, het cosmetisch resultaat en de patiënttevredenheid van de behandeling van LM met de ablatieve laser gevolgd door zes weken lokaal imiquimod 5% crème. De gegevens van patiënten met histologisch bewezen LM, die tussen 2008 en 2014 waren behandeld met de ablatieve laser gevolgd door imiquimod crème, werden verzameld. Patiëntendossiers en pathologie verslagen werden gebruikt om histologisch bewezen recidieven, cosmetische resultaten (0-10), behandeling gerelateerd ongemak (0-10), en of patiënten de behandeling aan anderen zouden aanbevelen (ja/nee), te bepalen. Vijfendertig patiënten werden geïdentificeerd en geëvalueerd, met een mediane (IQR) follow-up tijd van 19 (13-38) maanden. Lokale recidieven werden gevonden in zes patiënten. Vijf van deze zes recidieven waren gelokaliseerd op de neus (van de in totaal 15 patiënten met LM op de neus). Patiënten gaven het cosmetisch resultaat een gemiddelde score van 8,5 (95% BI, 8,2 – 8,9) en het ongemak tijdens de behandeling een gemiddelde score van 4,9 (95% BI, 3,9 – 5,9). Negentig procent van de patiënten zou deze behandeling aanbevelen aan anderen. Geen van de patiënten ontwikkelden een LMM. We concludeerden dat ablatieve laserbehandeling gevolgd door imiquimod crème een redelijk alternatief is voor LM patiënten die ongeschikt zijn voor chirurgische behandeling of die deze behandeling weigeren, op lokalisaties anders dan de neus. De grootste voordelen zijn de cosmetische en functionele resultaten. Echter, totdat er meer gegevens beschikbaar zijn is het belangrijk om de patiënten te informeren over de potentiële voor- en nadelen van de behandeling en blijft het cruciaal om deze patiënten intensief te vervolgen.

In **hoofdstuk 4.3** onderzochten we histologische parameters die mogelijk gerelateerd zijn aan de eerder geobserveerde hogere incidentie van LM recidieven op de neus na ablatieve laser behandeling gevolgd door lokaal imiquimod crème. We selecteerden willekeurig materiaal van 22 chirurgische excisies van LM op de neus en 22 van LM op de wang. Histologische analyses werden uitgevoerd op hematoxyline en eosine en microphthalmia transcription factor immunohistochemisch gekleurde slides. Het aantal

haar-talgklier-complexen (pilosebaceous units [PSU]) per mm, de maximale extensie van atypische melanocyten langs de huidadnexen en de maximale diepte van de PSU zelf werden bepaald. De neus had een significant hogere dichtheid van PSU dan de wang. De atypische melanocyten breidden zich dieper uit langs de PSU op de neus, met een gemiddelde (SD) diepte van 1,29 mm (0,48) versus een gemiddelde diepte van 0,72 mm (0,30) op de wang ($P < 0{,}001$). De maximale diepte van de PSU op de neus was groter dan die op de wang, met een gemiddelde (SD) diepte van 2,28 mm (0,41) versus 1,65 mm (0,82) ($P = 0{,}003$). We concludeerden dat het hogere recidief risico voor LM op de neus na niet-chirurgische behandeling, welke we eerder observeerden in ons cohort, het meest waarschijnlijk wordt veroorzaakt door een hogere densiteit van atypische melanocyten en hun diepere extensie in de follikels. Deze resultaten geven meer inzicht in onze eerdere bevindingen, laten zien dat de anatomische locatie relevant is voor het recidief risico van LM na niet-chirurgische behandeling en hebben geleid tot een aanpassing van ons behandelprotocol.

In **hoofdstuk 4.4** bepaalden we retrospectief de recidiefkans van LM na gefaseerde micrografische chirurgie (volgens 'Breuninger'). Gegevens van alle patiënten met LM die waren behandeld met onze methode van gefaseerde chirurgische excisie tussen 2002 en 2011 werden verzameld. Om recidieven te identificeren gebruikten we het computer programma Sympathy®, wat gelinkt is aan PALGA, een nationaal netwerk en register van histo- en cytopathologie in Nederland. We identificeerde 100 patiënten die waren behandeld met gefaseerde chirurgische excisie met 100% immuno-histopathologische controle van de laterale marges. Na een gemiddelde follow-up duur van 60 maanden werd er bij vier patiënten een recidief gevonden, na 37, 58, 74 en 77 maanden follow-up. We concludeerden dat gefaseerde chirurgische excisie superieur is ten opzichte van ruime lokale excisie voor LM en zou moeten worden overwogen als eerste keus behandeling in nationale en internationale richtlijnen.

In **hoofdstuk 5** bediscussiëren we de belangrijkste bevindingen van de studies in dit proefschrift en plaatsen we deze in perspectief. Daarnaast bespreken we onze overwegingen voor de toekomst wat betreft de behandeling van wijnvlekken, van pijn en andere ongemakken tijdens laser behandelingen, van LM, en van het verbeteren van laserbehandelingen en laseronderzoek in het algemeen.

Lasers kunnen worden gebruikt voor vele dermatologische indicaties en nieuwe mogelijkheden voor laser als primaire behandeling of als aanvulling op andere behandelingen worden steeds ontwikkeld. Echter, het maken van beslissingen in de laserdermatologie is vaak gebaseerd op de ervaringen en meningen van experts. Om goed onderbouwde aanbevelingen te kunnen doen voor de dagelijkse klinische praktijk is een meer evidence-based medicine ('geneeskunde op basis van bewijs') benadering nodig. Het toepassen van bewijs van goed ontworpen en goed uitgevoerd onderzoek, inclusief RCTs, systematische reviews en meta-analyses, moet worden benadrukt.

Chapter 7

Appendices

Abbreviations
List of co-authors
List of publications
Curriculum Vitae
PhD portfolio
Dankwoord

ABBREVIATIONS

AKN	acne keloidalis nuchae
CCT	controlled clinical trial
CI	confidence interval
CIE	Commission International de l'Eclairage
CO_2	carbon dioxide
COX	cyclo-oxygenase
EAPC	estimated annual percentage change
EF	energy fluence
Er:YAG	erbium-doped yttrium aluminium garnet
ESR	European standardized rate
HE	hematoxylin and eosin
HIF-1α	hypoxia-inducible factor 1-alpha
HPV	human papilloma virus
ICDO	International Classification of Diseases for Oncology
IPL	intense pulsed light
IQR	interquartile range
KTP	potassium titanyl phosphate
LASER	light amplification by the stimulated emission of radiation
LM	lentigo maligna
LMM	lentigo maligna melanoma
LP	lidocaine/prilocaine
LT	lidocaine/tetracaine
MITF	microphthalmia transcription factor
MMS	Mohs micrographic surgery
mTOR	mammalian target of rapamycin
NCR	Netherlands cancer registry
Nd:YAG	neodymium-doped yttrium aluminium garnet
NRS	numeric rating scale
NSAID	non-steroidal anti-inflammatory drug
pCR	pathological complete response
PDL	pulsed dye laser
PDT	photo dynamic therapy
pKa	acid dissociation constant
PSF	pneumatic skin flattening
PSU	pilosebaceous unit
PWS	port wine stain
RCT	randomized controlled trial

RPM	rapamycin
SD	standard deviation
SSE	staged surgical excision
TLR	Toll-like receptor
TRT	thermal relaxation time
USSR	United States standardized rate
VAS	visual analog scale
VEGF	vascular endothelial growth factor
WSR	World standardized rate

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CURRICULUM VITAE

Karin Greveling werd geboren op 20 maart 1987 in Breda. In 2005 behaalde zij haar gymnasium diploma aan het Stedelijk Gymnasium te Breda. Hetzelfde jaar startte zij haar studie geneeskunde aan de Universiteit van Maastricht. Het was tijdens haar oudste coschap (GEZP) op de afdeling Dermatologie van het Maastricht Universitair Medisch Centrum, dat haar interesse voor het vak dermatologie werd bevestigd. Nadat zij in 2012 haar artsexamen behaalde is zij, om algemene ervaring op te doen, gaan werken als arts niet in opleiding tot specialist (ANIOS) Interne Geneeskunde in het Amphia Ziekenhuis te Breda. In 2013 startte zij met haar promotieonderzoek op de afdeling Dermatologie van het Erasmus Medisch Centrum, onder begeleiding van haar promotor Prof. Prens en copromotor dr. van Doorn. Naast haar promotie werkte zij mee aan meerdere klinische trials. Sinds januari 2017 is zij in opleiding tot dermatoloog in het Erasmus Medisch Centrum te Rotterdam.

PHD PORTFOLIO

Name PhD student: Karin Greveling
Erasmus MC Department: Dermatology
PhD period: 2013-2017
Promotor: Prof. dr. E.P. Prens
Copromotor: Dr. M.B.A. van Doorn

1.PhD training	**Year**	**Workload (Hours / ECTS)**
Courses		
• NIHES: Biostatististical Methods I: Basic Principles Part A	2016	1.0 ECTS
• Research Integrity	2015	0.3 ECTS
• Biomedical English Writing and Communication	2015	3.0 ECTS
• Laser course (preconference), Innsbruck	2014	3 hours
• Molmed: The Basic introduction course on SPSS	2014	1.0 ECTS
• Molmed: Biomedical English Writing	2014	2.0 ECTS
• MolMed: Research management for PhD students	2014	1.0 ECTS
• MolMed: Photoshop and Illustrator CS6 Workshop	2014	0.3 ECTS
• Basiscursus Regelgeving en Organisatie voor Klinische onderzoekers (BROK)	2013	20 hours
• Systematisch literatuuronderzoek in Pubmed	2013	5 hours
• Systematisch literatuuronderzoek andere databases	2013	3.5 hours
• EndNote	2013	3.5 hours
• Open Clinica	2013	8 hours
Oral presentations		
• Erasmus Aesthetics Congres, Rotterdam, The Netherlands. *Pijn management bij laser therapie*	2016	1.0 ECTS
• Skintermezzo, Rotterdam, The Netherlands. *EMLA vs. Pliaglis – Resultaten van de OPTICA trial*	2016	1.0 ECTS
• Wetenschappelijke jaarvergadering NVED, Lunteren, The Netherlands. *Epidemiology of Lentigo Maligna and Lentigo Maligna Melanoma in the Netherlands, 1989 – 2013*	2016	1.0 ECTS
• Wetenschappelijke vergadering NVDV, Rotterdam, The Netherlands. *Lentigo Maligna en Cosmetiek*	2015	1.0 ECTS
• 3rd PhD weekend, Wassenaar, The Netherlands. *Lasers – werkingsmechanisme*	2015	1.0 ECTS
• 2nd PhD weekend, Maastricht, The Netherlands. *Pre-trial*	2014	1.0 ECTS
• Skintermezzo, Rotterdam, The Netherlands. *Laser assisted drug delivery in dermatology*	2013	1.0 ECTS
Conferences (attending)		
• 4th PhD weekend, Antwerp, Belgium (organizing committee)	2016	1.0 ECTS
• AAV research day Erasmus MC	2015	6 hours
• National PhD day, Den-Haag	2014	1.0 ECTS

• EADV, Amsterdam, The Netherlands	2014	1.0 ECTS
• Laser Innsbruck, Innsbruck, Austria	2014	1.0 ECTS
• Wetenschappelijke jaarvergadering NVED, Lunteren, The Netherlands	2014	1.0 ECTS
Other		
• Research meetings and Journal Clubs, Department of Dermatology, Erasmus MC	2013-2016	2.0 ECTS
• Skintermezzo meetings, Department of Dermatology, Erasmus MC, Rotterdam, The Netherlands	2013-2016	1.0 ECTS
2. Teaching		
• ICK education medical students, Erasmus MC, Rotterdam	2016	6 hours
• Supervising small research projects Liu Liu	2016	0.5 ECTS
• Supervising Master's thesis Neeltje ten Bosch	2015	2.0 ECTS

DANKWOORD

Het is af! Tot slot wil ik graag nog een aantal mensen bedanken.

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