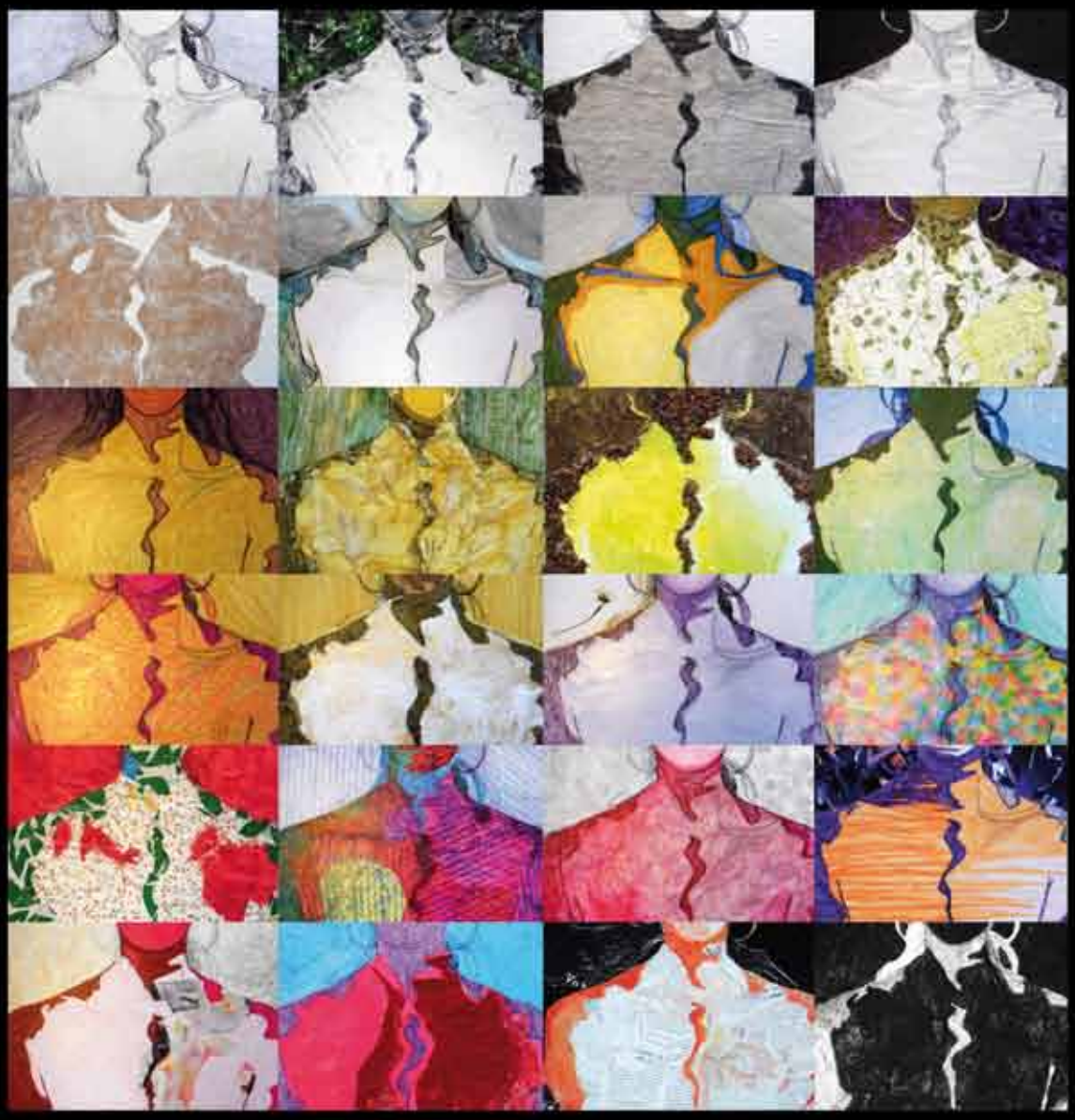
PHOTO- AND LASER THERAPY IN PIGMENT DISORDERS



B.S. Wind

PHOTO- AND LASER THERAPY IN PIGMENT DISORDERS

Bas Sander Wind

Photo- and lasertherapy in pigment disorders

ISBN: 978-94-90371-84-5

Cover painting: 'vitiligo' by Bea Peter (www.beapeter.nl)

Layout and printing: Offpage, www.offpage.nl

Publication of this thesis was financially supported by: ABBOTT Immunology, Afdeling Huidziekten, ALK-Abelló, Actavis BV, Astellas Pharma BV, BAP-medical BV, Bauerfeind Benelux BV, Beiersdorf NV, Huidstichting Chanfleury van IJsselstein, Dalton Medical Laser & Light Technology, Fagron, Galderma SA, GlaxoSmithKline BV, Laser Medico BVBA, Laservision Instruments BV, La Roche-Posay, LEO Pharma BV, Louis Widmer NL, Landelijke Vereniging voor Vitiligo-Patiënten, Medi Nederland BV, Medizorg, Philips Lighting BV, Pfizer BV, Solta Medical, Stichting Nederlands Instituut voor Pigmentstoornissen, Stichting EIS, Tobrix, Universiteit van Amsterdam, Van der Bend BV, Waldmann BV

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ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
Prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties ingestelde
commissie, in het openbaar te verdedigen in de Agnietenkapel
op dinsdag 14 juni 2011, te 14.00 uur

door

Bas Sander Wind
geboren te Groningen

PROMOTIECOMMISSIE:

Promotor:	Prof. dr. J.D. Bos
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Overige leden:	Prof. dr. W.R. Faber Prof. dr. N. van Geel Prof dr. M. Hædersdal Prof. dr. C.M.A.M. van der Horst Dr. G.N. Relyveld

Faculteit der Geneeskunde

CONTENTS

1

GENERAL INTRODUCTION

INTRODUCTION

This thesis deals with new therapies for the cutaneous pigment diseases vitiligo, melasma, and Becker's nevus. In this introduction, they are shortly described as to what they are and what the present status of therapy is.

VITILIGO

Vitiligo is a chronic skin disease, characterized by destruction of skin melanocytes leading to sharply depigmented macules. This acquired pigment disorder is common in all races, regardless of age and sex and affects 1-2% of the world population.(1) The course of the disease is unpredictable, but is often slowly progressive. Depigmented patches may be present in a localized asymmetric form with a focal or segmental (dermatomal) distribution or in a generalised symmetric form with an acrofacial, disseminated or universal distribution.(2)

To date, no curative treatment is available. Non-surgical modalities, considered as first-line therapy, include topical corticosteroids. However, the effect of topical therapy alone is poor.(3)

Secondly, narrowband ultraviolet B (NB-UVB) therapy is another often used modality.(1,4-6) Outpatient clinic based NB-UVB therapy often is, however, a considerable burden for the patient. Classical institutional NB-UVB is effective, but time consuming as it requires two visits each week during six to 12 months. Because of these disadvantages of outpatient UVB therapy, home UVB therapy was introduced in 1979, primarily for psoriasis (7-11), and in the early 1990s for vitiligo as well.(12)

UVA has also been advocated for the treatment of vitiligo, however efficacy has shown to be poor.(3) Repeated multiple exposures to either UVA or NB-UVB result in a marked increase in the number and functional state of active melanocytes, and they are considered as some of the strongest stimuli to induce repigmentation.(13,14) However, irradation probably promotes photoageing and photocarcinogenesis.(15-17)

Recently, the red Helium-Neon (HeNe) laser (632.8 nm) was introduced as a possible inducer of repigmentation in vitiligo. The mechanism of action thereof remains unclear, but as the HeNe laser is a low energy laser, thermal effects on the irradiated tissues are considered to be minute and effects are generally attributed to direct biostimulation of exposed cells.(15,16)

Finally, in therapy-resistant non-progressive vitiligo, autologous punch grafting is widely used; it is relatively inexpensive, easy to perform, and successful.(17,18) Post-operative irradiation (natural sun exposure, UVA, NB-UVB) has been suggested to improve pigment cell outgrowth and pigment production after skin transplantation, but is time consuming and could induce photoageing and photocarcinogenesis.(15-17)

1 MELASMA

Melasma is a common pigment disorder, characterized by symmetric hyperpigmented patches on the face, which often causes significant emotional and psychosocial suffering, thus negatively influencing the patient's quality of life.(20) Melasma is encountered in all skin types, but (of course) particularly in ethnic skin.(21) The pathogenesis of melasma is not fully understood. Genetic background and sun exposure seem to be the most important etiologic factors besides pregnancy, systemic drugs, hormonal medication and phototoxic or photoallergic cosmetics.(22)

Melasma is often difficult to manage because of its refractory and recurrent nature. Current treatments include topical bleaching creams, chemical peels and laser therapy. However, results are often disappointing. Treatment of choice is triple topical therapy, a combination of topical bleaching agents that was first introduced in 1975 as the 'Kligman formula'. It contains hydroquinone, tretinoin and dexamethasone. Nowadays, dexamethasone is frequently replaced by various other moderately potent to potent corticosteroids.(23,24) The results of laser therapy and intense pulsed light therapy in melasma are generally disappointing and treatment is limited by adverse effects such as postinflammatory hyperpigmentation, especially in dark-skinned patients. Therefore, these approaches are controversial.(25,26)

Recently, non-ablative fractional laser therapy at 1550 nm was reported as a promising treatment for melasma.(27,28) At this wavelength water absorption is predominant. In fractional laser therapy multiple small sized coagulated zones are separated by surrounding untreated tissue.(29) It was reported that these microscopic treatment zones allow transport and extrusion of microscopic epidermal necrotic debris including melanin from melanocytes through a compromised dermal-epidermal junction.(29,30) Generally, a visible wound does not appear because these microscopic treatment zones have a diameter less than 100 micrometer.(29) The stratum corneum was found to be intact after 24 hours.(31,32) Moreover, as the microscopic treatment zones are surrounded by untreated tissue, recovery is relatively fast and inflammation is mild.

BECKER´S NEVUS

Becker's nevus is a relatively common skin disorder, characterized by the development of unilateral hyperpigmented patches, that eventually develop a slightly elevated, with a sometimes verrucous surface and often hypertrichosis in 56-70% of male cases.(33-35) The prevalence ranges from 0.25 to 2.5%, and is about five times more frequent in males than in females.(34-37) Little is known about the pathogenesis of the disorder, but increased androgen sensitivity of fibroblasts has been suggested as a possible etiologic factor.(38-42) Currently, no treatment is available for Becker's nevus. Studies on intense pulsed light and Quality-switched ruby laser showed disappointing

or even adverse effects.(43,44) Better results have been achieved with erbium yttrium-aluminium-garnet (YAG) laser and long-pulsed alexandrite laser.(44,45)

Recently, non-ablative fractional laser therapy was suggested as treatment option for Becker's nevus.(46) The main principle of fractional laser therapy is the coagulation or ablation of small columns of skin, leaving the surrounding tissue intact.(29) This enhances healing of the treated and coagulated skin after treatment, minimizing the risk for unwanted effects. Ablative fractional laser therapy at 10600 nm might be even more effective in the treatment of Becker's nevus than non-ablative fractional laser therapy, as complete ablation of microscopic treatment zones takes place instead of coagulation, preventing a possible reuptake of melanin from the microscopic treatment zones by dermal macrophages and keratinocytes.

AIMS OF THIS THESIS

Treatment of pigment disorders is certainly challenging. In non-segmental vitiligo, home UVB therapy and the necessity of post-operative irradiation after punch grafting are debated in this thesis. Furthermore, following the introduction of fractional laser devices in 2004, promising results in various pigment disorders, especially melasma, have been published. However, randomized clinical trials to definitely prove safety and effectiveness, have as yet not been published. Therefore, this thesis discusses (i) the efficacy and safety of home UVB therapy, (ii) the effect of different light sources on outgrowth of pigment from punch grafts, in non-segmental vitiligo; (iii-v) the efficacy and safety of non-ablative and ablative fractional laser therapy in melasma and in Becker's nevus, and finally (vi) the histopathological differences between non-ablative and ablative fractional laser.

Home UVB therapy for the treatment of vitiligo has been debated since its introduction in the early 1990s. Non-evidence based but understandable fear is often expressed about higher risks regarding inaccurate dosimetry, phototoxicity, suboptimal treatment, and unsupervised continuation of irradiations, photoageing and carcinogenicity. **Chapter 2** is the first study to provide pro's and cons of home UVB therapy versus outpatient UVB therapy in patients with non-segmental vitiligo.

Post-operative irradiation (UVA, NB-UVB, HeNe laser) has been suggested to improve pigment outgrowth after punch grafting, but is time consuming and UVA and NB-UVB could promote photoageing and photocarcinogenesis. In **Chapter 3** pigment outgrowth of punch grafts after irradiation with UVA, NB-UVB and HeNe laser is compared to no phototherapy in patients with non-segmental vitiligo.

Non-ablative fractional laser therapy at 1550 nm has been reported as an effective treatment for melasma, although there is minimal evidence for its efficacy and controlled trials were lacking. In **Chapter 4** is a randomized inter-patient study to assess efficacy and safety of non-ablative 1550 nm fractional laser therapy at 10 mJ/microbeam compared with the gold standard (triple topical therapy).

As non-ablative 1550 nm fractional laser therapy at 10 mJ/microbeam proved relatively safe and effective, in **Chapter 5** an intra-patient study was performed to compare non-ablative 1550 nm fractional laser therapy with triple topical therapy, using more aggressive settings and long term intermittent maintenance bleaching during follow-up.

Non-ablative fractional laser therapy has been suggested as a treatment option for Becker's nevus. Ablative fractional laser therapy might be even more effective, as complete ablation of microscopic treatment zones takes place instead of coagulation, preventing a possible reuptake of melanin from the microscopic treatment zones by dermal macrophages and keratinocytes. In **Chapter 6** efficacy and safety of ablative fractional laser therapy in the treatment of Becker's nevus was assessed.

Fractional laser therapy has become a widely accepted modality and creates multiple small sized coagulated zones, separated by surrounding untreated tissue. Histological studies have shown that permanent tissue damage is usually minimal or absent after either non-ablative or ablative fractional laser. However, histological comparisons between non-ablative and ablative fractional laser have not been published. In **Chapter 7** the histological outcome of non-ablative and ablative fractional laser was compared.

REFERENCE LIST

1. Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. Am J Clin Dermatol 2001;2(3): 167-81.
2. Mosher DB, Fitzpatrick TB, Ortonne JP. Disorders of pigmentation. In Fitzpatrick TB, Freedberg IM, editors. Fitzpatrick's dermatology in general medicine. 5th ed. New York: McGraw-Hill, Health Professions Division, 1999: 945-55
3. Westerhof W, Nieuweboer-Krobotová L, Mulder PG, Glazenburg EJ. Left-right comparison study of the combination of fluticasone propionate and UV-A vs. either fluticasone propionate or UV-A alone for the long-term treatment of vitiligo. Arch Dermatol 1999;135(9):1061-6.
4. Whitton ME, Ashcroft DM, Barrett CW, Gonzalez U. Interventions for vitiligo. Cochrane Database Syst Rev 2006;(1):CD003263.
5. Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PM. Nonsurgical repigmentation therapies in vitiligo. Meta-analysis of the literature. Arch Dermatol 1998;134(12):1532-40.
6. Grimes PE. New insights and new therapies in vitiligo. JAMA 2005;293(6):730-5.
7. Milstein HJ, Vonderheid EC, Van Scott EJ, Johnson WC. Home ultraviolet phototherapy of early mycosis fungoides: preliminary observations. J Am Acad Dermatol 1982;6(3):355-62.
8. Resnik KS, Vonderheid EC. Home UV phototherapy of early mycosis fungoides: long-term follow-up observations in thirty-one patients. J Am Acad Dermatol 1993;29(1):73-7.
9. Larko O, Swanbeck G. Home solarium treatment of psoriasis. Br J Dermatol 1979;101(1):13-6.
10. Jordan WP, Jr., Clarke AM, Hale RK. Long-term modified Goeckerman regimen for psoriasis using an ultraviolet B light source in the home. J Am Acad Dermatol 1981;4(5):584-91.
11. Lowe NJ. Home ultraviolet phototherapy. Semin Dermatol 1992;11(4):284-6.
12. Koster W, Wiskemann A. [Phototherapy with UV-B in vitiligo]. Z Hautkr 1990;65(11):1022-4, 1029.
13. Pathak MA. Effect of UV-A, UV-B and psoralen on in vivo human melanin pigmentation. Pigment Cell. 1976;3:291-8.

14. Miyamura Y, Coelho SG, Wolber R, Miller SA, Wakamatsu K, Zmudzka BZ, Ito S, Smuda C, Passeron T, Choi W, Batzer J, Yamaguchi Y, Beer JZ, Hearing VJ. Regulation of human skin pigmentation and responses to ultraviolet radiation. Pigment Cell Res. 2007;20(1):2-13.
15. Yu HS, Wu CS, Yu CL, Kao YH, Chiou MH. Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo. J Invest Dermatol. 2003;120(1):56-64.
16. Lan CC, Wu CS, Chiou MH, Hsie PC, Yu HS. Low-energy helium-neon laser induces melanocyte proliferation via interaction with type IV collagen: visible light as a therapeutic option for vitiligo. Br J Dermatol. 2009;161(2):273-80.
17. Falabella R. Grafting and transplantation of melanocytes for repigmenting vitiligo and other types of leukoderma. Int J Dermatol. 1989;28(6):363-9.
18. Westerhof W, Boersma B. The minigrafting test for vitiligo: detection of stable lesions for melanocyte transplantation. J Am Acad Dermatol. 1995;33(6):1061-2.
19. Lahiri K, Malakar S, Sarma N, Banerjee U. Repigmentation of vitiligo with punch grafting and narrow-band UV-B (311 nm)--a prospective study. Int J Dermatol. 2006;45(6):649-55.
20. Pawaskar MD, Parikh P, Markowski T, McMichael AJ, Feldman SR, Balkrishnan R. Melasma and its impact on health-related quality of life in Hispanic women. J Dermatol Treat 2007;18:5-9.
21. Gupta AK, Gover MD, Nouri K, Taylor S. Treatment of melasma: a review of clinical trials. J Am Acad Dermatol 2006;55:1048-65.
22. Grimes PE. Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995;131:1453-7.
23. Gano SE, Garcia RL. Topical tretinoin, hydroquinone, and betamethasone valerate in the therapy of melasma. Cutis 1979; 23:239-41.
24. Taylor SC, Torok H, Jones T, Lowe N, Rich P, Tschen E, et al. Efficacy and safety of a new triple-combination agent for the treatment of facial melasma. Cutis 2003;72:67-72.
25. Rendon M, Berneburg M, Arellano I, Picardo M. Treatment of melasma. J Am Acad Dermatol 2006;54(5 Suppl. 2):S272-81.
26. Picardo M, Carrera M. New and experimental treatments of cloasma and other hypermelanoses. Dermatol Clin 2007;25:353-62.
27. Tannous ZS, Astner S. Utilizing fractional resurfacing in the treatment of therapy-resistant melasma. J Cosmet Laser Ther 2005;7:39-43.
28. Rokhsar CK, Fitzpatrick RE. The treatment of melasma with fractional photothermolysis: a pilot study. Dermatol Surg 2005;31:1645-50.
29. Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004;34:426-38.
30. Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. J Biomed Opt 2006;11:1-9.
31. Laubach HJ, Tannous Z, Anderson R, Manstein D. Skin responses to fractional photothermolysis. Lasers Surg Med 2006;38:142-9.
32. Goldberg DJ, Berlin AL, Phelps R. Histologic and ultrastructural analysis of melasma after fractional resurfacing. Lasers Surg Med 2008;40:134-8.
33. Becker SW. Concurrent melanosis and hypertrichosis in distribution of nevus unius lateris. Arch Derm Syphilol 1949;60:155-60.
34. Tymen R, Forestier JF, Boutet B, Colomb D. Naevus tardif de Becker, a propos d'une série de 100 observations. Ann Dermatol Venereol 1981;108:41-6.
35. Ingordo V, Gentile C, Iannazzone SS, Cusano F, Naldi L. The 'EpiEnlist' project: a dermo-epidemiologic study on a representative sample of young Italian males. Prevalence of selected pigmentary lesions. J Eur Acad Dermatol Venereol 2007;21:1091-6.
36. McLean DI, Gallagher RP. "Sunburn" freckles, cafe-au-lait macules, and other pigmented lesions of schoolchildren: the Vancouver Mole Study. J Am Acad Dermatol 1995;32:565-70.
37. Kim YJ, Han JH, Kang HY, Lee ES, Kim YC. Androgen receptor overexpression in Becker nevus: histopathologic and immunohistochemical analysis. J Cutan Pathol 2008;35:1121-6.
38. Grande SH, Harris R, Hansen CD, Callis Duffin KP, Florell SR, Hadley ML. Androgen receptor expression patterns in Becker's nevi: an immunohistochemical study. J Am Acad Dermatol 2008;59:834-8.

39. Nirde P, Dereure O, Belon C, Lumbroso S, Guilhou JJ, Sultan C. The association of Becker nevus with hypersensitivity to androgens. Arch Dermatol 1999;135:212-4.
40. Formigon M, Alsina MM, Mascaro JM, Rivera F. Becker's nevus and ipsilateral breast hypoplasia--androgen-receptor study in two patients. Arch Dermatol 1992;128:992-3.
41. Person JR, Longcope C. Becker's nevus: an androgen-mediated hyperplasia with increased androgen receptors. J Am Acad Dermatol 1984;10:235-8.
42. Moreno Arias GA, Ferrando J. Intense pulsed light for melanocytic lesions. Dermatol Surg 2001;27:397-400.
43. Kopera D, Hohenleutner U, Landthaler M. Quality-switched ruby laser treatment of solar lentigines and Becker's nevus: a histopathological and immunohistochemical study. Dermatology 1997;194:338-43.
44. Trelles MA, Allones I, Moreno-Arias GA, Velez M. Becker's naevus: a comparative study between erbium:YAG and Q-switched neodymium:YAG; clinical and histopathological findings. Br J Dermatol 2005;152:308-13.
45. Choi JE, Kim JW, Seo SH, Son SW, Ahn HH, Kye YC. Treatment of Becker's Nevi With a Long-Pulse Alexandrite Laser. Dermatol Surg 2009:1105-8.
46. Glaich AS, Goldberg LH, Dai T, Kunishige JH, Friedman PM. Fractional resurfacing: a new therapeutic modality for Becker's nevus. Arch Dermatol 2007;143:1488-90.

2

HOME VERSUS OUTPATIENT NARROWBAND UVB THERAPY FOR THE TREATMENT OF NON-SEGMENTAL VITILIGO: A RETROSPECTIVE QUESTIONNAIRE STUDY

British Journal of Dermatology. 2010;162(5):1142-4.

ABSTRACT

Background: Generally, narrowband ultraviolet B (NB-UVB) therapy is carried out in an outpatient setting. Data on home NB-UVB therapy in vitiligo are lacking.

Objectives: To compare home with outpatient NB-UVB therapy for the treatment of non-segmental vitiligo.

Materials and Methods: 104 consecutive patients with non-segmental vitiligo who completed total body NB-UVB therapy were included from March 2008 until January 2009. Patients were asked to fill in a questionnaire on efficacy, side effects, and patients' satisfaction.

Results: The overall response rate was 86% (home patients 89%/outpatients 80%). Generally, repigmentation was reported to start in the first six months (home patients: 68%/outpatients: 65%; ns). Mean overall repigmentation was slightly higher in the home group (ns). Acral areas tended to repigment less. Occurrence of acute side effects was comparable (home patients 7%/outpatients 6%). Total cumulative dose was not significantly different (home patients: 107 J/cm^2/outpatients 123 J/cm^2; ns). Outpatients were significantly more satisfied with the result (59 vs 33%; $p<0.05$). Most patients in both groups would recommend their treatment to friends. Time investment per week was significantly less in the home group (31 vs 125 minutes; $p<0.001$).

Conclusions: This study shows that patient-reported outcomes of home and outpatient NB-UVB therapy are comparable with a similar pigmentation and occurrence of side effects. However, satisfaction with the result was significantly lower in the home group. Finally, time investment for home patients was significantly less. Therefore, we suggest that home NB-UVB therapy is a valuable alternative to outpatient NB-UVB therapy in the treatment of non-segmental vitiligo.

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INTRODUCTION

Vitiligo is a chronic skin disease, characterised by depigmented macules due to loss of melanocytes. This acquired pigment disorder is common in all races, regardless of age and sex and affects 1-2% of the world population.(1)

To date, no curative treatment is available. Non-surgical modalities, considered as first-line therapy, include topical therapy (mainly corticosteroids), and narrowband ultraviolet B (NB-UVB) therapy.(1-4)

NB-UVB therapy has substantial consequences for the patient because it is usually delivered in an outpatient clinic. NB-UVB therapy itself takes only a few minutes, but patients have to travel to the outpatient clinic during working hours twice a week. Because of the disadvantages of outpatient UVB therapy, home UVB therapy was first introduced in 1979, mainly for psoriasis (5-9), and in the early 1990s for vitiligo.(10)

In the Netherlands, two home care institutions provide UVB equipment and nursing supervision for about 200 vitiligo patients annually (215 patients, registry data 2007). Safety, effectiveness and compliance concerning home treatment have been debated. Non-evidence based but understandable fear is often expressed about higher risks regarding inaccurate dosimetry, phototoxicity, suboptimal treatment, and unsupervised continuation of irradiations after treatment has finished.(11-14) Home UVB therapy for psoriasis has been debated since its introduction thirty years ago.(15;16) Afore mentioned possible disadvantages might lead to ineffective therapy, higher occurrence of acute side effects and to increased total cumulative doses of UVB.

This study is the first to provide data on the pro's and cons of home UVB therapy versus outpatient UVB therapy in patients with non-segmental vitiligo.

MATERIALS AND METHODS

Patients from this study had all been referred to the Netherlands Institute for Pigment Disorders (SNIP), a specialized vitiligo clinic affiliated with the Department of Dermatology of the Academical Medical Center of the University of Amsterdam. From March 2008 until January 2009, all consecutive patients with generalized non-segmental vitiligo who completed total body NB-UVB therapy, either at the SNIP or at home, were included. All patients were mailed a package containing a questionnaire and a stamped return envelope. Patients were asked to return the completed questionnaires. In case a patient did not respond, the patient was reminded by phone with a maximum of five follow-up calls. Medical ethics approval was not required as in the Netherlands approval is only required for self-report questionnaires containing intrusive items.

Sample size

The sample size could not be calculated on the basis of assumed differences in effectiveness as (i) no data are available on possible differences in treatment effects, (ii) it is expected that the effectiveness of both therapies is similar.

NB-UVB therapy and equipment

Outpatient NB-UVB therapy was performed with circular Waldmann UV-1000 units (Waldmann Villingen-Schwenningen, Germany), comprised of TL-01 tubes. These units were scheduled in dosage (J/cm^2). The home NB-UVB therapy units were semi-circular Waldmann UV-100 units with TL-01 tubes. These units do not have an irradiation intensity indicator; hence treatments were prescribed in units of time (seconds). The devices were delivered and recollected by the home care institutions, of which trained nursing staff cared for instruction and supervision of NB-UVB treatment. The direct costs for NB-UVB treatment were comparable and covered by insurance companies. The treatment schedule was in line with our outpatient schedule, and consisted of (i) irradiation twice a week on non-consecutive days, (ii) when no direct suberythema directly or shortly after irradiation occurred, patients were advised to increase by 50 mJ/cm^2 for all skin-types, (iii) when suberythema occurred, an equal dose was administered, (iv) and when painful erythema occurred, one treatment was omitted. No minimal erythema dose was tested before start of NB-UVB therapy. Additional treatment was allowed if the dermatologist had decided it was necessary. Hence, this was no reason for exclusion. The SNIP and the home care institutions measured the light intensity of every unit before the first and after the last irradiation. Patients kept a record of their treatment times. For all patients standardised cumulative doses were calculated using the intensity measurements together with the individual treatment charts. Three-month follow-up visits were performed in all patients.

Outcome assessment

To answer the separate research questions, a short five-minute questionnaire was designed, as there were no comparable questionnaires available in the literature. The main outcome was assessed with six questions on patient characteristics, four questions on NB-UVB therapy, three questions on efficacy, three questions on safety, one question on patient reported repigmentation and six questions on satisfaction.

Statistical analysis

Means, standard deviations, two-tailed homoscedastic Student's t-tests, Chi square tests, Chi square association tests, Kruskal Wallis tests, Spearman rank correlation tests, ANOVA tests, and Mann Whitney U tests were performed with Statistical Package for the Social Sciences 12.0 (SPSS, Chicago, IL). Student's t-tests were used unless otherwise noted.

RESULTS

A total of 104 eligible patients with non-segmental vitiligo were sent a questionnaire. Fifty-seven of 64 home patients (89%) and 32 of 40 outpatients (80%) responded [overall response rate 86%; not significant (ns)]. Patient characteristics are described in Table 1. Home patients received significantly more treatments per week than outpatients (2.3 versus 1.9; $p<0.001$). However, total cumulative dose was not significantly different between the groups. No other significant differences were found in the NB-UVB regimen between the home and outpatient group (Table 2).

Generally, repigmentation was reported to start in the first six months (68% of home patients, and 65% of outpatients; ns) (Fig. 1). No association was found between the frequency of suberythema and start of repigmentation in both groups (home patients r_s=-0.67 (53); ns / outpatients r_s=0.11 (29); ns).

In both groups the majority of patients were satisfied or very satisfied with the supervision of nursing staff and physicians (ns; Table 3). Mean overall repigmentation

Table 1 Patient characteristics

	Home patients	Outpatients
Male:female ratio	1:2	1:1.3
Mean age ± SD (min-max)	39 ± 16 (8-68)	43 ± 14 (10-70)[#]
Disease duration > 5 years (%)	63	56[#†]
Skin type (%)		
I	0	0
II	40	19
III	53	16
IV	3	40
V	2	19
VI	2	6
Previous treatments (%)		
Total NB-UVB	68	69[#]
Home NB-UVB	46	19
Outpatient NB-UVB	22	50
Total in years	0.5	0.7[#]
UVA	0	0
PUVA	9	13[#]
Topical treatment	39	28[#§]
Transplantation	11	26[#§]

[#] ns
[†] Mann Whitney U test
[§] Chi square association test

Table 2 Characteristics and results of NB-UVB therapy.

	Home patients	Outpatients
Mean number of treatments per week	2.3	1.9*
12 months or more NB-UVB treatment (%)	69	71#
Mean total cumulative dose ± SD (J/cm²)	107±126	123±111#†
Missed - more than 10 treatments (%)	25	16#†
Suberythema - more than 20 times (%)	42	44#
Painful erythema - more than 10 times (%)	7	6#
Blistering (%)	0	3#

Home patients used NB-UVB therapy significantly more often than outpatients (p<0.001).
* p<0.001
# ns
† Mann Whitney U test

was slightly higher in the home group than in the outpatient group (ns) (Table 4). Acral areas tended to repigment less than other areas.

Outpatients were significantly more satisfied with the result after finishing NB-UVB therapy (59 vs 33%; p<0.05) (Fig. 2). No association was found between start of repigmentation and satisfaction in both groups (home patients r_s=0.02 (53); ns / outpatients r_s=0.01 (29); ns).

There was no relation between skin type and: (i) start of repigmentation (χ^2 (4)=0.30; ns), (ii) frequency of suberythema (χ^2 (4)=1.15; ns), (iii) satisfaction (F(4,82)=1.04; ns).

90% of home patients and 100% of outpatients reported to have been sufficiently educated before start of and during NB-UVB therapy (Fishers exact test; ns). Most patients would recommend their treatment to friends or other patients (96% of home patients, 90% of outpatients; ns). The mean time investment per week was significantly lower in the home group (31 versus 126 minutes) (p<0.001).

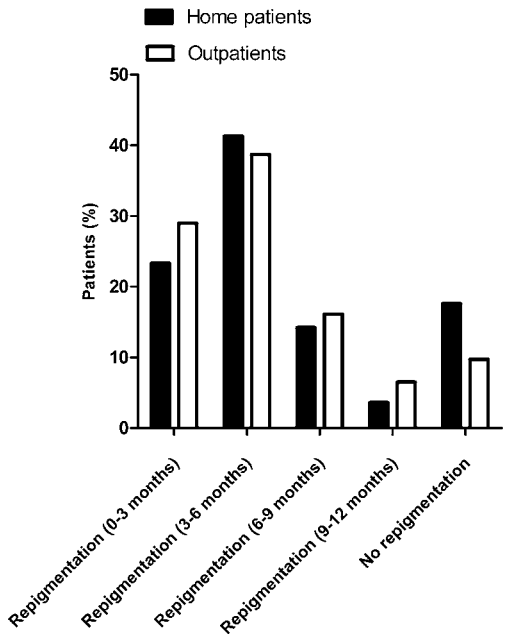

Fig 1. Onset of repigmentation per patient group. No significant differences were found between groups (Mann Whitney U test).

Table 3 Satisfaction about supervision by nursing staff and physicians (%) after finishing NB-UVB therapy.

	Home patients	Outpatients
(Very) satisfied	77	88
Moderately satisfied	9	9
Ambivalent	9	3
Moderately unsatisfied	2	0
(Very) unsatisfied	3	0

No significant differences were found between groups (Mann Whitney U test).

Table 4 Mean repigmentation (%) per treatment area after finishing NB-UVB therapy.

	Home patients	Outpatients
Face	45	30[#]
Neck	45	32[#]
Trunk	31	30[#]
Arms	38	33[#]
Hands	19	17[#]
Legs	37	30[#]
Feet	11	7[#]

[#] ns

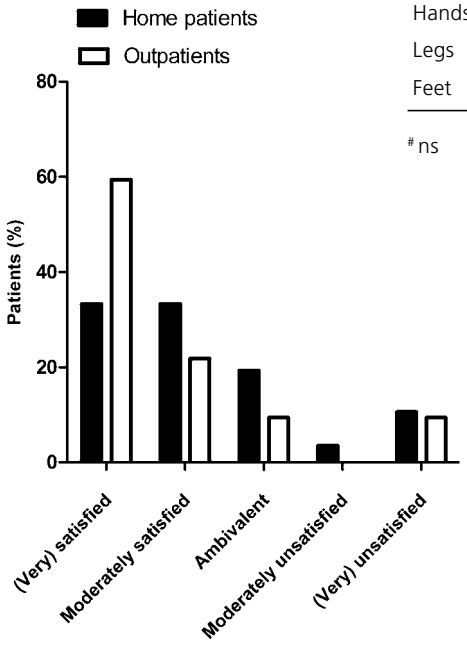

Fig 2. Satisfaction with result after one year NB-UVB therapy per patient group. Outpatients were significantly more satisfied with the result after one year (Mann Whitney U test: 659.5; $p<0.05$).

DISCUSSION

The present study shows that home and outpatient NB-UVB therapy are comparable with a similar occurrence of side effects. Therefore we regard home NB-UVB therapy as a valuable alternative to outpatient NB-UVB therapy in the treatment of non-segmental vitiligo.

To our knowledge, this is the first study comparing home and outpatient NB-UVB therapy for the treatment of vitiligo. Usually, NB-UVB therapy is performed in an outpatient setting. To date, one randomized and two observational studies on home NB-UVB therapy in psoriasis reported favourable results.(16-18) In a randomized study, Koek et al. recently showed that home NB-UVB therapy was equally safe and effective, and a useful alternative to outpatient NB-UVB therapy for the treatment of psoriasis.(16) This outcome is in line with our results.

Our study has its limitations as it is (i) not randomised and retrospective design, (ii) based on daily practice, (iii) unbalanced regarding skin types involved, and finally (iv) based primarily on patient reported outcomes.

The allocation of subjects to either home NB-UVB therapy or outpatient NB-UVB therapy was not random but based on the patient's choice and practical considerations. Patients residing nearby our department tended to receive outpatient NB-UVB therapy while patients from far off tended to have home NB-UVB therapy. As our institute is located in a multiethnic city with more than 50% of its inhabitants originating from non-Western societies, we noticed a marked difference in skin types between groups which may have biased our results. Furthermore, as this study was based on daily practice, additional treatments during NB-UVB therapy were allowed. This design ensures that our results can be generalised. Finally, this study lacked objective measures of disease severity and treatment results and therefore could show bias in these respects. However, the consistency of the data between groups and the agreement of our results with the literature on outpatient NB-UVB supports the value of our data. Accordingly, the self-reported repigmentation at different body sites is in line with those reported in the literature.

The concerns on home NB-UVB therapy expressed among dermatologists are (i) over-treatment, (ii), higher occurrence of short- and longterm side effects, (iii) and reduced treatment efficacy. Firstly, there was a significant difference in number of weekly treatments, but the outpatients were slightly less treated than the advised two treatments per week (1.9). Secondly, home and outpatient NB-UVB therapy were equally safe, as judged by the similar rate of acute side effects. Longterm side effects are expected to be comparable between groups, as total cumulative doses were not significantly different. The total cumulative dose in our study (home group: 107 J/cm^2 / outpatient group: 123 J/cm^2) was lower than previously reported in two other studies on NB-UVB outpatient therapy in vitiligo (155 and 201 J/cm^2 after one year, respectively).(19;20) Concerns about unsupervised continuation or restart of irradiations at home is not an issue in the Netherlands as the devices are provided

by home care institutions and are recollected at the end of treatment. Thirdly, start of repigmentation and total repigmentation were not significantly different between groups. Moreover, in both groups acral areas repigmented less than other areas, which is also found by others.(21)

In conclusion, this study shows that patient-reported outcomes of home and outpatient NB-UVB therapy are comparable with a similar repigmentation and occurrence of side effects. However, satisfaction with the result was significantly lower in the home group. Finally, time investment for home patients was significantly less. Therefore, we suggest that home NB-UVB therapy is a valuable alternative to outpatient NB-UVB therapy in the treatment of non-segmental vitiligo.

ACKNOWLEDGMENTS

We sincerely thank John de Korte PhD for critical revision of the questionnaire, and Tim R. Wind MSc for statistical support.

REFERENCES

1. Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. Am J Clin Dermatol 2001;2(3): 167-81.
2. Whitton ME, Ashcroft DM, Barrett CW, Gonzalez U. Interventions for vitiligo. Cochrane Database Syst Rev 2006;(1):CD003263.
3. Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PM. Nonsurgical repigmentation therapies in vitiligo. Meta-analysis of the literature. Arch Dermatol 1998 Dec;134(12):1532-40.
4. Grimes PE. New insights and new therapies in vitiligo. JAMA 2005 Feb 9;293(6):730-5.
5. Milstein HJ, Vonderheid EC, Van Scott EJ, Johnson WC. Home ultraviolet phototherapy of early mycosis fungoides: preliminary observations. J Am Acad Dermatol 1982 Mar;6(3):355-62.
6. Resnik KS, Vonderheid EC. Home UV phototherapy of early mycosis fungoides: long-term follow-up observations in thirty-one patients. J Am Acad Dermatol 1993 Jul;29(1):73-7.
7. Larko O, Swanbeck G. Home solarium treatment of psoriasis. Br J Dermatol 1979 Jul;101(1):13-6.
8. Jordan WP, Jr., Clarke AM, Hale RK. Long-term modified Goeckerman regimen for psoriasis using an ultraviolet B light source in the home. J Am Acad Dermatol 1981 May;4(5):584-91.
9. Lowe NJ. Home ultraviolet phototherapy. Semin Dermatol 1992 Dec;11(4):284-6.
10. Koster W, Wiskemann A. [Phototherapy with UV-B in vitiligo]. Z Hautkr 1990 Nov;65(11):1022-4, 1029.
11. Cameron H, Yule S, Moseley H, Dawe RS, Ferguson J. Taking treatment to the patient: development of a home TL-01 ultraviolet B phototherapy service. Br J Dermatol 2002 Nov;147(5):957-65.
12. Lowe NJ. Home ultraviolet phototherapy. Semin Dermatol 1992 Dec;11(4):284-6.
13. Sarkany RP, Anstey A, Diffey BL, Jobling R, Langmack K, McGregor JM, et al. Home phototherapy: report on a workshop of the British Photodermatology Group, December 1996. Br J Dermatol 1999 Feb;140(2):195-9.
14. Murphy GM, McCann P, O'Leary A, Rogers S. Guidelines for the use of phototherapy and photochemotherapy in Ireland. Ir J Med Sci 1997 Apr;166(2):92-7.
15. Koek MB, Buskens E, Steegmans PH, van Weelde H, Bruijnzeel-Koomen CA, Sigurdsson V. UVB phototherapy in an outpatient setting or at home: a pragmatic randomised single-blind trial designed to settle the discussion. The PLUTO study. BMC Med Res Methodol 2006;6:39.

16. Koek MB, Buskens E, van Weelde H, Steegmans PH, Bruijnzeel-Koomen CA, Sigurdsson V. Home versus outpatient ultraviolet B phototherapy for mild to severe psoriasis: pragmatic multicentre randomised controlled non-inferiority trial (PLUTO study). BMJ 2009;338:b1542.
17. Cameron H, Yule S, Moseley H, Dawe RS, Ferguson J. Taking treatment to the patient: development of a home TL-01 ultraviolet B phototherapy service. Br J Dermatol 2002 Nov;147(5):957-65.
18. Paul BS, Stern RS, Parrish JA, Arndt KA. Low-intensity selective UV phototherapy. A clinical trial in outpatient therapy for psoriasis. Arch Dermatol 1983 Feb;119(2):122-4.
19. Percivalle S, Piccino R, Caccialanza M, Forti S. Narrowband UVB phototherapy in vitiligo: evaluation of results in 53 patients. G Ital Dermatol Venereol 2008 Feb;143(1):9-14.
20. Chen GY, Hsu MM, Tai HK, Chou TC, Tseng CL, Chang HY, et al. Narrow-band UVB treatment of vitiligo in Chinese. J Dermatol 2005 Oct;32(10):793-800.
21. Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K. Guideline for the diagnosis and management of vitiligo. Br J Dermatol 2008;159:1051-76.

3

PUNCH GRAFT TESTING IN VITILIGO; EFFECTS OF UVA, NB-UVB AND 632.8 NM HELIUM-NEON LASER ON THE OUTCOME

J Eur Acad Dermatol Venereol. 2010. doi: 10.1111/j.1468-3083.2010.03874.x.

ABSTRACT

In a randomised controlled observer-blinded study including six patients with stable vitiligo the effects of UVA, NB-UVB and Helium-Neon laser irradiation on the outcome of punch-grafting were studied. In each of four 2x2 cm depigmented test-regions, four 1.5 mm pigmented punch grafts were placed. These test-regions were randomly allocated to the phototherapeutic modalities twice weekly versus no therapy at all during three months. In two patients the majority of punch grafts survived, whereas in the other four patients the majority of punch grafts depigmented. We concluded that stable vitiligo did not preclude failure of punch grafting. Intrinsic patient-related factors in the grafted area seem to determine outgrowth of pigment, while the phototherapeutic modalities have minor to no effect. The number of patients who showed pigment outgrowth was too small to compare the different modalities.

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INTRODUCTION

Vitiligo is an acquired disease characterised by progressive loss of melanocytes leading to the formation of white patches of the skin. It affects probably 1-2% of the world's population, regardless of age, sex and skin colour [8]. The disease can alter a patient's appearance dramatically, which often leads to social and psychological distress [6]. To date, non-segmental vitiligo cannot be cured, but with narrowband (NB) UVB phototherapy considerable repigmentation can be achieved [7]. In therapy-resistant non-progressive vitiligo, autologous punch grafting is widely used, as it is relatively inexpensive, easy to perform, and successful [1,9]. Post-operative irradiation (sunlight, UVA, NB-UVB) has been suggested to improve pigment outgrowth after punch grafting [4], but is unpractical and probably promotes photoageing and photocarcinogenesis. Recently, the red Helium-Neon (HeNe) laser (632.8 nm) was introduced as a safe potential inductor of repigmentation in vitiligo. The mechanism of action remains partly unclear, but as the HeNe laser is a low energy laser, thermal effects on the irradiated tissue are considered to be minute and effects are generally attributed to direct biostimulation of exposed cells [5,10]. Aim of this study was to evaluate pigment outgrowth of punch grafts after irradiation with UVA, NB-UVB and HeNe laser as compared to no phototherapy in patients with non-segmental vitiligo.

MATERIALS AND METHODS

The study protocol has been approved by the local medical ethical committee. This trial was conducted according to the Declaration of Helsinki Principles. A randomised controlled observer-blinded study was performed in six patients, older than 18 years, with non-segmental, stable vitiligo (VIDA score 0). Stability was defined as absence of progression during the last year. None of the patients had therapy for at least one year before inclusion. All patients were recruited from the outpatient clinic of the Netherlands Institute for Pigment Disorders at the Academic Medical Centre in Amsterdam. Written informed consent was obtained from all patients. In each patient four similar depigmented test-regions of approximately 2x2 cm were determined on the torso or proximal upper extremities. The test-regions were outlined on a transparent plastic sheet that was used to make a template from tin foil. In each of these depigmented test-regions four 1.5 mm pigmented punch grafts (with a surface area of 1.8 mm^2) from the hip area were placed (Fig. 1a-b). The four test-regions were randomly allocated to receive either no phototherapy, UVA, NB-UVB, or HeNe laser twice weekly on non consecutive days during three months, resulting in a total of 24 phototherapy sessions. All sessions were performed by the same physician. A UVA (BB-UVA; 320-400 nm) facial tanner (HB 171, Philips BV, Eindhoven, the Netherlands) was used at a power density of 8 mW/cm^2. The exposure time was increased from 4 minutes during the first phototherapy session to 8 minutes (second session) and 12

minutes (third session). From the fourth until the last session the exposure remained 14.5 minutes. A 311 nm NB-UVB hand-held device was used (Waldmann TL 01, Villingen-Schwenningen, Germany) at a power density of 8 mW/cm^2. Treatment was started at 0.1 J/cm^2. This dose was increased by 0.1 J/cm^2 at each treatment if no side effects were reported. In accordance to previous studies, a red continuous wave HeNe laser with an average power of 1.0 mW was used for 30 seconds per punch graft, at an average distance of 10 cm. During each phototherapeutic treatment a tin foil template was used to prevent irradiation of the other three test-regions. Directly after the last phototherapeutic treatment (T_1), and at three and six months after the last treatment (T_2 and T_3) a blinded physician measured the largest (d_1) and its perpendicular diameter (d_2) of each punch graft in all treated areas. For each punch graft, the (re)pigmented surface area A was calculated by $A = 0.25\ \pi\ d_1 d_2$.

RESULTS AND DISCUSSION

At the start of the phototherapy (T_0) each patient had a total of 16 pigmented grafts (1.8 mm^2) in four depigmented test regions. After 24 phototherapy sessions (T_1) we noticed that in two patients the majority of punch grafts survived (Fig. 1c), whereas in the other four patients the majority of punch grafts depigmented (Fig. 1d), irrespective of phototherapy. During follow-up no re-activation of vitiligo was found in any patient under study. In the two responding and four non responding patients, mean age was 32 and 48 years and mean disease duration was 16 and 23 years, respectively. One responding and one non responding patient had thyroid disease. The mean surface area per punch graft, for all patients, is shown in Fig. 2.

In the present study there was no apparent difference in pigment outgrowth between UVA, NB-UVB, HeNe laser and no phototherapy. The reason for the large number of depigmented grafts in stable vitiligo is unclear. We suggest that some patients with stable non-segmental vitiligo are characterised by sustained anti-melanocytic activity in the depigmented patches.

The main limitations of this study are (i) a small number of patients, (ii) a relatively short treatment period of three months, and (iii) a high number of depigmented grafts.

These data suggest that intrinsic patient-related factors in the grafted area seem to determine outgrowth of pigment, while the phototherapeutic modalities have minor to no effect. Secondly, the number of patients (two) who showed outgrowth of pigment was too small to compare the different modalities. Finally, stable vitiligo does not predict successful transplantation. A punch graft-test is probably essential to predict successful outcome of punch grafting in non-segmental vitiligo [2,3,9].

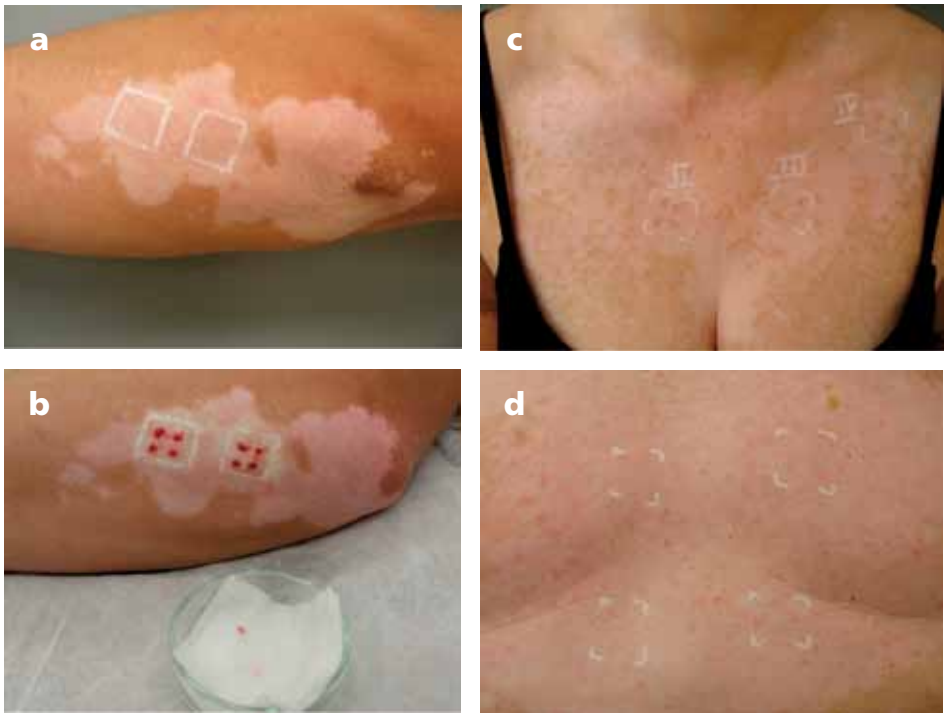

Fig. 1 On the left elbow of a patient two of the four 2x2 cm test-regions were drawn in the vitiligo laesion (**a**). In each of the four test-regions four 1.5 mm biopsies were punched out and pigmented minigrafts from the hip area were placed (**b**). Directly after 24 phototherapy sessions (T_1); pigment outgrowth in all 16 minigrafts was seen in one patient (**c**), whereas in another patient almost all minigrafts were depigmented (**d**).

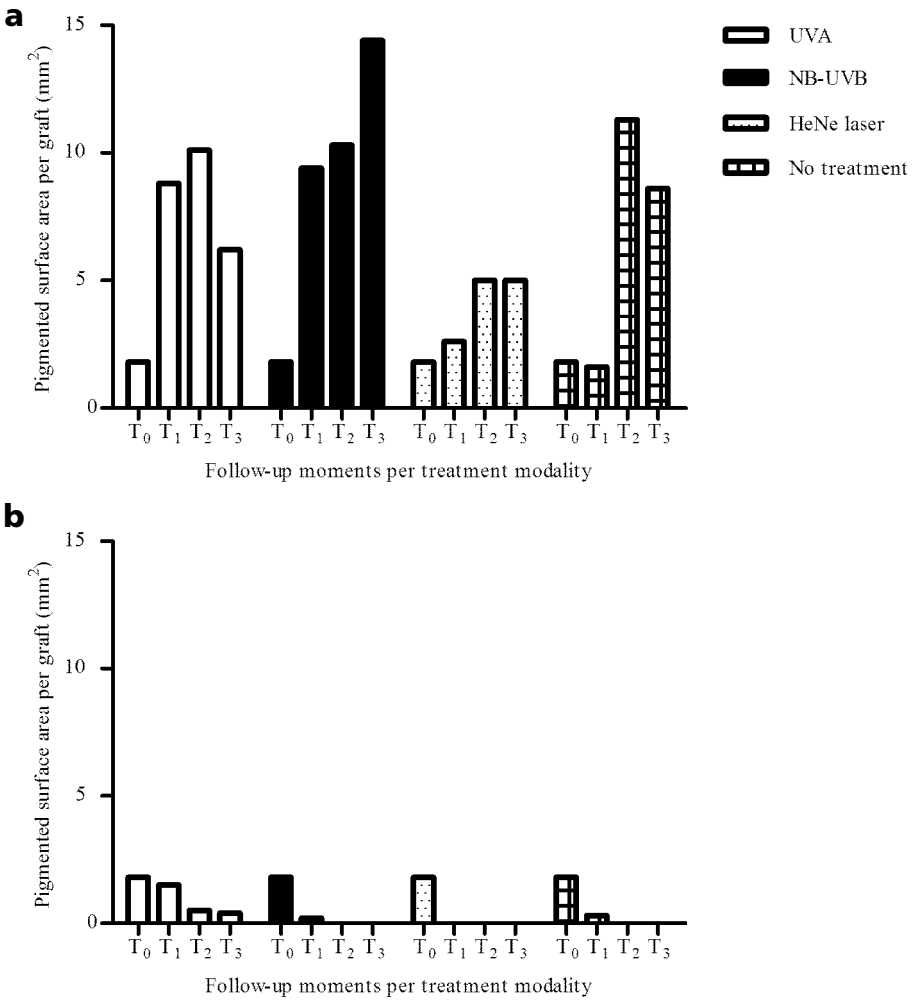

Fig. 2 Mean pigmented surface area per punch graft (mm^2) per treatment modality (UVA, NB-UVB, HeNe laser or no treatment) during the course of study; in the two patients of which the majority of punch grafts showed pigment outgrowth (**a**), and in the four patients of which the majority of punch grafts depigmented (**b**). At T_0 in all patients each of four test-regions consisted of four 1.8 mm^2 pigmented punch grafts.

T_0 - start of treatment
T_1 - directly after 24 phototherapeutic sessions
T_2 - three months after last phototherapeutic session
T_3 - six months after last phototherapeutic session

REFERENCES

Falabella R (1989) Grafting and transplantation of melanocytes for repigmenting vitiligo and other types of leukoderma. Int J Dermatol 28:363-369.

Falabella R (2003) Surgical treatment of vitiligo: why, when and how. J Eur Acad Dermatol Venereol 17:518-520.

Falabella R, Arrunategui A, Barona MI et al (1995) The minigrafting test for vitiligo: detection of stable lesions for melanocyte transplantation. J Am Acad Dermatol 32:228-232.

Lahiri K, Malakar S, Sarma N et al (2006) Repigmentation of vitiligo with punch grafting and narrow-band UV-B (311 nm)--a prospective study. Int J Dermatol 45:649-655.

Lan CC, Wu CS, Chiou MH et al (2009) Low-energy helium-neon laser induces melanocyte proliferation via interaction with type IV collagen: visible light as a therapeutic option for vitiligo. Br J Dermatol 161:273-280.

Linthorst Homan MW, Spuls PI, de Korte J et al (2009) The burden of vitiligo: patient characteristics associated with quality of life. J Am Acad Dermatol 61:411-420.

Njoo MD, Spuls PI, Bos JD et al (1998) Nonsurgical repigmentation therapies in vitiligo. Meta-analysis of the literature. Arch Dermatol 134:1532-1540.

Njoo MD, Westerhof W (2001) Vitiligo. Pathogenesis and treatment. Am J Clin Dermatol 2:167-181.

Westerhof W, Boersma B (1995) The minigrafting test for vitiligo: detection of stable lesions for melanocyte transplantation. J Am Acad Dermatol 33:1061-1062.

Yu HS, Wu CS, Yu CL et al (2003) Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo. J Invest Dermatol 120:56-64.

4

NON-ABLATIVE 1,550 NM FRACTIONAL LASER THERAPY VERSUS TRIPLE TOPICAL THERAPY FOR THE TREATMENT OF MELASMA: A RANDOMIZED CONTROLLED PILOT STUDY

J Am Acad Dermatol. 2011;64(3):516-23.

ABSTRACT

Background: Various treatments are available for melasma at present. However, results are often disappointing.

Objective: To assess the efficacy and safety of non-ablative 1,550 nm fractional laser therapy and compare results with those obtained with triple topical therapy (the gold standard).

Methods: Twenty female patients with moderate to severe melasma and Fitzpatrick skin types II-V were treated either with non-ablative fractional laser therapy or triple topical therapy (hydroquinone 5%, tretinoin 0.05% and triamcinolone acetonide 0.1% cream) once daily for eight weeks in a randomized controlled observer-blinded study. Laser treatment was performed every two weeks for a total of four times. Physician's global assessment (PhGA) was assessed at 3 weeks, 3 months and 6 months after the last treatment.

Results: The PhGA had improved ($p<0.001$) in both groups at 3 weeks. There was no difference in PhGA between the two groups. Mean treatment satisfaction and recommendation were significantly higher in the laser-group at three weeks ($p<0.05$). However, melasma recurred in five patients in both groups after 6 months. Side effects in the laser group were erythema, burning sensation, facial edema, and pain; in the triple group erythema, burning, and scaling.

Limitations: A small number of patients; only one set of laser parameters; and a possible difference in motivation between groups.

Conclusions: Non-ablative fractional laser therapy is safe and comparable in efficacy and recurrence rate to triple topical therapy. It may be a useful alternative treatment option for melasma when topical bleaching is ineffective or not tolerated. Different laser settings and long-term maintenance treatment should be tested in future studies.

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INTRODUCTION

Melasma is a common pigment disorder, which often causes significant emotional and psychosocial distress in patients. It adversely affects the patient's quality of life.[1] Melasma is encountered in all skin types, but is particularly seen in women with Fitzpatrick skin types IV-VI.[2] The pathogenesis of melasma is not fully understood. Genetic background and sun exposure seem to be the most important etiologic factors besides pregnancy, systemic drugs, hormonal medications and phototoxic or photoallergic cosmetics.[3]

Melasma is often difficult to manage because of its refractory and recurrent nature. Current treatments include topical bleaching creams, chemical peels and laser therapy. However, results are often disappointing. Treatment of choice is a triple topical therapy, a topical bleaching that was first introduced in 1975 as Kligman formula and consists of hydroquinone (HQ) 5%, tretinoin 0.1% and dexamethasone 0.1%. Nowadays, dexamethasone is frequently replaced by various other moderately potent to potent corticosteroids.[4,5] The results of laser therapy and intense pulsed light therapy in melasma are generally disappointing and treatment is limited by adverse effects such as the occurrence of postinflammatory hyperpigmentation, especially in dark-skinned patients. Therefore, the use of these devices is controversial.[6,7]

Recently, non-ablative fractional laser therapy at 1,550 nm was reported as a treatment for melasma.[8,9] At this wavelength water absorption is predominant. In fractional laser therapy multiple small sized coagulated zones are separated by surrounding untreated tissue.[10] It was reported that these microscopic treatment zones allow transport and extrusion of microscopic epidermal necrotic debris including melanin from melanocytes through a compromised dermal-epidermal junction.[10,11] Generally, a visible wound does not appear because these microscopic treatment zones have a diameter less than 100 micrometer.[10] The stratum corneum was found to be intact after 24 hours.[12,13] The recovery is relatively fast because only part of the skin surface is treated in one session.

Currently, non-ablative fractional laser therapy is regularly used in patients with melasma, although there is minimum evidence for its efficacy and controlled trials are lacking. The aim of the present study was to assess the efficacy and the safety of non-ablative 1,550 nm fractional laser therapy and to compare results with those obtained with the triple topical therapy (the gold standard) in the treatment of melasma.

PATIENTS AND METHODS

Study design / Patients

A randomized controlled observer-blinded study was performed in 22 female patients. Patients were older than 18 years, had Fitzpatrick skin types II-V and suffered from

moderate to severe melasma. They were recruited from the outpatient clinic of the Netherlands Institute for Pigment Disorders at the Academic Medical Center in Amsterdam (Table I). Two patients (one in each treatment group) did not start any treatment for reasons not related to the study, resulting in 10 patients in each group. The study protocol was approved by the local medical ethical committee and was registered in the ISRCTN trial register (ISRCTN84133969). Risks, benefits, and potential complications were communicated with the patients and informed written consent was obtained. None of the patients had used bleaching creams or topical steroid creams for at least four weeks prior to entering the study. Exclusion criteria

Table I Patient characteristics of laser group (**a**) and triple group (**b**)

a					
Patient	Age (years)	Skin type	Melasma type*	Previous therapy	Disease duration (years)
1	36	II	dermal	-	2
2	41	II	mixed	AA	2
3	36	III	epidermal	AA	2
4	36	III	epidermal	AA	4
5	49	III	epidermal	AA, P	6
6	36	III	epidermal	HQ, TTT, P	20
7	41	IV	epidermal	TTT	4
8	38	IV	epidermal	AA, TTT	3
9	52	IV	dermal	AA	20
10	55	V	dermal	AA	7
Mean	**35.3**				**7.2**
b					
11	25	II	epidermal	AA	0.3
12	34	II	epidermal	AA	3
13	47	II	epidermal	AA	14
14	31	III	epidermal	-	5
15	35	III	epidermal	TTT	7
16	31	III	epidermal	TTT	9
17	41	III	epidermal	AA, TTT	7.5
18	36	IV	mixed	-	2
19	36	IV	mixed	AA, P	4
20	37	V	dermal	AA, TTT, P	20
Mean	**42.0**				**7.0**

HQ, Hydroquinone; *TTT,* Triple Topical Therapy; *AA,* Azelaic Acid; *P,* Peeling
- No previous therapy
* As assessed by Wood's lamp examination

were: history of keloids, active eczema, active facial acne, history of facial eczema, suspected hypersensitivity to lidocaine or triple topical therapy, use of isotretinoin in the past six months (generally accepted practice in laser treatments), pregnancy and high exposure to sunlight or UV light (UVA or UVB). Type of melasma was assessed by Wood's lamp examination.[14,15] All patients were instructed to use sunscreen (SPF 50+) every three hours when outside.

The patients were enrolled and randomly assigned to the triple topical therapy or the laser therapy group using sealed envelopes numbered from 1-22 in which the allocation was indicated. The envelopes were opened in ascending order. The randomization was based on a digitally created random list (Graphpad Software Inc., La Jolla, CA).

Both groups started treatment at the beginning of November and treatment ended in December. Topical bleaching was started in the laser group when melasma recurred and restarted if this occurred in the triple therapy group. For these patients with a recurrence the study ended, and they were not included in the later follow-up.

Triple topical therapy

Ten patients received triple topical therapy (HQ 5%, tretinoin 0.05%, triamcinolone acetonide 0.1% cream) for 8 weeks. Patients were instructed to apply the cream once a day in the evening on all the hyperpigmented macules. They were contacted by telephone for the occurrence of any side effects 3 weeks after the start of the treatment. The follow-up visits were scheduled at 3 weeks, 3 months and 6 months after the last treatment day.

Non-ablative fractional laser therapy

Ten patients were treated in four non-ablative 1,550 nm fractional laser therapy sessions. The inter-treatment interval was 2 weeks. Anesthesia consisted of topical 2.5% lidocaine and 2.5% prilocaine ointment applied an hour before each treatment. A 1,550 nm Er:glass non-ablative laser (Fraxel Re:store laser, Reliant Technologies Inc., Mountain View, CA) was used at the recommended settings. Each treatment session involved eight fractional laser passes to create an estimated final density of ~2000-2500 microscopic treatment zones per cm^2. Four passes were made in one direction and four perpendicularly. The energy per microbeam was 10 mJ. Skin types II and III were treated at ~20% coverage (level 7) and skin types IV and V at ~14% coverage (level 5). The follow-up visits were scheduled at 3 weeks, 3 months and 6 months after the last treatment day.

Patient-reported outcomes

All side effects were documented and patients were asked to score erythema, edema, crusting and blistering on a scale from 0 to 3. Patients were asked to score the improvement of hyperpigmentation on a visual analogue scale from 0 to 10 with 0 as no improvement and 10 as the best possible improvement (Patient's Global Assessment, PGA). Patients were also asked whether they would recommend the

treatment to their friends and colleagues. Pain in the laser group was recorded on a scale from 0 to 10 (PGA) after the first and the third treatment session.

Reflectance spectroscopy

The melanin index was measured using reflectance spectroscopy (DermaSpectrometer, Cortex Technology, Hadsund, Denmark) at the start of the treatment and during follow-up to assess the improvement in hyperpigmentation. The mean of three measurements was taken. The darkest macule was selected for assessment and compared with the perilesional normal skin. These locations were documented using a charcoal pencil and digital photography and assessed during the follow-up visits.

Physician's Global Assessment and the melasma area and severity index

As recommended in the guidelines for clinical trials in melasma,[16] a blinded observer dermatologist performed the Physician's Global Assessment (PhGA) as the main outcome parameter. Photographs taken under standardized conditions with a digital camera (Canon G6, Canon Components Inc., Saitama, Japan) before treatment and during follow-up were used. The improvement of hyperpigmentation was scored on a scale from 0 to 6 (0: total clearance (100% improvement), 1: almost total clearance (90% improvement), 2: distinct clearance (75% improvement) 3: moderate clearance (50% improvement) 4: mild clearance (25% improvement) 5: no change, 6: worsening of hyperpigmentation).[16]

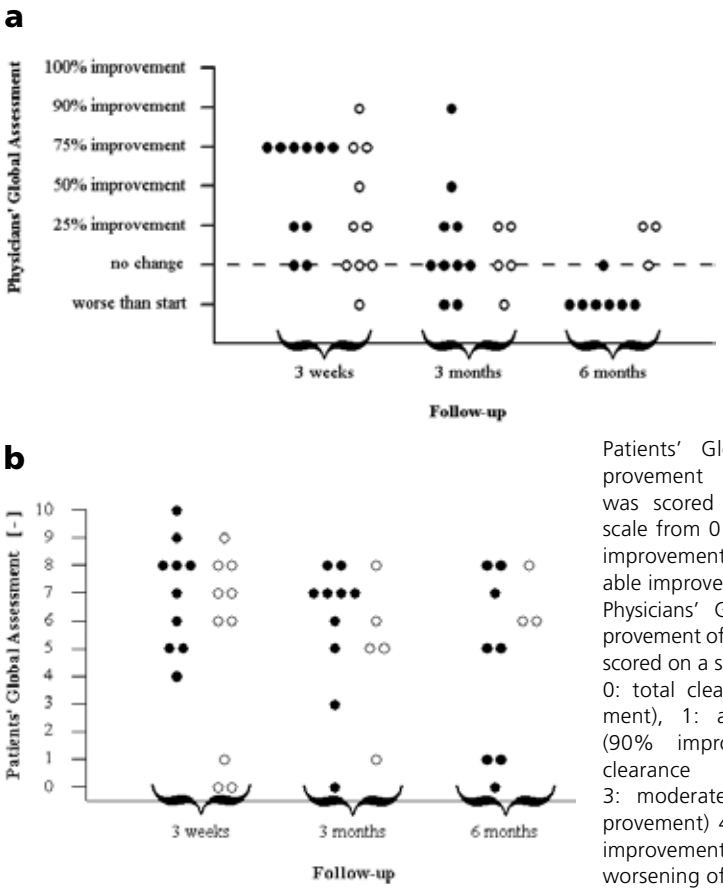
The blinded observer dermatologist also calculated the melasma area and severity index (MASI) from the digital photographs. The MASI quantifies the extent of the lesion, the darkness and the homogeneity of the hyperpigmentation. Each of the four areas (forehead, perioral region / chin, right malar and left malar regions) was scored. The sum of the severity rating for the darkness and the homogeneity is multiplied by the numerical value of the areas involved in order to calculate the MASI (range 0 to 48).[16]

Statistical analysis

We calculated that a sample size of 18 patients would have a power of 80% with an α of 0.05 to detect a difference of 10% in the PhGA assuming a standard deviation of 50%. Means, standard deviations, two-tailed homoscedastic Student's t-tests, Fisher's exact tests, binomial tests, and Chi-square tests were performed using Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL) in patients who complied with the protocol.

RESULTS

The characteristics of the twenty treated patients are listed in Table I. Mean energies per consecutive laser treatment were 1.7, 2.1, 2.1 and 1.9 kJ respectively. The PGA at three weeks follow-up was 7.0 for the laser group vs 5.2 for the triple therapy group (Figure 1a). This was not a statistically significant difference and also within the groups no significant difference was found. The PhGA showed a distinct improvement in both the groups at the 3 weeks follow-up ($p<0.001$; Fig. 2). The PhGA gradually returned in both groups to the baseline at the 3 months and 6 months follow-up (compared with the baseline, $p=0.9$ and $p=0.9$, respectively) (Figure 1b). There was no statistically significant difference between the groups with respect to either improvement or worsening. The same applied to the melanin index and the MASI. However, the mean treatment satisfaction (8.3 vs 5.3; $p<0.05$ (Table II)) and the



Patients' Global Assessment: improvement of hyperpigmentation was scored on a visual analogue scale from 0 to 10, with zero as no improvement and 10 as best imaginable improvement.
Physicians' Global Assessment: improvement of hyperpigmentation was scored on a scale from zero to six.
0: total clearance (100% improvement), 1: almost total clearance (90% improvement), 2: distinct clearance (75% improvement), 3: moderate clearance (50% improvement) 4: mild clearance (25% improvement) 5: no change, 6: worsening of hyperpigmentation

Fig 1. Patients' Global Assessment (**a**) and Physicians' Global Assessment (**b**). •laser group, ○ triple group.

Fig. 2 Clinical photographs of a patient before treatment (**a**), and three weeks after four sessions of non-ablative fractional laser (**b**)

Table II Melasma parameters of laser group (**a**) and triple group (**b**). Recurrence of melasma led to restarting topical bleaching. The study was terminated in these patients and they were not included in the later follow-up (see **Table III**).

a				
	Start	3 weeks	3 months	6 months
Patients at follow-up	-	10	10	8
Mean PGA[+]	-	7.0 ± 1.9	5.8 ± 2.3	4.4 ± 3.1
Mean satisfaction	-	8.3 ± 1.3 [¶]	7.1 ± 2.9	6.4 ± 3.4
Melanin index[‡]	5.0 ± 5.6	3.8 ± 4.6	4.6 ± 6.6	7.3 ± 7.4
MASI	8.5 ± 3.6	6.6 ± 3.8	8.7 ± 5.5	18.0 ± 10.4
b				
Patients at follow-up	-	10	5	3
Mean PGA[+]	-	5.2 ± 3.2	5.0 ± 2.6	6.7 ± 1.2
Mean satisfaction	-	5.3 ± 3.4 [¶]	5.5 ± 2.8	8.0 ± 1.0
Melanin index[‡]	2.6 ± 2.2	3.4 ± 3.5	2.4 ± 2.3	2.9 ± 1.0
MASI	9.8 ± 6.5	6.9 ± 5.9	8.5 ± 7.1	15.1 ± 12.6

[+]Patient's Global Assessment: improvement in hyperpigmentation was scored on a visual analogue scale from 0 to 10 with zero as no improvement and 10 as the best possible improvement.
MASI, Melasma area and severity index
[‡]Melanin index measured by spectroscopy
[¶] $p<0.05$

treatment recommendation (100% vs. 50%; $p<0.05$) were both significantly higher in the laser group at the 3 weeks follow-up.

Side effects in the laser group consisted of sunburn-like erythema (75%) and burning sensation (58%) both lasting one to three days. Moderate to severe facial

Table III Number of patients from laser group (**a**) and triple group (**b**) per visit.

a				
	Start	3 weeks	3 months	6 months
Patients at follow-up	-	10	10	8
Recurrence[#]	-	0	2	3
Total better than start	-	10	8	5
b				
Patients at follow-up	-	10	5	3
Recurrence[#]	-	2	3	0
Total better than start	-	8[§]	2[§]	3

[#]Recurrence of melasma led to restarting topical bleaching. The study was terminated in these patients and they were not included in the later follow-up.
[§]Three out of the eight patients did not appear at the 3 months follow-up. The two patients who were better at the 3 months follow-up than at the start were joined by one of these three patients resulting in three patients at the 6 months follow-up.

edema lasting up to four days was reported by 40% of them. Blistering, crusting, scarring and hypo- or hyperpigmentation were not observed. Patients reported an average pain score of 6.4 on a scale from 0 to 10. All patients returned to work or normal activity immediately after the laser treatment. Reported side effects in the triple group were erythema (25%) and burning sensation (20%) that lasted for 30 minutes to 24 hours. Scaling was a frequent complaint (55%). In both groups no hypo- or hyperpigmentation was observed. Melasma recurred in five patients in each group during the follow-up (Table III). No significant differences between the recurrences of both groups regarding skin type or type of melasma were observed (Table IV).

Table IV Results in patients with recurrence. No significant differences between the recurrence of melasma, the skin type and the type of melasma were observed in laser group (**a**) and triple group (**b**)

a			
Patient	Skin type	Melasma type*	Recurrence
3	III	epidermal	6 months
6	III	epidermal	6 months
7	IV	epidermal	6 months
8	IV	epidermal	3 months
9	IV	dermal	3 months
b			
14	III	epidermal	3 months
16	III	epidermal	3 months
18	IV	mixed	3 months
19	IV	mixed	3 weeks
20	V	dermal	3 weeks

*As assessed by Wood's lamp examination

DISCUSSION

Non-ablative 1,550 nm fractional laser therapy proved to be a safe treatment option for patients including those with darker skin types (Fitzpatrick skin types IV and V) suffering from melasma in this study. The patients considered non-ablative 1,550 nm fractional laser therapy to be a satisfactory and recommendable treatment. In both groups the PhGA showed a distinct improvement at the 3 weeks follow-up ($p<0.001$; Fig. 2). The PGA, melanin index and MASI showed no statistically significant differences neither within nor between the groups. Clinically, recurrence of melasma was encountered in the majority of both patient groups at the 6 months follow-up. These results indicate that triple topical therapy should still be considered as the gold standard in the treatment of melasma.[7,16] There is a large number of reported studies and a lot of clinical experience with triple topical therapy in melasma. The costs are lower and the treatment is less painful. Non-ablative 1,550 nm fractional laser therapy was safe and comparable in efficacy and recurrence rate to triple topical therapy. It may be a useful alternative treatment option for melasma when topical bleaching is ineffective or not tolerated.

At present, there are six reported uncontrolled studies involving a total of 31 patients with melasma who were treated with non-ablative fractional laser therapy using a 1,550 nm Fraxel Re:store laser (Reliant Technologies Inc.).[8,9,13,17-19] There are no reports on non-ablative fractional laser therapy using other lasers. In the studies using the Fraxel Re:store laser, settings ranged from 2,000 to 3,500 microthermal zones per cm^2 at six to 12 mJ per microbeam. The number of treatments ranged from one to six, with an interval of 1 to 4 weeks. A remarkable improvement of melasma, lasting up to six months post-treatment, was reported in one case study.[8] The other 30 patients had their last follow-up at 3 months with a remarkable improvement in 22 patients and some improvement in 8 patients.[9,13,17-19] The relatively short follow-up period in these studies as compared to that of our study may be an explanation for these better results.

Ultrastructural changes associated with non-ablative fractional laser therapy were evaluated in one of those studies in 10 of the 31 reported patients.[13] Biopsies obtained before and three months after four treatments showed a decrease in the number of melanocytes. The authors suggested that non-ablative fractional laser therapy may delay repigmentation because a decreased number of melanocytes was still noted after approximately three epidermal turn-over cycles. This is consistent with our results where eight of the 10 patients showed no signs of recurrence at three months followup. However, it seems unlikely that non-ablative fractional laser therapy delays repigmentation over a prolonged period since recurrence was noted in 5 of these 10 patients at the 6 months follow-up.

The side effects of triple therapy such as erythema, burning sensation and scaling are well known, and comparable with reports from literature. Although the patient's reported outcomes have their limitations and are not objective, the information on

these easily recognizable symptoms can be obtained via the telephone. Side effects of non-ablative 1,550 nm fractional laser therapy were also comparable with those reported by others with the exception of postinflammatory hyperpigmentation. None of our patients had signs of postinflammatory hyperpigmentation, whereas it was seen in two of the 31 cases described in the literature.[9,19] Overall, the incidence of this complication is approximately 5% including the results of this study. In this respect, it should be noted that we used relatively conservative laser settings for dark-skin patients. Our average pain score of 6.4 is also almost identical to the 6.3 (both on a scale from 0 to 10) reported by Rokhsar and colleagues.[9]

Two very different treatment modalities were compared in this study. Triple topical therapy is known to be effective for epidermal melasma. Less improvement is seen in dermal melasma, possibly because of the limited penetration of the drugs into the dermis. Non-ablative fractional laser therapy is able to eliminate dermal material, whereby it potentially can be used to treat deeper dermal pathology, such as dermal and mixed melasma.[11] A large cohort of patients will be necessary to prove this hypothesis, whereby an accurate assessment of the epidermal and the dermal melanin distribution is important. In our study, a general impression of the depth of the melanin distribution was obtained by Wood's lamp examination. However, this method is less reliable for darker skin types (Fitzpatrick skin types V and VI).[14,15] Other diagnostic modalities such as the spectrophotometric intracutaneous analysis (SIAscope™) are available for a more precise non-invasive assessment of melanin distribution. Recently, it was elegantly demonstrated that melanin distribution as assessed with this technique correlated extremely well with the depth dependent concentrations of eumelanin in 30 subjects with different skin types.[20] This modality may be useful in future studies for distinguishing reliably between dermal, epidermal and mixed melasma.

The main limitations of our study are (i) a small number of included patients, (ii) a sample size, which was powered for PhGA only, (iii) laser settings that may have been suboptimal, (iv) and a possible difference in the motivation and the therapy adherence between the two groups. Although our sample size analysis predicted that inclusion of 18 patients would be sufficient, the limited number of patients available for the analysis at the 6 months follow-up indicates that our assumptions at start of the study were perhaps too optimistic. A possible shortcoming is also the fact that the non-significant results in and between the two groups for PGA, melanin index and MASI may be the result of a sample size that was powered for PhGA only.

Experience with this new treatment modality in pigment disorders is limited. Currently, dose-response studies are lacking. We suggest that an increase in microbeam density may be beneficial in the treatment outcome; possibly at an increased risk of postinflammatory hyperpigmentation.[21] However, in this and other studies, the number of patients was too limited to test this hypothesis. In general, the included patients hoped to be randomized to the non-ablative 1,550 nm fractional laser therapy, a new treatment option for melasma that was free of charge in this study,

possibly resulting in skin rejuvenation. This may have resulted in biased outcomes in terms of PGA. Firstly, patients in the laser group were better motivated to appear at the follow-up. Secondly, patients in the triple group may have been less motivated to apply triple topical therapy, and sunscreen as a protective measure consistently every three hours when outside. This could be explained by the fact that patients in the laser group were seen for laser treatment four times during eight weeks, whereas the triple group was only called three weeks after start of the eight-week-treatment. This may have decreased compliance for both usage of sunscreen, as well as triple topical therapy. The value of the results and the comparisons at the 3- and 6 months follow-up were somewhat limited by the lower motivation of the patients in the triple group to appear at the follow-up and the termination of the study in patients with a recurrence.

The relatively high number of patients with a recurrence at the follow-up was not regarded as a limitation of this study because this is inherent to the natural course of melasma. All the patients were treated in the winter months in order to minimize the seasonal variations in pigmentation at baseline and to reduce the sun exposure during the study. Thus, the follow-up at 3 months was in early spring and the last visit was in early summer. This time course may confound the outcomes by promoting spontaneous resolution in the winter, but also by increasing the likelihood of recurrence at the end of the study in early summer.

Initially good results were seen at the short term follow-up. However, in both groups pigmentation worsened during the course of time. No significant differences were found between the two groups upon longer follow-up and the results at 6 months (in the summer, without any active treatment) were worse than those at baseline (winter). Interestingly, in spite of this overall outcome, all laser patients would recommend this treatment option to their friends and colleagues, whereas only half of the patients in the triple group would recommend their therapy to others.

In conclusion, in this study non-ablative 1,550 nm fractional laser therapy proved to be a safe treatment option for patients suffering from melasma, including those with darker skin types. Patients considered laser therapy to be satisfactory and recommendable. Topical bleaching should still be regarded as the gold standard in the treatment of melasma.[7,16] The costs are lower and treatment is less painful. Non-ablative 1,550 nm fractional laser therapy was safe and comparable in efficacy and recurrence rate to triple topical therapy. It may be a useful alternative when topical bleaching is ineffective or not tolerated.

In future studies a larger cohort of patients should be recruited in order to better compare treatment outcomes in epidermal and dermal melasma. Moreover, given the high recurrence rate and the absence of major side effects, optimization of laser parameters and long-term maintenance treatment with a bleaching regimen should be considered in future research.

ACKNOWLEDGMENTS

The laser equipment was kindly provided by Reliant Technologies Inc. (Mountain View, CA), exclusively for the purpose of this study. Recently, Reliant Technologies Inc. merged with Thermage. The new firm is Solta Medical Inc. (Hayward, CA).

REFERENCES

1. Pawaskar MD, Parikh P, Markowski T, McMichael AJ, Feldman SR, Balkrishnan R. Melasma and its impact on health-related quality of life in Hispanic women. J Dermatol Treat 2007;18:5-9.
2. Gupta AK, Gover MD, Nouri K, Taylor S. Treatment of melasma: a review of clinical trials. J Am Acad Dermatol 2006;55:1048-65.
3. Grimes PE. Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995;131:1453-7.
4. Gano SE, Garcia RL. Topical tretinoin, hydroquinone, and betamethasone valerate in the therapy of melasma. Cutis 1979; 23:239-241.
5. Taylor SC, Torok H, Jones T, Lowe N, Rich P, Tschen E, et al. Efficacy and safety of a new triple-combination agent for the treatment of facial melasma. Cutis 2003;72:67-72.
6. Rendon M, Berneburg M, Arellano I, Picardo M. Treatment of melasma. J Am Acad Dermatol 2006;54(5 Suppl. 2):S272-81.
7. Picardo M, Carrera M. New and experimental treatments of cloasma and other hypermelanoses. Dermatol Clin 2007;25:353-62.
8. Tannous ZS, Astner S. Utilizing fractional resurfacing in the treatment of therapy-resistant melasma. J Cosmet Laser Ther 2005;7:39-43.
9. Rokhsar CK, Fitzpatrick RE. The treatment of melasma with fractional photothermolysis: a pilot study. Dermatol Surg 2005;31:1645-50.
10. Manstein D, Herron GS, Sink RK , Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004;34:426-38.
11. Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. J Biomed Opt 2006;11:1-9.
12. Laubach HJ, Tannous Z, Anderson R, Manstein D. Skin responses to fractional photothermolysis. Lasers Surg Med 2006;38:142-9.
13. Goldberg DJ, Berlin AL, Phelps R. Histologic and ultrastructural analysis of melasma after fractional resurfacing. Lasers Surg Med 2008;40:134-8.
14. Gilchrest B, Fitzpatrick T, Anderson R, Parrish J. Localization of melanin pigmentation in the skin with Wood's lamp. Br J Dermatol 1977;96:245-8.
15. Sanchez N, Pathak M, Sato S. Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. J Am Acad Dermatol 1981;4:698-710.
16. Pandya A, Berneburg M, Ortonne JP, Picardo M. Guidelines for clinical trials in melasma. Pigmentation Disorders Academy. Br J Dermatol 2006;156(Suppl. 1):S21-8.
17. Karsai S, Raulin C. Fraktionierte photothermolyse, eine neue Option in der Behandlung des Melasma? Hautarzt 2008;59(2):92-100.
18. Laubach HJ, Manstein D. Fraktionierte photothermolyse. Hautarzt 2007;58:216-23.
19. Naito SK. Fractional photothermolysis treatment for resistant melasma in Chinese females. J Cosmet Laser Ther 2007;9:161-3.
20. Matts PJ, Dykes PJ, Marks R. The distribution of melanin in skin determined in vivo. Br J Dermatol 2007;156:620-8.
21. Chan HH, Manstein D, Yu CS, Shek S, Kono T, Wei WI. The prevalence and risk factors of post-inflammatory hyperpigmentation after fractional resurfacing in Asians. Lasers Surg Med 2007;39:381-5.

5

NON-ABLATIVE 1,550 nm FRACTIONAL LASER THERAPY VERSUS TRIPLE TOPICAL THERAPY FOR THE TREATMENT OF MELASMA: A SPLIT-FACE STUDY

Lasers Surg Med. 2010;42(7):607-12.

ABSTRACT

Background: Melasma is a chronic, often relapsing skin disorder, with poor long term results from all current therapies.

Objective: To assess efficacy and safety of non-ablative 1,550 nm fractional laser therapy (FLT) as compared to the gold standard, triple topical therapy (TTT).

Study design: Twenty-nine patients with melasma were included in a randomized controlled observer-blinded study with split-face design. Each side of the face was randomly allocated to either 4-5 non-ablative FLT sessions (15 mJ/microbeam, 14-20% coverage) or TTT (hydroquinone 5%, tretinoin 0.05%, triamcinolone acetonide 0.1% cream). TTT was applied once daily for 15 weeks until the last FLT session. After this last treatment, patients were asked to apply TTT twice weekly on both sides of the face during follow-up. Improvement of melasma was assessed by patient's global assessment (PGA), patient's satisfaction, physician's global assessment (PhGA), melanin index, and lightness (L-value) at three weeks, and at three and six months after the last treatment.

Results: Mean PGA and satisfaction were significantly lower at the FLT side ($p<0.001$). PhGA, melanin index and L-value showed a significant worsening of hyperpigmentation at the FLT side. At the TTT side, no significant change was observed. At six months follow-up, most patients preferred TTT. Side-effects of FLT were erythema, burning sensation, edema, and pain. Nine patients (31%) developed PIH after two or more laser-sessions. Side-effects of TTT were erythema, burning sensation and scaling.

Conclusions: Given the high rate of postinflammatory hyperpigmentation, non-ablative 1,550 nm fractional laser at 15 mJ/microbeam is not recommendable in the treatment of melasma. TTT remains the gold standard treatment.

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INTRODUCTION

Melasma is a common cause of hyperpigmentation and is hallmarked by irregular brown macules on the sun-exposed parts of the face, primarily the cheeks, forehead, upper lip, nose and chin. It frequently poses a substantial emotional and psychosocial burden on patients, and adversely affects patient's quality of life.[1] Melasma is found in all skin types but is especially seen in women with Fitzpatrick skin types IV-VI.[2] The pathogenesis is not fully understood, but genetic background and sun exposure seem to be the most important etiologic factors besides pregnancy, systemic drugs, hormonal medications and phototoxic or photoallergic cosmetics.[3]

Because of its refractory and recurrent nature, melasma is difficult to manage. Current treatments include topical bleaching creams, chemical peels and laser therapy. However, results are often disappointing.

Treatment of choice is triple topical therapy (TTT), that was first introduced in 1975 as the Kligman formula consisting of hydroquinone (HQ) 5%, tretinoin 0.1% and dexamethasone 0.1%. Nowadays, different concentration of HQ and tretinoin are combined with various moderately potent to potent corticosteroids.[4,5]

In melasma, results of lasers and intense pulsed light systems are generally disappointing and treatment is limited by adverse effects, mainly the occurrence of postinflammatory hyperpigmentation (PIH), especially in dark-skinned patients. As a result, the use of these devices is controversial.[6,7]

Recently, non-ablative fractional laser therapy (FLT) at 1,550 nm was suggested as a treatment for melasma.[8,9,14] At this wavelength water absorption is predominant. FLT generates multiple small sized coagulated zones, separated by surrounding untreated tissue.[10] It has been suggested that these microscopic treatment zones allow transport and extrusion of microscopic epidermal necrotic debris including melanin from melanocytes through a compromised dermal-epidermal junction.[10,11] Generally, a visible wound does not appear because these microscopic treatment zones have a diameter less than 100 micrometer.[10] The stratum corneum was found to be intact after 24 hours.[12,13] As only part of the skin surface is treated in one session, recovery is relatively fast.

Currently, non-ablative FLT is regularly used in patients with melasma, although evidence for efficacy is poor. In a previous randomized parallel group study conducted at our institute, non-ablative FLT at 10 mJ per microbeam proved to be a safe and potentially useful alternative treatment option for melasma.[14] Given the lack of serious side-effects and relative poorer clearance of melasma in skin types IV and V, optimization of laser dosimetry was suggested.[14] Moreover, a high recurrence rate was observed at six months follow-up. The aim of the present study was to compare non-ablative 1,550 nm FLT and TTT for the treatment of melasma in a split-face design, using more aggressive settings for FLT and long term intermittent maintenance bleaching during follow-up.

PATIENTS AND METHODS

Study design / Patients

A randomized controlled observer-blinded study with a split-face design was performed in 29 patients. Patients older than 18 years with Fitzpatrick skin type II-V and melasma were included from the outpatient clinic of the Netherlands Institute for Pigment Disorders at the Academic Medical Center in Amsterdam (Table 1).

The study protocol has been approved by the local medical ethics committee and registered in the clinicaltrials.gov trial register (clinicaltrials.gov identifier: NCT01085279). Written and verbal information including risks, benefits, and potential complications was given to the patients, and written informed consent was obtained. None of the patients had used bleaching creams or topical steroid creams for at least four weeks prior to study entry. Exclusion criteria were: history of keloid, active eczema, active acne in the face, history of facial eczema, suspected hypersensitivity to lidocaine or TTT, use of isotretinoin in the past six months, pregnancy and high exposure to sunlight or UV light (UVA or UVB). Type of melasma was assessed by Wood's lamp examination.[15,16] All patients were instructed to use sunscreen (SPF 50+) every two hours when outside.

Table 1 Patient characteristics

Male:female ratio	2:27
Mean age	41 (29-59)
Skin type	
II	6
III	12
IV	8
V	3
Melasma type*	
Epidermal	21
Mixed	8
Disease duration (years)	5 (1-17)
Oral anticonception during study	5
Previous therapy	
Corticosteroid	1
Azelaic acid	13
Hydroquinone	3
Triple topical therapy	25
Peeling	11
Intense pulsed light	1
Fractional laser therapy	4

* As assessed by Wood's lamp examination

On the day of the first treatment each side of the face was randomly allocated to either non-ablative 1,550 nm FLT or TTT. The randomization procedure involved sealed envelopes in which the allocation was indicated. The sealed envelopes were numbered from 1 to 29. Envelopes were opened in ascending order. The randomization was based on a digitally created random list (Graphpad Software Inc., La Jolla, CA) generated by the independent cooperator. Treatment started in March 2009 and ended in May 2009. Follow-up visits at our institute were scheduled at three weeks, three and six months after the last laser treatment. Hence, follow-up ended November 2009.

Triple topical therapy

In all patients, one side of the face was treated with triple topical therapy (HQ 5%, tretinoin 0.05%, triamcinolone acetonide 0.1% cream) for 15 weeks. Patients were instructed to apply cream once a day in the evening on all hyperpigmented macules of one side of the face. After this last treatment, patients were asked to apply TTT twice weekly on both sides of the face during follow-up.

Fractional laser therapy

The side of the face allocated to FLT was treated with a 1,550 nm Er:glass non-ablative laser (Fraxel Re:store laser, Solta Medical Inc., Hayward, CA). One treatment session involved eight fractional laser passes to create an estimated final density of ~2000-2500 microscopic treatment zones per cm^2. Four passes were made in one direction and four perpendicularly. The energy per microbeam was 15 mJ. Patients with skin type II were treated during four sessions with ~20% coverage (level 7), patients with skin types III and IV during five sessions with ~17% coverage (level 6) and patients with skin type V during five sessions with ~14% coverage (level 5). During treatment, cooling of the skin was achieved using a Zimmer Cryo 6 Cold Air Device (Phoenix Medical Inc., Phoenix, AZ). Anesthesia consisted of topical 2.5% lidocaine and 2.5% prilocaine ointment one hour prior to each treatment.

Patient-reported outcomes

The occurrence of side effects was assessed at each FLT visit and at three weeks follow-up. All side effects were documented and patients were asked to score erythema, edema, crusting, and blistering on a scale from 0 to 3. Patients were asked to score the improvement of hyperpigmentation at both sides of the face separately on a visual analogue scale from 0 to 10, with 0 as no improvement and 10 as total clearance (Patient's Global Assessment, PGA). Treatment satisfaction was also scored on a visual analogue scale from 0 to 10. Furthermore, patients were asked which treatment they preferred and which treatment they would recommend to friends or colleagues. Pain was recorded on a scale from 0 to 10 after the first and third treatment.

Reflectance spectroscopy and melanin index

Improvement of hyperpigmentation was assessed by color measurement through reflectance spectroscopy (Microflash 200 d, Datacolor International, Lawrenceville, GA) by a blinded investigator. This instrument, with an aperture of 4 mm, determines color by measuring the intensity of reflected light of particular wavelengths. In this study, the obtained L-value, indicating the lightness of the measured area of skin, was used. In addition, melanin index was measured using a chromameter (Derma-Spectrometer, Cortex Technology ApS, Hadsund, Denmark) in order to assess changes in the amount of dermal and epidermal melanin. Measurements were performed on

a selected homogenous macule at both treated and control site and at normal skin before the first treatment and at follow-up.

At start, location of measurements was documented using a charcoal pencil and digital photography. The same locations were assessed at follow-up.

Physician's Global Assessment

As recommended in the guidelines for clinical trials in melasma,[16] a blinded observer dermatologist assessed the Physician's Global Assessment (PhGA) as main outcome parameter using photographs that were taken under standardized conditions with a digital camera (Canon G6, Canon Components Inc., Saitama, Japan) before treatment and at follow-up. Improvement of hyperpigmentation was scored on a scale from 0 to 6 (0: total clearance (100% improvement), 1: almost total clearance (90% improvement), 2: distinct clearance (75% improvement) 3: moderate clearance (50% improvement) 4: mild clearance (25% improvement) 5: no change, 6: worsening of hyperpigmentation).[16]

Statistical analysis

Standard deviations of the difference in response of matched pairs (σ) regarding triple therapy and non-ablative fractional laser are not reported in the literature. However, we estimated that the difference would be a mean of 1 with a standard deviation of 1.5 on the PhGA scale. A sample size of 20 patients was calculated to have a power of 80% with an alpha of 0.05. To correct for potential drop out we aimed to recruit 30 patients.

Means, standard deviations, two-tailed homoscedastic Student's t-tests, ANOVA-tests, and Chi-square tests were performed with Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL).

RESULTS

The characteristics of the 29 treated patients are listed in Table 1. Twenty-three patients completed the trial. Mean energy per laser treatment was 0.74 kJ. The laser settings are summarized in Table 2.

An intention to treat analysis was performed. Mean PGA and treatment satisfaction were significantly lower at the FLT side ($P<0.001$, Table 3). At six months follow-up, a significantly higher number of patients preferred TTT.

Assessment by the blinded dermatologist (PhGA) showed a significant worsening of hyperpigmentation of the FLT side compared to baseline during follow-up ($P<0.05$). Treatment with TTT did not result in significant changes (Fig. 1).

Melanin index and L-value showed a significant increase of hyperpigmentation at the FLT side compared to baseline during follow-up ($P<0.05$). At the TTT side, no significant improvement or worsening was observed.

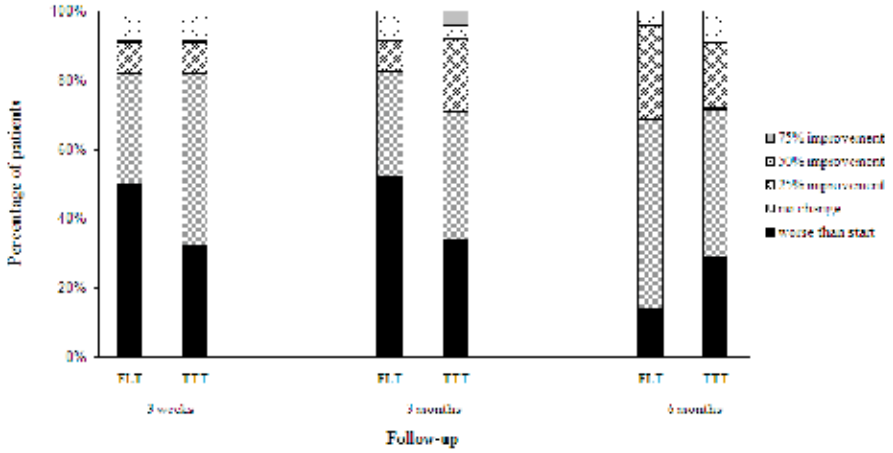

Fig 1 Blinded physician's global assessment of non-ablative 1,550 nm fractional laser therapy and triple topical therapy during follow-up. Significant worsening of melasma was seen during follow-up at the FLT side ($F(1,18)=7.84$, $p<0.05$).

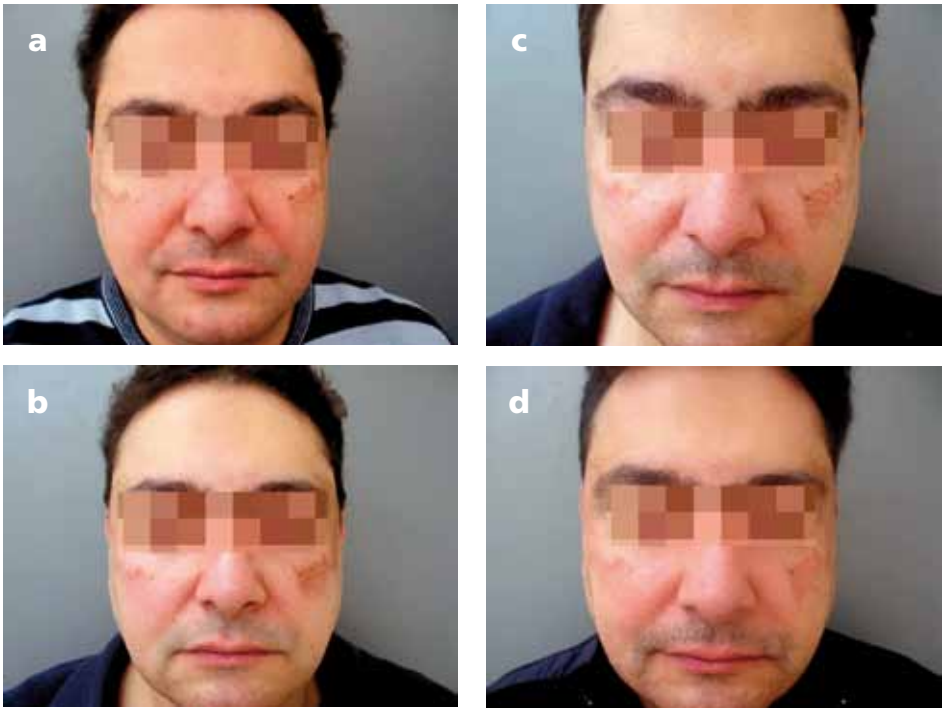

Fig 2 Clinical photographs of a patient before treatment (**a**), at three weeks (**b**), three months (**c**) and six months (**d**). The right side of the face was treated with triple topical therapy for 15 weeks. The left side of the face was treated only two times with non-ablative 1,550 nm fractional laser. Postinflammatory hyperpigmentation persisted during follow-up.

Table 2 Settings of non-ablative 1,550 nm fractional laser

Pulse energy	15 mJ
Level	
skin type II	level 7 (~20% coverage), 4 sessions
skin type III and IV	level 6 (~17% coverage), 5 sessions
skin type V	level 5 (~14% coverage), 5 sessions
Number of passes per session	8
Mean number of treatments	3.6 (1-5)
Mean energy per treatment	0.74 kJ

Table 3 Patient reported outcomes

	3 weeks	3 months	6 months
Patient's Global Assessment (VAS)			
FLT	5.7 (0-10)*	4.9 (0-9)*	4.7 (0-10)*
TTT	5.0 (0-9)	5.7 (1-10)	6.1 (0-9)
Patient's satisfaction (VAS)			
FLT	5.7 (0-10)*	3.5 (0-8)*	5.3 (1-10)*
TTT	5.1 (0-8)	5.5 (1-10)	6.2 (0-8)
Advise to friends/colleagues (%)			
FLT	50	37	26
TTT	28	42	48
No preference	22	21	26

* $p<0.001$

PhGA, melanin index and L-value were not significantly influenced by the use of oral anticonceptives.

Side effects at the FLT side consisted of sunburn-like erythema (99%) with an average duration of four days and burning sensation (86%) with an average duration of one day. Sixty percent of patients reported moderate to severe facial edema with an average duration of two days. Crusting and blistering were reported by 6% and 4% of patients, respectively. Patients reported an average pain score of 5.4 on a scale from 0-10 (table IV). All patients returned to work or normal activity immediately after the laser treatment. Nine patients (31%) developed PIH at the FLT side after two or more laser treatments. All these patients had Fitzpatrick skin type III or higher. PIH occurred in both epidermal and mixed type melasma with a comparable frequency (33% and 25% respectively). Patients who developed PIH were excluded for further laser treatments. Hypopigmentation and scarring were not observed. Reported side effects at the TTT side were erythema (46%) and burning sensation (19%), which

was occasionally continuous as long as treatment was applied. Forty-seven percent of patients reported scaling. One patient was forced to stop TTT after six weeks because of severe erythema. This patient was treated with triamcinolone acetonide 0.1% instead and later with hydroquinone 5% and triamcinolone 0.1%.

DISCUSSION

Using 15 mJ/microbeam, non-ablative 1,550 nm FLT was not safe and effective in the treatment of melasma. Maintenance treatment at the FLT side did not result in improved clearance of melasma. At the TTT side, no significant improvement or worsening was observed. At six months follow-up, a significantly higher number of patients preferred TTT.

To date, there are five uncontrolled studies involving a total of 51 patients with melasma who were treated with non-ablative FLT using a 1,550 nm Fraxel Re:store laser (Solta Medical Inc., Hayward, CA).[8,9,13,18,19] Only one randomized trial has been performed involving 10 patients with melasma treated with the Fraxel Re:store laser and 10 patients treated with TTT.[14] In one uncontrolled study, three patients with melasma were treated with a 1,440 nm Affirm laser (Cynosure Inc., Westford, MA).[20] In the studies using the Fraxel Re:store laser, settings ranged from 2,000 to 3,500 microthermal zones per cm^2 at six to 15 mJ/microbeam. The number of treatments ranged from one to six. Follow-up ranged from zero to six months. In one study, an improvement of 20-50% was reported by all six patients shortly after the last treatment session.[18] At three months follow-up, a mild to excellent clinical improvement was noted in 20 of 23 patients.[9,13,20] Furthermore, in 10 of these 23 patients, histological analysis showed a significant improvement of hyperpigmentation.[13] A remarkable improvement of melasma up to six months post-treatment in one patient was reported by Tannous et al..[8] In contrast, the two larger studies with a six month follow-up showed a gradual recurrence of melasma during follow-up.[14,19]

The reported side effects such as erythema, burning sensation and scaling of the TTT are well known. In our study, side effects of non-ablative 1,550 nm FLT were comparable with those reported by others. The average pain score of 5.4 is comparable with the 6.3 and 6.4 (both on a scale from 0 to 10) reported by Rokhsar et al. and Kroon et al..[9,14] However, the high rate of PIH after non-ablative 1,550 nm FLT found in this study (31%) contrasts with the findings in other studies. In the literature, the occurrence of PIH ranges up to 17%.[9,13,14,18,19] In two studies, involving a total of 20 patients treated with non-ablative 1,550 nm FLT, PIH was not noted at all.[13,14]

Non-ablative 1,550 nm FLT is widely used in melasma and the risk for development of PIH is generally thought to be minimal. However, in the present settings the risk of PIH is substantial.

Firstly, treatment in spring may have led to a high sun exposure of the laser treated site, increasing the risk of laser induced PIH. This may partially explain the high rate of PIH, although patients were instructed to use sunscreen every two hours when outside. In addition, as sun exposure is a risk factor for the development and worsening of melasma, the limited efficacy of both non-ablative 1,550 nm FLT and TTT might be due to the treatment in spring and follow-up in summer.

Furthermore, the relatively high laser settings used in this study might be responsible for the occurrence of PIH. In comparison to most other studies, patients were treated with a relatively high energy per microbeam (15 mJ). Although some authors state that the occurrence of PIH is primarily determined by the density of microscopic treatment zones and not the energy per microbeam, or that it is not dependent on laser parameters at all, there are reasons to suppose that the energy per microbeam does play an important role in the development of PIH.[21,22] In a previous randomized study using the same device, we observed no PIH when treating with an energy of 10 mJ/microbeam.[14] This is in sharp contrast with our present finding of PIH in 31% of patients. It should be noted that the present study was performed in spring and and an energy of 15 mJ/microbeam was applied. The latter does not necessarily lead to such a high rate of PIH. Using the same laser settings, PIH was found in 13% of 25 patients with skin type III or IV in a study by Lee et al.[19]

A minor limitation might be the effect of cooling on the efficacy and safety of non-ablative 1,550 nm FLT. Although cooling is supposed to minimize patient's discomfort during treatment, it also negatively influences the size of microscopic treatment zones and therefore compromizes treatment efficacy.[23,24] Moreover, cold air cooling has been suggested to increase the risk for PIH.[25]

Although TTT did not a show a significant improvement during treatment and follow-up, possibly due to treatment in spring, it remains the gold standard for the treatment of melasma. There is abundant clinical experience and evidence for the efficacy of TTT in the treatment in melasma.[4-7] Costs are lower and the treatment is safer and less painful.

In conclusion, non-ablative 1,550 nm FLT is not effective in the treatment of melasma using 15 mJ/microbeam in spring time. Given the relatively high rate of postinflammatory hyperpigmentation, caution is advocated in the usage of non-ablative 1,550 nm FLT at 15 mJ/microbeam.

ACKNOWLEDGMENTS

The laser equipment and disposables were kindly provided by B&Co Laser Medico (Herzele, Belgium) and Solta Medical Inc. (Hayward, CA) exclusively for the purpose of this study.

REFERENCES

1. Pawaskar MD, Parikh P, Markowski T, McMichael AJ, Feldman SR, Balkrishnan R. Melasma and its impact on health-related quality of life in Hispanic women. J Dermatol Treat 2007; 18:5-9.
2. Gupta AK, Gover MD, Nouri K, Taylor S. Treatment of melasma: a review of clinical trials. J Am Acad Dermatol 2006; 55:1048-1065.
3. Grimes PE. Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995; 131:1453-1457.
4. Gano SE, Garcia RL. Topical tretinoin, hydroquinone, and betamethasone valerate in the therapy of melasma. Cutis 1979; 23:239-241.
5. Taylor SC, Torok H, Jones T, Lowe N, Rich P, Tschen E, Menter A, Baumann L, Wieder JJ, Jarratt MM, Pariser D, Martin D, Weiss J, Shavin J, Ramirez N. Efficacy and safety of a new triple-combination agent for the treatment of facial melasma. Cutis 2003; 72:67-72.
6. Rendon M, Berneburg M, Arellano I, Picardo M. Treatment of melasma. J Am Acad Dermatol 2006; 54(5 Suppl. 2):S272-281.
7. Picardo M, Carrera M. New and experimental treatments of cloasma and other hypermelanoses. Dermatol Clin 2007; 25:353-362.
8. Tannous ZS, Astner S. Utilizing fractional resurfacing in the treatment of therapy-resistant melasma. J Cosmet Laser Ther 2005; 7:39-43.
9. Rokhsar CK, Fitzpatrick RE. The treatment of melasma with fractional photothermolysis: a pilot study. Dermatol Surg 2005; 31:1645-1650.
10. Manstein D, Herron GS, Sink RK , Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004; 34:426-438.
11. Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. J Biomed Opt 2006; 11:1-9.
12. Laubach HJ, Tannous Z, Anderson R, Manstein D. Skin responses to fractional photothermolysis. Lasers Surg Med 2006; 38:142-149.
13. Goldberg DJ, Berlin AL, Phelps R. Histologic and ultrastructural analysis of melasma after fractional resurfacing. Lasers Surg Med 2008; 40:134-138.
14. Kroon MW, Wind BS, Beek JF, Van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Non-ablative fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled pilot study. J Am Acad Dermatol 2010, in press.
15. Gilchrest B, Fitzpatrick T, Anderson R, Parrish J. Localization of melanin pigmentation in the skin with Wood's lamp. Br J Dermatol 1977; 96:245-248.
16. Sanchez N, Pathak M, Sato S. Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. J Am Acad Dermatol 1981; 4:698-710.
17. Pandya A, Berneburg M, Ortonne JP, Picardo M. Guidelines for clinical trials in melasma. Pigmentation Disorders Academy. Br J Dermatol 2006; 156(Suppl. 1):S21-28.
18. Naito SK. Fractional photothermolysis treatment for resistant melasma in Chinese females. J Cosmet Laser Ther 2007; 9:161-163.
19. Lee HS, Won CH, Lee DH, An JS, Chang HW, Lee JH, et al. Treatment of melasma in Asian skin using a fractional 1,550-nm laser: an open clinical study. Dermatol Surg 2009; 35:1499-1504.
20. Karsai S, Raulin C. Fraktionierte photothermolyse, eine neue Option in der Behandlung des Melasma? Hautarzt 2008; 59(2):92-100.

21. Chan HH, Manstein D, Yu CS, Shek S, Kono T, Wei WI. The prevalence and risk factors of post-inflammatory hyperpigmentation after fractional resurfacing in Asians. Lasers Surg Med 2007; 39:381-385.
22. Graber EM, Tanzi EL, Alster TS. Side effects and complications of fractional laser photothermolysis: experience with 961 treatments. Dermatol Surg 2008; 34:301-305.
23. Fisher GH, Kim KH, Bernstein LJ, Geronemus RG. Concurrent use of a handheld forced cold air device minimizes patient's discomfort during fractional photothermolysis. Dermatol Surg 2005; 31:1242-1243.
24. Laubach H, Chan HH, Rius F, Anderson RR, Manstein D. Effects of skin temperature on lesion size in fractional photothermolysis. Lasers Surg Med 2007; 39:14-18.
25. Manuskiatti W, Eimpunth S, Wanitphakdeedecha R. Effect of cold air cooling on the incidence of postinflammatory hyperpigmentation after Q-switched Nd:YAG laser treatment of acquired bilateral nevus of Ota like macules. Arch Dermatol 2007; 143:1139-1143.

6

ABLATIVE FRACTIONAL LASER THERAPY AS TREATMENT FOR BECKER'S NEVUS; A RANDOMIZED CONTROLLED PILOT STUDY

J Am Acad Dermatol. 2010, in press

ABSTRACT

Background: Becker's nevus (BN) is an uncommon pigment disorder characterized by hyperpigmentation and sometimes hypertrichosis. To date, no effective treatment is available.

Objectives: To assess efficacy and safety of ablative 10,600 nm fractional laser therapy (FLT) in the treatment of BN.

Methods: Eleven patients with BN, older than 18 years, were included in a prospective randomized controlled, observer-blinded split-lesion trial. In each patient two similar square test regions were randomized to either ablative FLT at 10 mJ/microbeam, coverage 35-45%, and topical bleaching (to prevent laser-induced postinflammatory hyperpigmentation, PIH); or topical bleaching alone (to allow comparison of the regions). At three and six months follow-up, clearance of hyperpigmentation was assessed by physician's global assessment (PhGA), reflectance spectroscopy, melanin index, patient's global assessment (PGA), patient's satisfaction and histology.

Results: At six months follow-up, PhGA improved in the FLT region ($p<.05$). Reflectance spectroscopy, melanin index, number of melanocytes and amount of dermal melanin did not significantly differ between both regions. PGA and patient's satisfaction were 5.0 and 5.9 (visual analogue scale, 0-10). Side-effects were PIH (n=3), erythema (n=3), burning sensation (n=3), crusting (n=3), edema (n=2) and blistering (n=2).

Limitations: Small number of patients, treatment in spring, possibly suboptimal laser settings, and the combined usage of FLT and a bleaching agent.

Conclusion: Ablative FLT was moderately effective in some patients with BN. However, postinflammatory hyperpigmentation and relatively negative patient reported outcomes still preclude ablative FLT from being a standard therapy. Larger studies with different laser settings will be required to optimize this treatment modality.

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INTRODUCTION

Becker's nevus (BN) is an uncommon acquired skin disorder, characterized by the development of unilateral hyperpigmented patches, that eventually develop a slightly elevated, verrucous surface and often hypertrichosis (56-70% of male cases).[1-3] The prevalence ranges from 0.25 to 2.5%, and is about five times more frequent in males than in females.[2-4] Little is known about the pathogenesis of the disorder, but increased androgen sensitivity has been suggested as a possible etiologic factor.[5-9] Currently, no treatment is available for BN. Studies on intense pulsed light source and Q-switched ruby laser showed disappointing or even counterproductive effects.[10, 11] Better results have been achieved with erbium YAG (Er:YAG) laser and long-pulsed alexandrite laser.[12, 13]

Recently, non-ablative fractional laser therapy (FLT) was suggested as treatment option for BN.[14] The main principle of FLT is the coagulation or ablation of small columns of skin, leaving the surrounding tissue intact.[15] This enhances healing of the treated skin after treatment, minimizing the risk of unwanted effects. As the microscopic treatment zones are only 50-150 µm in diameter, a visible wound does usually not occur.[16] Ablative FLT might be even more effective in the treatment of BN than non-ablative FLT, as complete ablation of microscopic treatment zones takes place instead of coagulation, preventing a possible reuptake of melanin from the microscopic treatment zones by dermal macrophages and keratinocytes.

The aim of this study was to assess efficacy and safety of ablative FLT in the treatment of BN.

METHODS

Study design / Patients

A randomized controlled observer-blinded study was performed in 11 patients. Patients older than 18 years, with BN and Fitzpatrick skin types II-V were recruited from the outpatient clinic of the Netherlands Institute for Pigment Disorders at the Academic Medical Center in Amsterdam. The study protocol has been approved by the local medical ethical committee and registered in the clinicaltrials.gov trial register (ClinicalTrials.gov Identifier: NCT01083498). Verbal and written information regarding risks, benefits, and potential complications was given to the patients, and written informed consent was obtained. Exclusion criteria were: use of bleaching creams during the past six weeks, history of keloid, history of herpes infection, active eczema, suspected hypersensitivity to lidocaine or hydroquinone, use of isotretinoin in the past six months, and recent high exposure of the lesion to sunlight or UV light (UVA or UVB). Patients with BN in sun exposed areas were instructed to use sunscreen (sun protecting factor 50+) every two hours when being outside.

In each patient, two similar square test regions of approximately 3x3 cm were randomized to receive either ablative FLT in combination with intermittent topical bleaching (to prevent laser-induced postinflammatory hyperpigmentation, PIH) or the same intermittent regimen of topical bleaching alone (to allow comparison of the regions). The randomization procedure involved sealed envelopes in which the allocation was indicated. The sealed envelopes were numbered from 1-11. Envelopes were opened in ascending order. The randomization was based on a digitally created random list (Graphpad Software Inc., La Jolla, CA) generated by an independent cooperator. Treatment started in March 2009. Follow-up visits at our institute were scheduled at three and six months after the last laser treatment. Follow-up ended December 2009.

All patients received the same intermittent regimen of triple topical therapy (hydroquinone 5%, tretinoin 0.05%, triamcinolone acetonide 0.1% cream), a compound that does not influence the hyperpigmentation of BN by itself. Patients were instructed to apply the cream once a day in the evening on both treatment and control site from the second week after each treatment to the next laser treatment.

Fractional laser therapy

FLT consisted of three sessions; with an interval of six weeks after the first session, and four weeks after the second. A 10,600 nm ablative fractional laser (Fraxel re:pair laser, Solta Medical Inc., Hayward, CA) was used. One treatment session involved four FLT passes to create an estimated final coverage of 45% (level 11) in patients with skin type II, and 35% (level 9) in patients with skin type III-V. Two passes were made in one direction and two perpendicularly. The energy per microbeam was 10 mJ (Table I). Anesthesia consisted of topical 2.5% lidocaine and 2.5% prilocaine ointment two hours prior to each treatment. After each laser treatment, patients were instructed to clean the laser treated area with a 1% acetic acid solution three to five times a day for the first three days. In addition, patients were advised to use an indifferent cream to prevent itching and burning sensation. Patients who developed PIH were excluded for further laser treatment. These patients were advised to use triple topical therapy once a day until three months follow-up.

Table I. Settings of ablative fractional laser used in this study

Pulse energy per microbeam	10 mJ/microbeam
Level	
skin type II	level 11 (45% coverage)
skin type III-V	level 9 (35% coverage)
Number of passes per session	4
Mean treated area per patient (cm^2)	9
Mean number of treatments	2.7 (1-3)
Mean energy per treatment	0.11 kJ

Patient-reported outcomes

All side effects were documented and patients were asked to score erythema, burning, edema, crusting, and vesicles on a scale from 0 to 3. In addition, the duration of these side effects was registered. Patients were asked to score the improvement of hyperpigmentation on a visual analogue scale (VAS) from 0 to 10 (Patient's Global Assessment, PGA) at all follow-up moments. Moreover, pain and patient's satisfaction were scored on a VAS from 0 to 10.

Physician's Global Assessment

A blinded observer dermatologist assessed the Physician's Global Assessment (PhGA), as proposed by the Pigmentary Disorders Academy,[17] as main outcome parameter, using photographs that were taken under standardized conditions with a digital camera (Canon G6, Canon Components Inc., Saitama, Japan) before treatment and at follow-up. Improvement of hyperpigmentation was scored on a scale from zero to six (0: total clearance (100% improvement), 1: almost total clearance (90-99% improvement), 2: distinct clearance (75-89% improvement) 3: moderate clearance (50-74% improvement) 4: mild clearance (25-49% improvement) 5: no change, 6: worsening of hyperpigmentation).

Reflectance spectroscopy and melanin index

Improvement of hyperpigmentation was assessed by color measurement through reflectance spectroscopy (Microflash 200 d, Datacolor International, Lawrenceville, GA). This instrument, with an aperture of 4 mm, determines color by measuring the intensity of reflected light of particular wavelengths. In this study, the obtained L value, reflecting the lightness of the measured area of skin, was used. In addition, melanin index was measured using a spectrophotometer (Derma-Spectrometer, Cortex Technology ApS, Hadsund, Denmark) in order to assess changes in the amount of dermal and epidermal melanin.

Measurements were performed at the optical center of both treated and control site before the first treatment and at follow-up.

Histopathologic assessment

At three months follow-up, 2 mm punch biopsies were taken for light microscopic evaluation of the treatment induced changes. Of each patient, a biopsy was taken from the optical center of both the treated site and of the control site of the lesion. Biopsies were fixed in buffered formalin, and after paraffin embedding 5 μm sections were stained with haematoxylin & eosin stain for routine histologic observations and with Fontana stain for evaluation of the site and extent of melanin pigmentation. Additionally, one section was immunostained with Melan A monoclonal antibody (clone A103, dilution 1:50, Monosan) which specifically stains melanocytes. Number of melanocytes in each biopsy was counted per high power field (magnification x400)

using a Zeiss Axioskop 2 microscope (Carl Zeiss MicroImaging Inc, Thornwood, NY). All stained sections were screened by a pathologist who was blinded to information on the site (control or treated) of which the biopsies were taken.

Statistical analysis

As there are no reported data in the literature, a sample size calculation could not be performed. Means, standard deviations, two-tailed homoscedastic Student's t-tests, and ANOVA tests were performed with Statistical Package for the Social Sciences 16.0 (Chicago, IL).

6

RESULTS

Treatments were given from March to June 2009 and follow-up ended in December 2009. A total of 11 patients were randomized in this trial. The characteristics of the 11 treated patients are described in Table I. Mean energy per laser treatment was 0.11 kJ (Table II). One patient was lost to follow-up for reasons not related to this study. Thus, 10 patients completed the study.

Table II. Characteristics of 11 patients with Becker's nevus

Male:female ratio	1.75:1
Mean age	31 (18-58)
Age of onset	13 (8-20)
Skin type	
II	6
III	3
V	2
Localization	
Head	1
Torso	5
Upper extremity	4
Lower extremity	1
Hypertrichosis	6
Hypertrophy	0
Mean surface area of whole lesion (cm^2)	164 (28-340)
Previous treatments	
Dermabrasion	1
Laser (unknown)	1
None	8

At three and six months follow-up, mean PGA was 3.8 and 5.0 and mean patient's satisfaction was 5.1 and 5.9 respectively (VAS 0-10). Four of ten patients would recommend FLT to other patients at six months follow-up. Direct side effects of FLT were mild to moderate and consisted of erythema (n=3), burning sensation (n=3) and crusting (n=3) lasting up to seven days and, less frequently, edema (n=2) or blistering (n=2). Patients reported an average pain score of 4.0 (VAS 0-10).

The results of the blinded dermatologist assessment (PhGA) showed a significant improvement of hyperpigmentation at the region treated with FLT at three and six months follow-up compared to baseline (Fig 1 and 2).

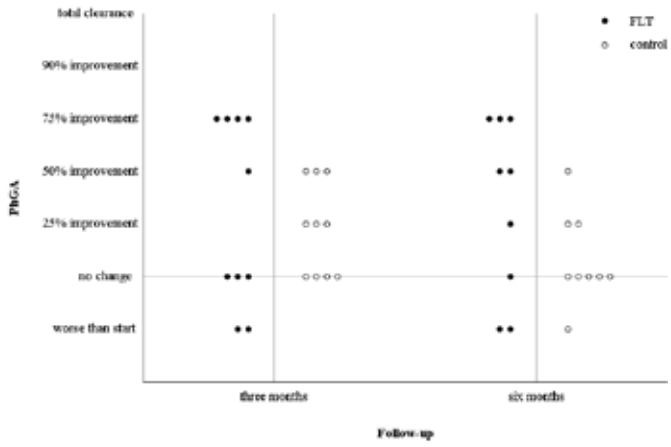

Fig 1. Physician's global assessment of FLT and control at three and six months follow-up.

Three male patients, two with skin type II and one with skin type III developed PIH after one, two and three laser treatments, as assessed by the treating physician. During follow-up, PIH resolved in one of the three patients. According to PhGA, all other patients showed an average improvement of hyperpigmentation of 50 and 58% at three and six months follow-up ($p<.05$). BN which developed PIH were located on the face, axilla and waist respectively.

Reflectance spectroscopy and melanin index did not significantly differ within and between both test regions (Table III). Histologically, number of melanocytes and amount of dermal melanin did not significantly differ between both test regions (Fig 3).

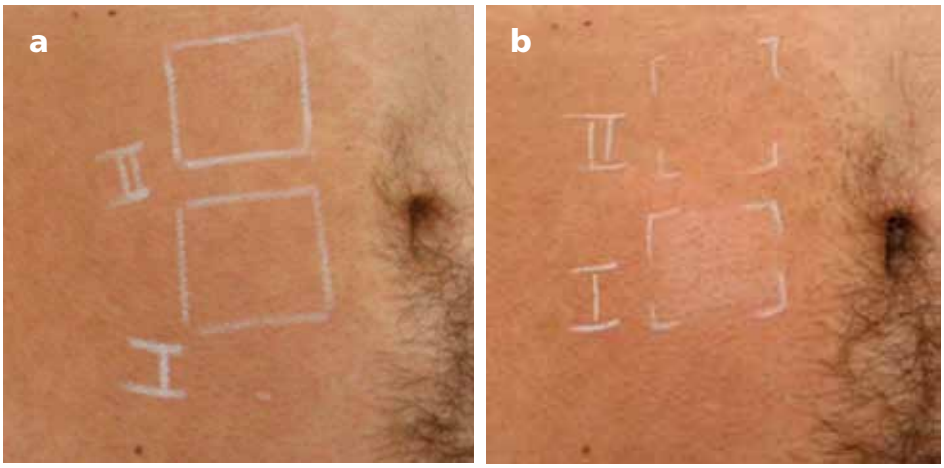

Fig 2. Clinical photographs of a BN on the abdomen before (a), and 6 months after the last ablative FLT (b), showing marked improvement of hyperpigmentation in square I versus no improvement in untreated control (square II).

Table 3. Objective measurements at three and six months follow-up. No significant differences in Melanin Index, L-value or number of melanocytes were found

	three months	six months
Melanin Index		
FLT	40	37
control	40	37
L-value		
FLT	54	55
control	53	56
Number of melanocytes per HPF		
FLT	13	-
control	13	-

HPF – high power field, x400
FLT – Fractional laser therapy

DISCUSSION

Becker's nevus is generally regarded to be resistant to laser therapy. In this study, ablative 10,600 nm FLT proved to be moderately effective in some patients, regardless of skin type. PhGA, the main outcome parameter, improved at three and six months follow-up. However, three out of 11 patients developed laser-induced PIH. Patient

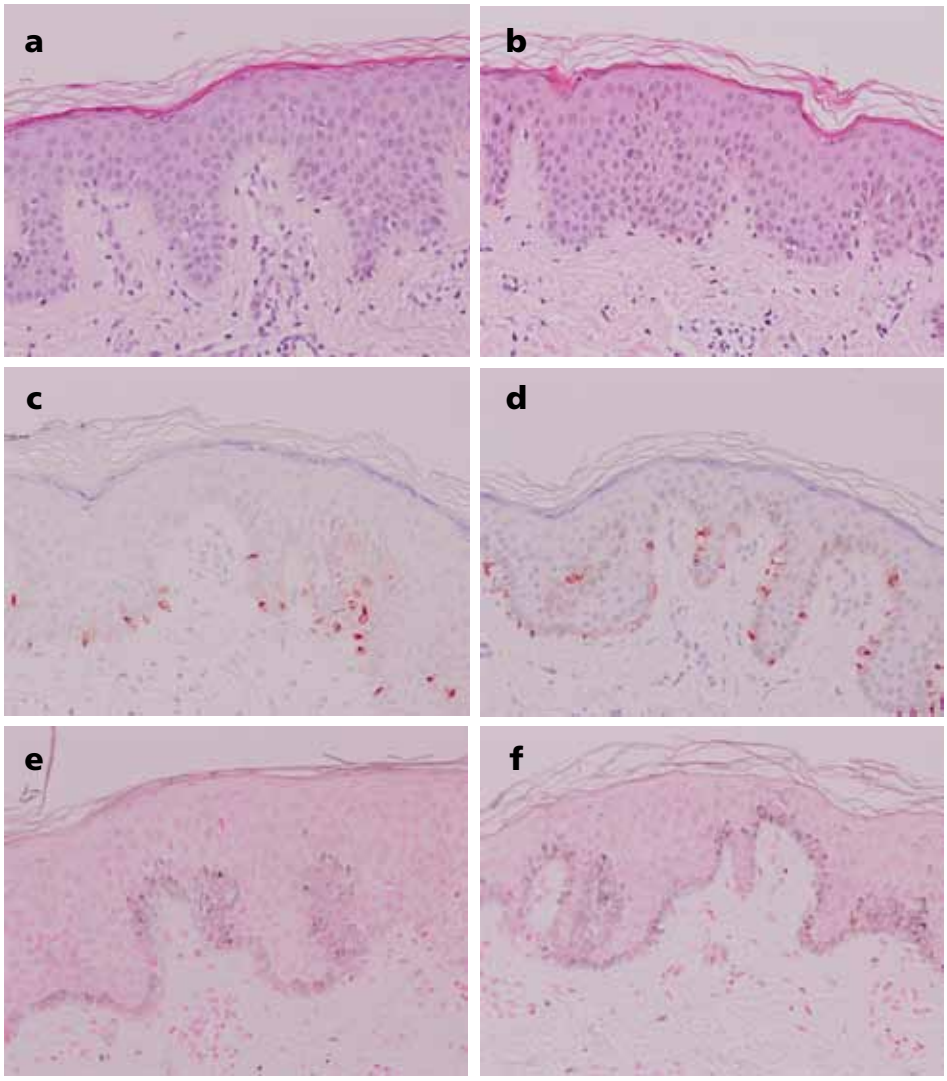

Fig. 3 Light microscopic images of a BN treated with ablative FLT (panel a, c, e) and control (b, d, f), at three months follow-up. There were no significant differences noted in structure of the epidermis (panel a, b, Haematoxylin &Eosin stain), in the numbers of melanocytes (panel c, d, Melan A immunostain) or in amounts or distribution of melanin pigmentation (panel e, f, Fontana stain) in both instances. All magnifications are X20.

reported outcomes were relatively negative. While PhGA improved significantly, there was no significant improvement in objective color measurement and histopathologic assessment. Possibly a larger number of subjects is necessary to reach statistical significance of such endpoints.

In the literature one report has been published of two patients with BN treated successfully with non-ablative FLT.[14] Side effects consisted of mild pain and moderate to severe erythema and edema. Crusting was not observed. To date, there are four studies on BN treated by other laser modalities. One paper is an observer blinded trial comparing Er:YAG laser and Q-switched neodymium YAG (Nd:YAG) laser in 22 patients with BN. Treatment with Er:YAG resulted in at least 50% clearance in all patients at two year follow-up. Although this rate was also achieved in the Nd:YAG laser group, repigmentation rate was higher. Side effects were comparable to those that occurred in our study and consisted mainly of erythema and crusting. No PIH was observed.[12] Contradicting results have been reported after treatment with a Q-switched ruby laser.[11, 18] A recent study by Choi et al. showed a fair to excellent clinical response in 11 patients treated with long-pulsed alexandrite laser with mild to moderate side effects in some patients, consisting of hypopigmentation, skin texture change and formation of a hypertrophic scar.[13]

The main limitations of this study are (i) the small number of patients, (ii) treatment in spring (iii) laser settings that may have been suboptimal, (iv) and the combined usage of FLT and a bleaching agent. The number of patients included in this study was too small to note any difference in treatment results between the patients with different Fitzpatrick skin types and between patients with different clinical characteristics of BN. However, the randomized, split-lesion design of this study enhanced the reliability of the data, as it enabled us to achieve significant results with a relatively small group of patients.

Secondly, treatment in spring and summer may have led to high sun exposure of the laser treated site, which could have increased the risk of laser-induced PIH. In one of the three patients who developed PIH, the BN was located on the face. Friction induced by arm-body movement and tight clothing might have contributed to the other two cases of PIH (axilla and waist).

Furthermore, experience with this treatment modality in BN is lacking. Dose-response studies have not been conducted so far. We suggest that treatment with more conservative laser settings, especially in the treatment of sun exposed skin, might reduce the risk of laser-induced PIH, possibly at the expense of reduced treatment efficacy. Treatment in fall and winter may also reduce the risk of laser-induced PIH.

Finally, as a combination of FLT and topical bleaching was compared to topical bleaching alone, the effect may not completely be contributed to laser treatment alone.

In conclusion, ablative 10,600 nm fractional laser therapy proved to be moderately effective in some patients, regardless of skin type. The PhGA improved at three- and six months follow-up. However, three of 11 patients developed laser-induced PIH and patient-reported outcomes were relatively negative. Therefore, ablative FLT should not be regarded as the definitive therapy for the treatment of BN.

A larger cohort of patients should be recruited in future investigations in order to compare treatment outcomes in different skin types and different characteristics of BN. Moreover, given the high rate of laser-induced PIH, optimization of laser parameters is mandatory in future research.

ACKNOWLEDGMENTS

The laser equipment and disposables were kindly provided by B&Co Laser Medico (Herzele, Belgium) and Solta Medical Inc. (Hayward, CA), exclusively for the purpose of this study. B&Co Laser Medico and Solta Medical Inc. had no role in design and conduct of this study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript.

REFERENCES

1. Becker SW. Concurrent melanosis and hypertrichosis in distribution of nevus unius lateris. Arch Derm Syphilol 1949;60:155-60.
2. Tymen R, Forestier JF, Boutet B, Colomb D. Naevus tardif de Becker, a propos d'une série de 100 observations. Ann Dermatol Venereol 1981;108:41-6.
3. Ingordo V, Gentile C, Iannazzone SS, Cusano F, Naldi L. The 'EpiEnlist' project: a dermo-epidemiologic study on a representative sample of young Italian males. Prevalence of selected pigmentary lesions. J Eur Acad Dermatol Venereol 2007;21:1091-6.
4. McLean DI, Gallagher RP. "Sunburn" freckles, cafe-au-lait macules, and other pigmented lesions of schoolchildren: the Vancouver Mole Study. J Am Acad Dermatol 1995;32:565-70.
5. Kim YJ, Han JH, Kang HY, Lee ES, Kim YC. Androgen receptor overexpression in Becker nevus: histopathologic and immunohistochemical analysis. J Cutan Pathol 2008;35:1121-6.
6. Grande SH, Harris R, Hansen CD, Callis Duffin KP, Florell SR, Hadley ML. Androgen receptor expression patterns in Becker's nevi: an immunohistochemical study. J Am Acad Dermatol 2008;59:834-8.
7. Nirde P, Dereure O, Belon C, Lumbroso S, Guilhou JJ, Sultan C. The association of Becker nevus with hypersensitivity to androgens. Arch Dermatol 1999;135:212-4.
8. Formigon M, Alsina MM, Mascaro JM, Rivera F. Becker's nevus and ipsilateral breast hypoplasia--androgen-receptor study in two patients. Arch Dermatol 1992;128:992-3.
9. Person JR, Longcope C. Becker's nevus: an androgen-mediated hyperplasia with increased androgen receptors. J Am Acad Dermatol 1984;10:235-8.
10. Moreno Arias GA, Ferrando J. Intense pulsed light for melanocytic lesions. Dermatol Surg 2001;27:397-400.
11. Kopera D, Hohenleutner U, Landthaler M. Quality-switched ruby laser treatment of solar lentigines and Becker's nevus: a histopathological and immunohistochemical study. Dermatology 1997;194:338-43.
12. Trelles MA, Allones I, Moreno-Arias GA, Velez M. Becker's naevus: a comparative study between erbium: YAG and Q-switched neodymium:YAG; clinical and histopathological findings. Br J Dermatol 2005;152:308-13.
13. Choi JE, Kim JW, Seo SH, Son SW, Ahn HH, Kye YC. Treatment of Becker's nevi with a long-pulse alexandrite laser. Dermatol Surg 2009;35:1105-8.
14. Glaich AS, Goldberg LH, Dai T, Kunishige JH, Friedman PM. Fractional resurfacing: a new therapeutic modality for Becker's nevus. Arch Dermatol 2007;143:1488-90.
15. Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004;34:426-38.
16. Hantash BM, Mahmood MB. Fractional photothermolysis: a novel aesthetic laser surgery modality. Dermatol Surg 2007;33:525-34.
17. Pandya AG, Guevara IL. Disorders of hyperpigmentation. Dermatol Clin 2000;18:91-8, ix.
18. Tse Y, Levine VJ, McClain SA, Ashinoff R. The removal of cutaneous pigmented lesions with the Q-switched ruby laser and the Q-switched neodymium: yttrium-aluminum-garnet laser. A comparative study. J Dermatol Surg Oncol 1994;20:795-800.

7

INCREASED FORMATION OF FIBROSIS AFTER TREATMENT WITH ABLATIVE VERSUS NON-ABLATIVE FRACTIONAL LASER THERAPY

Submitted

ABSTRACT

Background and objective: Fractional laser therapy (FLT) has become a widely accepted modality for skin rejuvenation, and has also been used in various other skin diseases. Aim of this study was to compare long-term histological effects of non-ablative and ablative FLT in the treatment of pigment disorders.

Materials and Methods: A randomized controlled observer-blinded study was performed in 18 patients with pigment disorders. In each patient, two similar test-regions were randomized to receive either FLT with intermittent topical bleaching (to prevent laser-induced postinflammatory hyperpigmentation) or topical bleaching alone (to allow comparison of the regions). Patients with ashy dermatosis and postinflammatory hyperpigmentation were treated with non-ablative 1,550 nm FLT (15 mJ/microbeam, 14-20% coverage), whereas patients with Becker's nevus were treated with ablative 10,600 nm FLT (10 mJ/microbeam, 35-45% coverage), for a total of three to five sessions. Biopsies were obtained three months after the last treatment, and analyzed by a blinded dermatopathologist.

Results: Clinical results have been described in two previous studies. At follow-up, dermal fibrosis was observed in four patients treated with ablative FLT. This complication was not observed in patients treated with non-ablative FLT. Thus development of fibrosis was seen significantly more often in patients treated with ablative as compared to non-ablative FLT ($p<.05$).

Conclusion: With these laser settings, ablative fractional laser therapy induces formation of fibrosis, whereas treatment with non-ablative fractional laser therapy does not. Whether formation of fibrosis has to be regarded as dermal remodeling or a subtle subclinical form of scarring should be investigated in future research.

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INTRODUCTION

Fractional laser therapy (FLT) has become a widely accepted modality for skin rejuvenation and the treatment of scars. Dermal remodeling and neocollagenesis is a key feature that results from ablative and non-ablative modalities of FLT. Non- ablative FLT generates multiple small sized coagulated zones, separated by surrounding untreated tissue.[1] It has been suggested that the microscopic treatment zones involved allow transport and extrusion of microscopic epidermal necrotic debris including melanin and dermal material through a compromised dermal–epidermal junction, making non-ablative FLT a potentially useful treatment option for pigment disorders.[1,2] In ablative FLT, additional to coagulation, ablation of microscopic treatment zones takes place, that potentially enhances dermal remodeling. Generally, a visible wound does not appear because the microscopic treatment zones have a diameter <100 µm.[1] Because only part of the skin surface is treated in one session, recovery is relatively fast. Histological studies have shown that permanent tissue damage is usually minimal or absent after either non-ablative or ablative FLT.[3,4] Only one study reported increased collagen deposition and fibrosis after ablative FLT in human skin, and histological comparisons between non-ablative and ablative FLT have not been published.[5]

The aim of this study was to compare the histological outcome of non-ablative and ablative FLT three months after four to five treatments.

PATIENTS, MATERIALS AND METHODS

Patients

Skin biopsies were obtained from 18 of 25 patients participating in two randomized clinical trials, with the non-ablative and ablative fractional laser for the treatment of pigment disorders. Patients, materials, methods and results have been described extensively in two previous studies (Kroon et al., submitted).[6]

Briefly, 18 patients with ashy dermatosis (AD; n=6), postinflammatory hyperpigmentation (PIH; n=4), and Becker's nevus (BN; n=8) were enrolled in this randomized controlled observer-blinded study. Patients older than 18 years with Fitzpatrick skin types II-V were recruited from the outpatient clinic of the Netherlands Institute for Pigment Disorders at the Academic Medical Center in Amsterdam. Verbal and written information was given. The study protocol was approved by the local medical ethics committee.

In each patient, two similar square test regions of approximately 3x3 cm were randomized to receive either FLT in combination with intermittent topical bleaching (to prevent laser-induced PIH) or the same intermittent regimen of topical bleaching alone (to allow comparison of the regions). Patients with AD and PIH were treated

with 1,550 nm non-ablative FLT, whereas patients with BN were treated with ablative 10,600 nm FLT. The randomization involved sealed envelopes opened in ascending order in which the allocation was indicated. This list was based on a digitally created random list (GraphPad Software, Inc., La Jolla, CA) generated by an independent cooperator. All patients received the same intermittent regimen of triple topical therapy (hydroquinone 5%, tretinoin 0.05%, triamcinolone acetonide 0.1%) cream. Patients were instructed to apply the cream once a day in the evening on both treatment and control site from the second week after each treatment until the next laser treatment.

Fractional laser therapy

Patients with AD and PIH were treated with a 1,550 nm non-ablative fractional laser (Fraxel re:store laser, Solta Medical Inc., Hayward CA) at four to five sessions. One treatment session involved eight FLT passes to create an estimated final coverage of 20% (corresponding to level 7) in patients with skin type II, 17% (corresponding to level 6) in patients with skin type III or IV, and 14% (corresponding to level 5) in patients with skin type V. Four passes were made in one direction and four perpendicularly. The energy per microbeam was 15 mJ (Table 1). During treatment, cooling of the skin was achieved using a Zimmer Cryo 6 Cold Air Device (Phoenix Medical, Inc., Phoenix, AZ).

Patients with BN were treated with a 10,600 nm ablative fractional laser (Fraxel re:pair laser, Solta Medical Inc., Hayward, CA) at three sessions. One treatment session involved four FLT passes to create an estimated final coverage of 45% (corresponding to level 11) in patients with skin type II, and 35% (corresponding to level 9) in patients with skin type III-V. Two passes were made in one direction and two perpendicularly. The energy per microbeam was 10 mJ (Table 1).

We assumed that the type of pigment disorder had no influence on dermal remodelling and the induction of fibrosis.

In all patients, anesthesia consisted of topical 2.5% lidocaine and 2.5% prilocaine ointment (EMLA cream ®) two hours prior to each treatment.

Histopathological assessment

Three months after the last laser treatment, 2 mm punch biopsies were taken for light microscopic evaluation of the treatment induced changes. Of each patient, a biopsy was taken from the optical center of both the treated site and the control site of the lesion. Biopsies were fixed in buffered formalin, and after paraffin embedding, 5 µm sections were stained with hematoxylin and eosin (H&E) stain for routine histological observations. In each of these biopsies the extent of fibrosis was determined per high power field using a Zeiss Axioskop 2 microscope (magnification x400; Carl Zeiss MicroImaging Inc, Thornwood, NY). Extent of fibrosis was scored as absent (0), minimally present (1) or present (2). All stained sections were screened by a dermatopathologist who was blinded to information on the site (control or treated) of which the biopsies were taken.

Table 1 Settings of non-ablative and ablative fractional laser therapy (FLT) used in this study

	1,550 nm non-ablative FLT	10,600 nm ablative FLT
Pulse energy per microbeam	15 mJ/microbeam	10 mJ/microbeam
Level		
skin type II	level 7 (20% coverage)	level 11 (45% coverage)
skin type III	level 7 (20% coverage)	level 9 (35% coverage)
skin type IV	level 6 (17% coverage)	level 9 (35% coverage)
skin type V	level 5 (14% coverage)	level 9 (35% coverage)
Number of passes per session	8	4
Mean treated area per patient (cm^2)	9	9
Mean number of treatments	4.1 (2-5)	2.7 (1-3)
Mean energy per treatment	0.17 kJ	0.11 kJ

Levels of fractional laser correspond to a certain density of treatment.

Statistical analysis

Means, standard deviations, and two-tailed homoscedastic Student's t-tests were performed with Statistical Package for the Social Sciences 16.0 (SPSS, Chicago, IL).

RESULTS

Skin biopsies were obtained from 18 of 25 patients participating in the clinical trials. With the used laser settings, non-ablative FLT was not effective for the treatment of ashy dermatosis and postinflammatory hyperpigmentation (Kroon et al., (submitted). However, ablative FLT was moderately effective in some patients with BN. Of note, postinflammatory hyperpigmentation was a frequently observed side effect.[6] No clinical signs of a scar or fibrosis were seen in all patients.

Patient characteristics are described in Table 2. Mean energy was 0.16 kJ per non-ablative and 0.11 kJ per ablative FLT session.

Non-ablative fractional laser

H&E staining revealed varying degrees of dermal or epidermal hyperpigmentation, consistent with AD and PIH. At three months follow-up, H&E staining showed no definitive evidence of microscopic treatment zones at the region treated with non-ablative FLT; rete ridges were normal and no microscopic epidermal necrotic debris was present. No significant differences in collagen deposition between the region treated with non-ablative FLT and the control region were observed. No signs of fibrosis were seen at the region treated with non-ablative FLT (Fig. 1a).

Table 2 Characteristics of 18 patients with Becker's nevus (BN), ashy dermatosis (AD) and postinflammatory hyperpigmentation (PIH)

	PIH (N=4)	AD (n=6)	BN (n=8)
Male:female ratio	00:04	03:03	05:03
Mean age	41	41	27
Disease duration (y)	18	6	11
Skin type			
II	-	-	5
III	-	1	2
IV	1	5	1
V	3	-	-
Localization			
Head/neck	1	1	-
Torso	1	5	5
Upper extremity	1	-	3
Lower extremity	-	-	-
Previous treatments			
Laser (unknown)	-	-	2
Bleaching creams	4	2	-
Corticosteroid creams	-	5	-
None	-	-	6

Ablative fractional laser

H&E staining revealed elongated, blunted rete ridges with basal epidermal hyperpigmentation characteristic for BN at both treated and control region at three months follow-up. In addition, smooth muscle hypertrophy in the dermis was observed. No definitive evidence of microscopic treatment zones at the region treated with non-ablative FLT was found. However, four out of eight patients (extent of fibrosis score: 2) developed dermal fibrosis due to increased collagen deposition at the region treated with ablative FLT compared to the control region (Fig. 1b). Clinically, no scar formation was noted. Three of these patients had skin type II, and one patient skin type IV. Two of these four BN were located on the torso, and two on the upper extremities.

Comparing non-ablative and ablative FLT, treatment with ablative FLT was associated with a significantly higher occurrence rate of dermal fibrosis ($p<.05$).

DISCUSSION

In this study, formation of dermal fibrosis at three months follow-up was observed in half of the patients treated with ablative FLT, whereas no fibrosis was observed in patients treated with non-ablative FLT. The occurrence of fibrosis after ablative FLT might be merely a sign of transient dermal remodeling which is supposed to be responsible for the skin tightening effect generally attributed to FLT.[7] In that case, our data would suggest that dermal remodeling following ablative FLT is more rigorous than following non-ablative FLT, which may enhance treatment efficacy. On the other hand, if fibrosis is persistent, it may be indicative of subtle subclinical scar formation due to FLT.

To date, no studies have been conducted comparing the histological effects of non-ablative and ablative FLT. However, the long-term histological effects of non-ablative and ablative FLT have been described in several studies separately. Three months after non-ablative FLT, histological evidence of laser induced microscopic treatment zones is usually absent and the initial inflammatory infiltrate has resolved. Newly formed collagen in the treated areas has become continuous with preexistent collagen in the untreated areas and the occurrence of fibrosis has not been reported so far.[1,3,4] In addition, clinically manifest scarring has not been observed. On the other hand, an increase in the undulation of rete ridge patterns suggestive of skin tightening has been demonstrated three months after treatment.[1]

In contrast, after ablative FLT, signs of dermal remodeling have been demonstrated even at long-term follow-up. An increase in heat shock protein 47 was found consistent with continued diffuse collagen synthesis and remodeling three months after ablative FLT.[7] These findings were confirmed in a study by Helbig et al., although follow-up was only two weeks.[8] In addition, formation of dermal fibrosis was observed eight weeks after ablative FLT in a study by Berlin and colleagues using H&E staining.[5] Clinically evident scarring in the neck due to ablative FLT has been reported in a limited number of cases.[8,9]

The main limitations of the present study are: (i) patients were not randomly allocated to either non-ablative or ablative FLT as we report results from two within-patient randomized trials; (ii) no pretreatment biopsies were taken, (iii) the number of patients was small, (iv) H&E staining for routine histology, and (v) follow-up may have been too short to assess if fibrosis was persistent.

Firstly, patients were not randomized to receive either non-ablative or ablative FLT. Instead, allocation was determined by the patient's skin disorder. However, none of the patients had any clinical textural changes or histological dermal changes besides smooth muscle hypertrophy in BN, therefore we assumed that patients with BN have a similar dermal response to injury as patients with AD and PIH.

Furthermore, no pretreatment biopsies were obtained, so that comparison of histological characteristics within the same region before and after FLT was not possible. However, our study has a randomized split-lesion design, i.e. in each patient

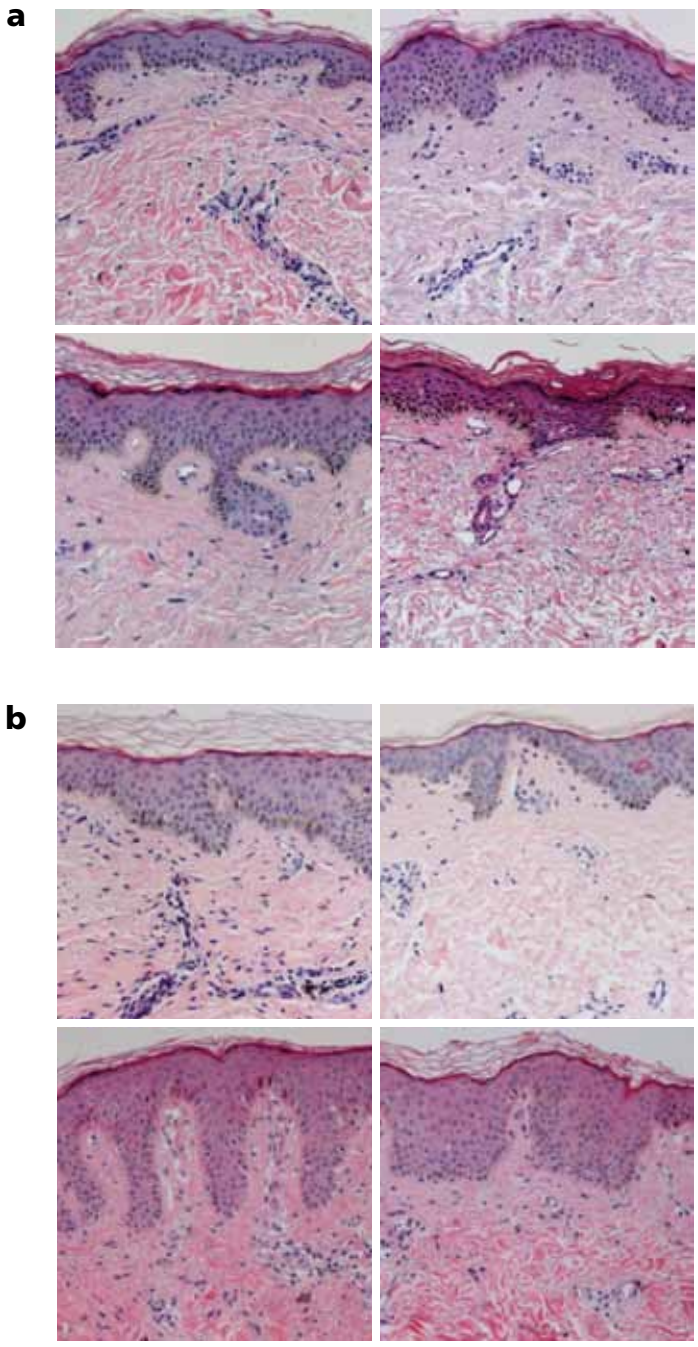

Fig 1. Biopsy specimens from four patients at three months follow-up. No fibrosis was seen in the specimens treated with non-ablative FLT (right panel) versus their control biopsies (left panel). (**a**). Formation of fibrosis was seen in patients treated with ablative FLT (left panel) versus their control biopsies (right panel) (**b**).

two similar test regions were randomized to receive either a combination of FLT and topical bleaching, or topical bleaching alone. As a result, a reliable comparison could be made between treated and untreated lesional skin. As biopsies were routinely stained with H&E and were only 2 mm in diameter, there was unfortunately not enough material left to extra stain with picrosirius red. Although this might have been even more informative, a more sensitive staining like picrosirius red may reveal additional cases with limited fibrosis and therefore demonstrate an even higher proportion of patients with fibrosis.

It should be noted that the energy per microbeam was higher in the patients treated with non-ablative FLT. However, the coverage was 14-20% in non-ablative FLT, but 35-45% in ablative FLT. These differences in settings may contribute to the formation of fibrosis. Recent work has shown that only minimal differences in dermal remodeling were observed between lower and higher microbeam energy settings (15 versus 70 mJ/microbeam) in non-ablative FLT.[10] Therefore, differences in energy settings in the non-ablative FLT group do not seem responsible for the observed differences in fibrosis. Due to the small number of patients included in this study, we could not demonstrate an association between the occurrence of fibrosis and specific patient characteristics or laser settings. Therefore, it is not clear why certain patients treated with ablative FLT developed dermal fibrosis, and others did not.

In conclusion, the treatment of ablative fractional laser therapy at 10 mJ and coverage of 35-45% predisposes to the formation of fibrosis, whereas treatment with non-ablative fractional laser therapy at 15 mJ is probably not associated with the formation of fibrosis. It is unclear whether formation of fibrosis should be attributed to the process of dermal remodeling and is thus a desirable effect, or if it should be regarded as a subtle subclinical form of scarring. In this respect, further research with a larger cohort of patients is mandatory to assess for specific patient associated factors, which may increase the risk of the formation of fibrosis after FLT. Finally, the long-term clinical implications of fibrosis observed in histological specimens should be investigated in future studies.

ACKNOWLEDGMENTS

The laser equipment and disposables were kindly provided by B&Co Laser Medico (Herzele, Belgium) and Solta Medical Inc. (Hayward, CA), exclusively for the purpose of this study. B&Co Laser Medico and Solta Medical Inc. had no role in design and conduct of this study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript.

REFERENCES

1. Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR (2004) Fractional photothermolysis: A new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med;34:426–438.
2. Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF (2006) Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. J Biomed Opt;11:1–9.
3. Laubach HJ, Tannous Z, Anderson RR, Manstein D (2006) Skin responses to fractional photothermolysis. Lasers Surg Med;38:142–149.
4. Dainichi T, Ueda S, Fumimori T, Kiryu H, Hashimoto T (2010) Skin tightening effect using fractional laser treatment II: A pilot animal study on skin remodeling. Dermatol Surg;36:71-75.
5. Berlin AL, Hussain M, Phelps R, Goldberg DJ (2009) A prospective study of fractional scanned nonsequential carbon dioxide laser resurfacing: a clinical and histopathologic evaluation. Dermatol Surg;35:222-228.
6. Meesters AA, Wind BS, Kroon MW, Beek JF, Van der Veen JPW, Nieuweboer-Krobotová L, Van der Wal AC, Bos JD, Wolkerstorfer A (2010) Ablative 10,600 nm fractional laser therapy as treatment for Becker's nevus; a pilot study. J Am Acad Dermatol; in press.
7. Hantash BM, Bedi VP, Kapadia B, Rahman Z, Jiang K, Tanner H, Chan KF, Zachary CB (2007) In vivo histological evaluation of a novel ablative fractional resurfacing device. Lasers Surg Med;39:96-107.
8. Fife DJ, Fitzpatrick RE, Zachary CB (2009) Complications of fractional CO_2 laser resurfacing: four cases. Lasers Surg Med;41:179-184.
9. Avram MM, Tope WD, Yu T, Szachowicz E, Nelson JS (2009) Hypertrophic scarring of the neck following ablative fractional carbon dioxide laser resurfacing. Lasers Surg Med;41:185-188.
10. Orringer JS, Rittié L, Baker D, Voorhees JJ, Fisher G (2010) Molecular mechanisms of nonablative fractionated laser resurfacing. Br J Dermatol;163:757-768.

SUMMARY, CONCLUSIONS, AND DISCUSSION
SAMENVATTING, CONCLUSIES EN DISCUSSIE
DANKWOORD
LIST OF ABBREVIATIONS
LIST OF PUBLICATIONS
BIBLIOGRAPHY

SUMMARY, CONCLUSIONS, AND DISCUSSION

This thesis deals with some therapeutical aspects of the pigment disorders non-segmental vitiligo, melasma, and Becker's nevus.

In non-segmental vitiligo, home UVB therapy and the necessity of post-operative irradiation after punch grafting are debated.

Furthermore, following the introduction of fractional lasers in 2004, promising results in various pigment disorders such as melasma and Becker's nevus, have been published. However, randomized clinical trials to definitely prove safety and efficacy were lacking.

Therefore, this thesis considers (i) the efficacy and safety of home UVB therapy in non-segmental vitiligo, (ii) the effect of different UV sources on outgrowth of punch grafts in non-segmental vitiligo; (iii-v) the efficacy and safety of non-ablative and ablative fractional laser therapy in melasma and in Becker's nevus, and finally (vi) the histopathological differences between non-ablative and ablative fractional laser.

Chapter 1 - General introduction and aims of thesis

Chapter 1 is a general introduction on the etiology, pathogenesis and current treatment options in vitiligo, melasma and Becker's nevus.

Chapter 2 - 'Home versus outpatient narrowband UVB therapy for the treatment of non-segmental vitiligo: a retrospective questionnaire study'

Chapter 2 shows the results of a retrospective questionnaire study in which we compared home versus outpatient NB-UVB therapy for the treatment of non-segmental vitiligo. NB-UVB therapy is usually delivered in an outpatient clinic. Therefore, vitiligo patients have to visit the clinic twice a week often during working hours and often for more than six months. In the early 1990s home NB-UVB therapy was introduced in the Netherlands. Since its introduction, safety, effectiveness and compliance concerning home treatment have been debated. This study was the first to provide data on the pro's and cons of home UVB therapy versus clinic based UVB therapy in patients with non-segmental vitiligo.

104 consecutive patients with non-segmental vitiligo who completed total body NB-UVB therapy between March 2008 and January 2009 were asked to fill out a questionnaire on safety, efficacy and satisfaction. 64 patients got clinical UVB while 40 patients had home UVB-treatment. The overall response rate was 86%.

We concluded that patient-reported outcomes of home and clinical NB-UVB therapy were comparable. The rate of pigmentation and occurrence of side effects were similar and time investment was significantly lower in the home group. Contrary to what you expect, satisfaction with the result in the home group was significantly lower.

Home NB-UVB therapy appeared to be a valuable and safe alternative for hospital-based NB-UVB therapy for patients with vitiligo.

Chapter 3 - 'Punch graft testing in vitiligo; effects of UVA, NB-UVB and 632.8 nm Helium-Neon laser on the outcome'

Post-operative UV-irradiation has been suggested to improve pigment outgrowth after punch grafting in vitiligo, but is time consuming while UVA and NB-UVB could promote photoageing and photocarcinogenesis. Helium-Neon laser treatment has been advocated in segmental vitiligo and may be a safe alternative for UV-irradiation.

Chapter 3 describes a randomised controlled observer-blinded study where the effects of UVA, NB-UVB and Helium-Neon laser irradiation on outcome of punch-grafting were studied in six patients with stable vitiligo. In each of four 2x2 cm depigmented test-regions, four 1.5 mm pigmented punch grafts were placed. These test-regions were randomly allocated to one of the phototherapeutic modalities twice weekly versus no therapy at all during three months. All patients were followed up for 6 months. In two patients the majority of punch grafts showed outgrowth of pigment in all treatment modalities, whereas in the other four patients the majority of punch grafts depigmented.

We concluded that stable vitiligo did not preclude failure of punch grafting. Intrinsic patient-related factors in the grafted area seemed to determine outgrowth of pigment, while the phototherapeutic modalities had minor to no effect. The number of patients who showed pigment outgrowth was too small to compare the different modalities.

Chapter 4 – 'Non-ablative 1550 nm fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled pilot study'

Melasma is a common pigment disorder, which is often difficult to manage because of its refractory and recurrent nature. Treatment of choice is triple topical therapy; a topical bleaching that was first introduced in 1975 as Kligman formula and consists of hydroquinone, tretinoin and a moderately potent to potent corticosteroid. Recently, non-ablative fractional laser therapy at 1550 nm was reported as a treatment for melasma.

In chapter 4 we assessed the efficacy and safety of non-ablative 1550 nm fractional laser therapy in melasma and compared these results with the gold standard, triple topical therapy. Twenty female patients with moderate to severe melasma and Fitzpatrick skin types II-V were randomized and included in an observer-blinded study with two arms. Ten patients were treated four times with non-ablative 1550 nm fractional laser therapy at 10 mJ/microbeam while the other 10 patients were treated once daily for eight weeks with a modified triple topical therapy (hydroquinone, tretinoin and triamcinolone acetonide cream). Improvement was determined using

the physician's global assessment (PhGA) at 3 weeks, 3 months and 6 months after the last treatment.

The PhGA had improved in both groups at 3 weeks. However, there was no difference in the PhGA between the two groups. The mean treatment satisfaction and treatment recommendation were significantly higher in the laser group at 3 weeks. However, melasma recurred in five patients in both groups after 6 months.

We concluded that non-ablative 1550 nm fractional laser therapy was safe and comparable in efficacy and recurrence rate to triple topical therapy. Furthermore we stated that it may be a useful alternative treatment option for melasma when topical bleaching is ineffective or not tolerated.

Chapter 5 – 'Non-ablative 1550 nm fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled split-face study'

As non-ablative 1550 nm fractional laser therapy at 10 mJ/microbeam proved relatively safe and effective in the previous study, in **chapter 5** an intra-patient study was performed to compare non-ablative 1550 nm fractional laser therapy with modified triple topical therapy, using higher settings (15 mJ/microbeam) and long term intermittent maintenance bleaching during follow-up. Twenty-nine patients with melasma were included in a randomized controlled observer-blinded study with split-face design. Each side of the face was randomly allocated to either 4-5 non-ablative 1550 nm fractional laser therapy sessions (15 mJ/microbeam, 14-20% coverage) or triple topical therapy (hydroquinone 5%, tretinoin 0.05%, triamcinolone acetonide 0.1% cream). Triple topical therapy was applied once daily for 15 weeks until the last laser session. After this last treatment, patients were asked to apply triple topical therapy twice weekly on both sides of the face during follow-up. Improvement of melasma was assessed by patient's global assessment (PGA), patient's satisfaction, physician's global assessment (PhGA), melanin index, and lightness (L-value) at 3 weeks, and at 3 and 6 months after the last treatment.

Mean PGA and satisfaction were significantly lower at the fractional laser side. PhGA, melanin index and L-value showed a significant worsening of hyperpigmentation as well. At 6 months follow-up, most patients preferred triple topical therapy. Moreover, 9 patients (31%) developed postinflammatory hyperpigmentation after two or more laser-sessions.

We concluded that given the high rate of postinflammatory hyperpigmentation, non-ablative 1550 nm fractional laser at 15 mJ/microbeam was not recommendable for the treatment of melasma. As laser treatment was partly in summer, this may have influenced the negative outcome.

Chapter 6 – 'Ablative fractional laser therapy as treatment for Becker's nevus; a randomized controlled pilot study'

Becker's nevus is an acquired skin disorder, characterized by the development of unilateral hyperpigmented patches that eventually develop a slightly elevated surface and often hypertrichosis. Prevalence ranges from 0.25-2.5%.

To date, no effective treatment is available. Recently, non-ablative fractional laser therapy was suggested as treatment option for Becker's nevus. Ablative fractional laser therapy might be even more effective as it results in complete ablation of microscopic treatment zones instead of coagulation, preventing a possible reuptake of melanin from the microscopic treatment zones by dermal macrophages and keratinocytes.

In chapter 6, 11 patients with Becker's nevus, older than 18 years were included in a prospective randomized controlled, observer-blinded split lesion trial. In each patient two similar square test regions were randomized to receive either ablative fractional laser therapy at 10 mJ/microbeam, coverage 35-45%, in combination with topical bleaching (to prevent laser induced postinflammatory hyperpigmentation or topical bleaching alone (to allow comparison of the regions). Three and 6 months after the last treatment, clearance of hyperpigmentation was assessed by physician's global assessment (PhGA), melanin index, reflectance spectroscopy, patient's global assessment (PGA), patient's satisfaction and histology.

At 6 months follow-up, PhGA improved in the laser treated region. Reflectance spectroscopy, melanin index, number of melanocytes and amount of dermal melanin did not significantly differ between both regions. PGA and patient's satisfaction were 5.0 and 5.9 (visual analogue scale, 0-10). Three patients developed postinflammatory hyperpigmentation.

We concluded that ablative fractional laser therapy was moderately effective in some patients with Becker's nevus. However, postinflammatory hyperpigmentation and relatively negative patient reported outcomes still preclude ablative fractional laser therapy from being a standard therapy.

Chapter 7 – 'Increased formation of fibrosis after treatment with ablative versus non-ablative fractional laser therapy'

Fractional laser therapy has become a widely accepted modality and creates multiple small sized coagulated zones, separated by surrounding untreated tissue. Histological studies have shown that permanent tissue damage is usually minimal or absent after either non-ablative or ablative fractional laser. However, histological comparisons between non-ablative and ablative fractional laser have not been published. The aim of this study was to compare the histological outcome of non-ablative and ablative fractional laser.

In chapter 7, in a randomized controlled observer-blinded study, biopsies of 18 patients (ashy dermatosis n=4, postinflammatory hyperpigmentation n=6, Becker's nevus n=8) were treated with ablative or non-ablative fractional laser were compared.

In each patient, two similar test-regions were randomised to receive either fractional laser with intermittent topical bleaching (to prevent laser-induced postinflammatory hyperpigmentation) or topical bleaching alone (to allow comparison of the regions). Patients with ashy dermatosis and postinflammatory hyperpigmentation were treated with non-ablative 1550 nm fractional laser (15 mJ/microbeam, 14-20% coverage), whereas patients with Becker's nevus were treated with ablative fractional laser (10 mJ/microbeam, 35-45% coverage), for a total of three to five sessions. Biopsies were obtained 3 months after the last treatment, and analyzed by a blinded dermatopathologist.

At follow-up, development of fibrosis was seen significantly more often in patients treated with ablative as compared to non-ablative fractional laser. We concluded that ablative fractional laser therapy may have induced formation of fibrosis, whereas treatment with non-ablative fractional laser therapy did not.

Further research whether this fibrosis is due to the used laser settings or the disorder is mandatory. Also, whether formation of fibrosis has to be regarded as dermal remodeling or a subtle subclinical form of scarring should be investigated in future histopathologic studies.

DISCUSSION

Vitiligo

To date, NB-UVB therapy is the most effective therapy for non-segmental vitiligo. We showed that patient-reported outcomes of home and outpatient NB-UVB therapy were comparable with regard to repigmentation and occurrence of side effects. Therefore, home NB-UVB therapy is a valuable alternative to clinic based NB-UVB therapy. Concerns among dermatologists about higher risks regarding inaccurate dosimetry, phototoxicity, suboptimal treatment, and unsupervised continuation, proved to be unfounded. Moreover, as time investment was significantly less and patients can perform NB-UVB therapy at any time at their own home, it means a significant step forward in the treatment of non-segmental vitiligo.

Secondly, we showed that in patients treated with autologous minigrafting, intrinsic patient-related factors in the grafted area seemed to determine outgrowth of pigment, while the phototherapeutic modalities had minor to no effect. Moreover, we concluded that stable vitiligo did not preclude failure of punch grafting. Since no real time parameters for disease activity in vitiligo are known, a minigraft-test probably still is the best way to predict the outcome of minigrafting

In future research, a prospective comparison between home versus outpatient NB-UVB therapy, and the necessity of post-treatment procedures after punch grafting, should be investigated.

Melasma

In patients with melasma, non-ablative 1550 nm fractional laser therapy at 10 mJ/microbeam was safe and comparable in efficacy and recurrence rate to triple topical therapy (50%). Therefore, it may be a useful alternative treatment option for melasma when topical bleaching is ineffective or not tolerated. However, given the high rate of postinflammatory hyperpigmentation, non-ablative 1550 nm fractional laser at 15 mJ/microbeam was not recommendable in the treatment of melasma. Caution should be advocated using non-ablative 1550 nm fractional laser in melasma, especially in spring and summer. As shown in chapter 4 and 5, more aggressive settings inherently lead to more side-effects. Non-ablative fractional laser is not a final solution nor a quick fix for melasma, as melasma is known to be recalcitrant and recurrent.

Because these two papers are the first randomized trials comparing fractional laser to triple topical therapy, more randomized studies are mandatory. As there is only one paper on long term treatment with triple topical therapy, in future studies, the long term intermittent treatment of triple topical therapy combined with non-ablative fractional laser may be considered.

Becker´s nevus

Ablative fractional laser therapy was moderately effective in some patients with Becker's nevus. However, postinflammatory hyperpigmentation and relatively negative patient reported outcomes still preclude ablative fractional laser therapy from being a standard therapy.

In future research, a larger cohort of patients should be recruited in order to compare treatment outcomes in different skin types and different characteristics of Becker's nevus. Moreover, given the high rate of laser-induced postinflammatory hyperpigmentation, optimization of laser parameters is mandatory in future research.

SAMENVATTING, CONCLUSIES EN DISCUSSIE

Dit proefschrift bevat studies over enkele therapeutische aspecten van de pigmentaandoeningen vitiligo vulgaris, melasma en naevus van Becker.

Bij vitiligo vulgaris is zowel lichttherapie in de thuissituatie als de noodzaak van nabelichten na minigrafttransplantatie een onderwerp van discussie. Daarnaast zijn er sinds de introductie van fractionele lasers in 2004 goede resultaten beschreven over de behandeling van verschillende pigmentstoornissen, in het bijzonder melasma. Gerandomiseerde studies om de veiligheid en effectiviteit te bevestigen, bleven tot op heden echter uit.

In dit proefschrift is daarom gekeken naar de (i) effectiviteit en veiligheid van lichttherapie in de thuissituatie, (ii) het effect van verschillende licht- en laserbronnen op de uitgroei van pigment na minigrafttransplantatie bij vitiligo vulgaris, (iii-v) de effectiviteit en veiligheid van non-ablatieve en ablatieve fractionele laserbehandeling bij melasma en de naevus van Becker, en ten slotte (vi) de histopathologische verschillen na behandeling met non-ablatieve en ablatieve fractionele laser.

Hoofdstuk 1 – Algemene introductie en doelen van dit proefschrift

Hoofdstuk 1 is een algemene introductie over de etiologie, pathogenese en huidige behandelingen van vitiligo, melasma en de naevus van Becker.

Hoofdstuk 2 - 'Een vergelijking van UVB lichttherapie in het ziekenhuis of in de thuissituatie voor de behandeling van vitiligo vulgaris: een retrospectieve vragenlijststudie'

Hoofdstuk 2 laat resultaten zien van een retrospectieve vragenlijststudie bij patiënten met vitiligo vulgaris waar UVB lichttherapie in de thuissituatie is vergeleken met poliklinische behandeling in het ziekenhuis.

UVB lichttherapie voor vitiligo vindt meestal poliklinisch plaats. Hierbij dient de patiënt gedurende enige maanden tot een jaar twee tot driemaal per week voor belichting naar het ziekenhuis te komen, wat in de regel niet buiten kantooruren mogelijk is. Begin jaren '90 werd UVB lichttherapie in de thuissituatie geïntroduceerd. Sindsdien is er discussie of dit wel veilig en effectief is en of dit niet zou leiden tot verminderde compliantie bij patiënten. Dit is de eerste studie waarbij gekeken is of UVB thuisbehandeling voor patiënten met vitiligo vergelijkbaar is met behandeling in het ziekenhuis.

Hiervoor werden 104 patiënten (64 ziekenhuisbelichters en 40 thuisbelichters) die hun lichttherapie hadden beëindigd tussen maart 2008 en januari 2009 benaderd om een vragenlijst omtrent effectiviteit, veiligheid en tevredenheid in te vullen. 86% van de 104 patiënten namen deel aan het onderzoek.

Wij concludeerden dat UVB lichttherapie in de thuissituatie en in het ziekenhuis vergelijkbaar was qua effectiviteit en het vóórkomen van bijwerkingen. Als voordeel

van de thuisbelichting kwam - zoals te verwachten - naar voren, dat het een aanzienlijke tijdsbesparing voor de patiënt opleverde. Opmerkelijk was, dat de tevredenheid bij de groep met thuisbelichting toch geringer was. UVB thuisbehandeling blijkt in ieder geval een veilig en gelijkwaardig alternatief te zijn voor de poliklinische UVB lichttherapie voor vitiligo vulgaris.

Hoofdstuk 3 - 'De minigraft-test in vitiligo vulgaris: effecten van UVA, kortband UVB en 632,8 nm Helium-Neon laser op de uitgroei'

Na een transplantatieprocedure bij vitiligo wordt veelal nabehandeld met UV-belichting met als achterliggende gedachte, dat hiermee deling en migratie van melanocyten wordt bevorderd.

Deze nabelichting is echter kostbaar en tijdrovend, daarnaast kan UV-straling ook negatieve effecten op de huid teweeg brengen. Als alternatief voor UV lichttherapie bij vitiligo is in enkele studies behandeling middels Helium-Neon laser gesuggereerd. Een voordeel zou zijn, dat hiermee in ieder geval geen biologische schade aan de huid wordt veroorzaakt.

Hoofstuk 3 beschrijft een gerandomiseerde gecontroleerde studie, geblindeerd voor de onafhankelijk arts, waarbij gekeken is naar het effect van UVA, smalband UVB en Helium-Neon laser op de uitgroei van pigment na minigrafttransplantatie in zes patiënten met stabiele vitiligo vulgaris. In elke patiënt werden vier vakjes van twee bij twee centimeter afgetekend, waarin 1,5 mm grote gepigmenteerde biopten geplaatst werden. Deze vakjes werden gerandomiseerd voor UVA, smalband UVB, Helium-Neon laser of geen nabehandeling. Lichttherapie werd tweewekelijks gegeven gedurende drie maanden, en patiënten kwamen tot zes maanden na de laatste behandeling terug voor controle. In twee patiënten vertoonde een meerderheid van alle biopten uitgroei, echter in de andere vier patiënten verdween de meerderheid van de melanocyten.

Wij concludeerden dat stabiele vitiligo geenszins een goede minigrafttransplantatie garandeert. Intrinsieke patiënt-gerelateerde factoren lijken in het getransplanteerde gebied de uitgroei van pigment te bepalen, waarbij aanvullende lichttherapie weinig tot geen effect leek te hebben. Het aantal geïncludeerde patiënten was te laag om een goede vergelijking tussen de effectiviteit van UVA, smalband UVB en Helium-Neon laser te kunnen maken.

Hoofdstuk 4 – 'Non-ablatieve 1550 nm fractionele laserbehandeling versus topicale triple therapie voor de behandeling van melasma: een gerandomiseerde gecontroleerde pilot-studie'

Melasma is een veel voorkomende pigmentstoornis, die lastig te behandelen is gezien het frequent recidiveren van de laesies. Voorkeursbehandeling is de topicale triple therapie, een melanogenese-remmende behandeling die geïntroduceerd is

in 1975 als Kligman formule (hydrochinon 5%, tretinoïne 0,1% en een matig tot sterk corticosteroïd). Onlangs is non-ablatieve 1550 nm fractionele laserbehandeling geïntroduceerd als behandeling voor melasma.

In **hoofdstuk 4** is gekeken naar de effectiviteit en veiligheid van non-ablatieve 1550 nm fractionele laserbehandeling. Twintig vrouwelijke patiënten met matig tot ernstig melasma en Fitzpatrick huidtype II-V werden gerandomiseerd voor of viermaal een behandeling met non-ablatieve 1550 nm fractionele laser (10 mJ/microbeam) dan wel gemodificeerde topicale triple therapie (hydrochinon 5%, tretinoïne 0,05%, triamcinolon acetonine 0.1% crème) eenmaal daags gedurende acht weken. Vermindering van de hyperpigmentatie werd beoordeeld door een onafhankelijk arts (physician's global assessment (PhGA)), drie weken, drie en zes maanden na de laatste behandeling.

De PhGA verbeterde in beide groepen bij de controle na drie weken. Tussen beide behandelingen waren geen significante verschillen in effectiviteit en bijwerkingen. De met de laser behandelde patiënten waren tevredener en meer geneigd de behandeling ook aan derden aan te raden. Bij vijf patiënten in beide groepen werd echter na zes maanden een recidief van het melasma waargenomen.

De non-ablatieve 1550 nm fractionele laserbehandeling bleek veilig en vergelijkbaar in effectiviteit met de gouden standaard, de topicale triple therapie, en kan in bepaalde gevallen een alternatief zijn bij de behandeling van melasma.

Hoofdstuk 5 – 'Non-ablatieve 1550 nm fractionele laserbehandeling versus topicale triple therapie voor de behandeling van melasma: een gerandomiseerde gecontroleerde split-face studie'

Aangezien non-ablatieve 1550 nm fractionele laserbehandeling bij 10 mJ/microbeam relatief veilig en matig effectief bleek in de voorgaande studie, vergeleken wij in **hoofdstuk 5** in een split-face studie, non-ablatieve 1550 nm fractionele laserbehandeling met een hogere instelling (15 mJ/microbeam) met topicale triple therapie. Daarnaast testten wij een onderhoudsbehandeling met topicale triple therapie om recidief van melasma te voorkomen.

Negenentwintig patiënten met melasma werden geïncludeerd. Na randomisatie kreeg één zijde van het gezicht 4-5 non-ablatieve fractionele laserbehandelingen (15 mJ/microbeam, 14-20% dekking) en de andere zijde van het gezicht topicale triple therapie. Deze crème werd dagelijks gedurende 15 weken gesmeerd tot de laatste laserbehandeling. Na deze behandeling werd patiënten gevraagd de crème tweemaal per week te smeren op beide kanten van het gezicht gedurende de nacontrole. Het effect van de therapie werd beoordeeld door de patiënt zelf (patient's global assessment (PGA), tevredenheid van de patiënt), en een onafhankelijk arts (physician's global assessment (PhGA)). Daarnaast werden objectieve kleurmetingen (melanine index, en de mate van lichtheid (L-waarde)) uitgevoerd bij drie weken, drie en zes maanden na de laatste behandeling.

Gemiddeld PGA en patiënttevredenheid waren significant lager aan de met de laser behandelde zijde van het gezicht. PhGA, melanine index en de L-waarde lieten tevens een significante verergering van de hyperpigmentatie zien. Negen patiënten (31%) ontwikkelden postinflammatoire hyperpigmentatie na twee of meer laserbehandelingen. Bij de laatste controle na zes maanden gaven de meeste patiënten de voorkeur aan de topicale triple therapie.

Wij concludeerden dat, gezien de hoge frequentie van postinflammatoire hyperpigmentatie, non-ablatieve 1550 nm fractionele laser bij een instelling van 15 mJ/microbeam geen plaats zou moeten hebben in de behandeling van melasma. Mogelijk zou de tijd van het jaar ook van invloed geweest kunnen zijn, daar behandeling ten dele plaats vond in de zomer.

Hoofdstuk 6 – 'Ablatieve fractionele laserbehandeling als behandeling voor de naevus van Becker; een gerandomiseerde gecontroleerde pilot studie'

De naevus van Becker is een zeldzame huidaandoening, gekarakteriseerd door de ontwikkeling van gehyperpigmenteerde maculae die een licht verheven oppervlak kunnen hebben met hypertrichose. De prevalentie varieert van 0,25 tot 2,5%.

Tot op heden is er geen effectieve therapie beschikbaar. Recent echter werd non-ablatieve fractionele laserbehandeling als behandelingsoptie gesuggereerd. Ablatieve fractionele laserbehandeling zou mogelijk effectiever zijn, omdat er in plaats van coagulatie, complete ablatie is van microscopische kolommen. Hierdoor is de heropname van melanine door dermale macrofagen en keratinocyten nagenoeg uitgesloten.

In de hoofdstuk 6 beschreven studie zijn 11 patiënten met een naevus van Becker geïncludeerd in een prospectief gerandomiseerde gecontroleerde splitlaesie studie, geblindeerd voor de onafhankelijke arts. In elke patiënt werden twee rechthoekige vakjes gerandomiseerd voor ablatieve fractionele laser (10 mJ/microbeam, 35-45% dekking) aangevuld met topicale triple therapie ter voorkoming van postinflammatoire hyperpigmentatie, of topicale triple therapie alleen om vergelijking van de vakjes mogelijk te maken.

Drie en zes maanden na de laatste behandeling werd de verbetering van hyperpigmentatie gescoord door een onafhankelijke arts (physician's global assessment (PhGA)), en door de patiënt zelf (mate van tevredenheid en patient's global assessment (PGA)). Daarnaast werden objectieve parameters gescoord (melanine index, L-waarde) en werden biopten voor histologisch onderzoek afgenomen.

Bij controle na zes maanden liet de PhGA een significante verbetering zien in het met de laser behandelde gebied. De melanine index, de L-waarde en histologie lieten geen significante verschillen zien tussen beide vakjes. De PGA en de patiënt-tevredenheid waren 5,0 and 5,9 (visueel analoge schaal, 0-10). Drie patiënten ontwikkelden postinflammatoire hyperpigmentatie.

Wij concludeerden dat ablatieve fractionele laserbehandeling redelijk effectief was in een deel van de patiënten met een naevus van Becker. Gezien de mate van voorkomen

van postinflammatoire hyperpigmentatie en de relatief lage patiënttevredenheid moet de behandeling vooralsnog niet gezien worden als standaardtherapie.

Hoofdstuk 7 – 'Verhoogde formatie van fibrose na behandeling middels ablatieve fractionele laser vergeleken met non-ablatieve fractionele laser'

Behandeling middels fractionele laser wordt veelvuldig toegepast en kenmerkt zich door de vorming van multipele gecoaguleerde kolommetjes die omgeven zijn door onbehandeld weefsel. Histologische studies toonden aan dat er nagenoeg geen permanente weefselschade is na zowel non-ablatieve als ablatieve fractionele laser. Vergelijkende histopathologische studies tussen non-ablatieve en ablatieve fractionele laser zijn echter nooit gepubliceerd.

Hoofdstuk 7 beschrijft een gerandomiseerde gecontroleerde studie, geblindeerd voor de onafhankelijke arts, waarin biopten van 18 patiënten, behandeld met ablatieve dan wel non-ablatieve fractionele laser, met elkaar werden vergeleken. Bij iedere patiënt, werden twee gelijke rechthoekige vakjes gerandomiseerd voor fractionele laser aangevuld met topicale triple therapie ter voorkoming van postinflammatoire hyperpigmentatie, dan wel topicale triple therapie alleen. Patiënten met ashy dermatose en postinflammatoire hyperpigmentatie werden behandeld met non-ablatieve 1550 nm fractionele laser (15 mJ/microbeam, dekking 14-20%), en patiënten met een naevus van Becker met een ablatieve fractionele laser (10 mJ/microbeam, dekking 35-45%). In totaal werden patiënten drie tot vijf maal behandeld. Drie maanden na de laatste behandeling werden biopten afgenomen van beide vakjes en geanalyseerd door een geblindeerd dermatopatholoog.

Fibrose werd significant vaker gezien bij patiënten die waren behandeld met de ablatieve fractionele laser. Wij concludeerden dat de ablatieve fractionele laser in de hier gebruikte instelling bij een naevus van Becker fibrose lijkt te induceren, terwijl dat niet gevonden werd bij de met de non-ablatieve fractionele laser behandelde aandoeningen.

Nader onderzoek is nodig om vast te stellen of bovengenoemde fibrose afhankelijk is van de in deze studie gebruikte laserinstellingen dan wel bepaald werd door de aard van de behandelde aandoening. Ook is nog niet duidelijk of deze fibrose gezien moet worden als remodellering van de huid of als subtiele subklinische littekenvorming.

DISCUSSIE

Vitiligo

Tot op heden is UVB lichttherapie de meest effectieve therapie voor non-segmentale vitiligo. Wij lieten zien dat lichtherapie thuis vergelijkbaar is met poliklinische lichttherapie wat betreft effectiviteit en vóórkomen van bijwerkingen.

Thuisbehandeling kan derhalve beschouwd worden als een volwaardig alternatief voor poliklinische lichtbehandeling.

De grote tijdsbeparing en het gemak om de behandeling op elk gewenst moment in eigen huis te kunnen uitvoeren, maakt UVB lichttherapie in de thuissituatie voor veel vitiligo-patiënten de beste of zelfs enige mogelijkheid om hun ziekte effectief te behandelen.

In een tweede onderzoek lieten wij zien dat bij patiënten behandeld met autologe huidtransplantaties, intrinsieke patiëntgerelateerde factoren de uitgroei van pigment bepaalden, en niet zozeer het type lichtbron dat als nabehandeling gebruikt werd. Anamnestisch stabiele vitiligo leek geenszins het succes van transplantatie te bepalen, vandaar dat de minigraft-test van belang blijft als aanvullende prognostische parameter.

In de toekomst zou een prospectieve vergelijking tussen lichttherapie thuis en in het ziekenhuis en de noodzaak van nabehandeling bij minigrafting nader onderzocht moeten worden.

Melasma

In patiënten met melasma bleek non-ablatieve 1550 nm fractionele laser in een dosering van 10 mJ/microbeam qua effectiviteit en veiligheid vergelijkbaar te zijn met topicale triple therapie. Deze laser zou daarom een waardevol alternatief kunnen zijn voor de behandeling van melasma wanneer deze niet effectief of te irritatief zou zijn. Zoals wij echter in hoofdstuk 4 en 5 lieten zien, leiden agressievere laserinstellingen tot meer bijwerkingen zoals postinflammatoire hyperpigmentatie.

Aangezien deze twee artikelen de eerste gerandomiseerde studies zijn waarbij fractionele laser vergeleken wordt met lokale triple therapie, zijn meer gerandomiseerde studies vereist. Bovendien is er slechts één studie die kijkt naar langetermijnresultaten van topicale triple therapie.

Bij deze aandoening, die na staken van therapie praktisch altijd recidiveert, is het van belang te komen tot een veilige en effectieve onderhoudsbehandeling, waarbij zowel de fractionele lasertherapie als topicale triple therapie op intermitterende wijze toegepast, een rol zouden kunnen spelen.

Naevus van Becker

Ablatieve fractionele lasertherapie was matig effectief bij sommige patiënten met een naevus van Becker. Echter door het optreden van postinflammatoire hyperpigmentatie en de relatief negatieve beoordeling door de patiënten zelf, kan ablatieve fractionele lasertherapie vooralsnog niet gezien worden als standaardbehandeling voor de naevus van Becker.

In de toekomst, zou een groter cohort patiënten met verschillende huidtypen bekeken moeten worden en is, gezien de hoge frequentie van postinflammatoire hyperpigmentatie, optimalisering van de laserparameters essentieel.

DANKWOORD

Zo…, Af!

Graag wil ik iedereen bedanken die heeft bijgedragen aan dit proefschrift. Een aantal personen dank ik graag in het bijzonder:

Prof. dr. J.D. Bos, mijn promotor en opleider. Bedankt voor de mogelijkheid die u gegeven heeft dit proefschrift te schrijven. Tijdens de gehele periode bent u er, ondanks alles, altijd geweest om mij bij te staan met raad en daad. Ik heb erg veel geleerd van uw adviezen en kritische commentaar gedurende mijn promotie. Ik wil u hier heel hartelijk voor danken.

Dr. J.P.W. van der Veen. Wietze, hartelijk dank voor de mogelijkheid binnen de SNIP te promoveren en je kritische commentaren en adviezen gedurende de vorming van dit proefschrift. Ik heb dit zeer gewaardeerd.

Dr. A. Wolkerstorfer. Albert, ontzettend bedankt voor al je tijd, moeite, energie, en aanstekelijke enthousiasme voor wetenschap en lasers. De altijd zeer inspirerende koffiesessies en buitenlandse tripjes zullen mij nog lang heugen. Het is een voorrecht je eerste PhD student (geweest) te zijn. ASLMS 2012?

Dr. J.F. Beek. Johan, onderzoeker-pur-sang. Niets was té veel of té laat: 'goed-beter-best' is met recht je credo. Een groot deel van dit proefschrift komt op jouw conto. Bedankt voor de fijne samenwerking!

Prof. dr. W.R. Faber, bedankt dat u tijdens uw emeritaat, vaak in uw vrije tijd, de thesebesprekingen overnam en mij bij stond waar nodig. Bedankt voor uw scherpte en goede adviezen.

De leden van de promotiecommissie: prof. dr. W.R. Faber, prof. dr. N. Van Geel, prof. dr. M. Hædersdal, prof. dr. C.M.A.G. van der Horst, en dr. G.N. Relyveld. Ik wil u allen danken voor uw deelname aan de commissie en de kritische beoordeling van het manuscript.

Marije Kroon, mijn promotiemaatje, en paranimf. Bedankt dat je in 2008 naar de SNIP kwam, dat we samen zoveel leuk onderzoek gedaan hebben, tripjes hebben mogen maken en natuurlijk dat je mijn paranimf bent. Binnenkort jouw promotie?!

Arne Meesters, superstudent. Ongelofelijk, wat een werk je hebt verzet tijdens je 'wetenschappelijke stage'. Een volledig uit de hand gelopen project, dat je omzette in zes co-publicaties en een eigen publicatie. Enorm bedankt voor je ongelofelijke inzet en slimheid. Je gaat een top-wetenschappelijke carrière tegemoet (ik hoop binnen de dermatologie)!

Tim Wind, paranimf en tweelingbroer. '¡Claro que si!'; thanks voor je statistische en 'psychologische' ondersteuning, waar nodig.

L. Nieuweboer-Krobotová. Inka, erg bedankt voor je hulp bij het scoren van de zoveelste 'Physician's Global Assessment' en je scherpe klinische blik tijdens het schrijven.

Dr. A.C. van der Wal, hartelijk dank voor uw beoordeling van de biopten in de laserstudies.

Het SNIP-team: Carmen, Bernice, Alisa, Meike, Mireille, Linda, Daphne, Angabeen, Emma, Renee, Gaidic, Karin, Angela. Geweldig om met zo'n leuk team te hebben mogen werken. Een warme deken van lieve collega's: bedankt voor alles. Ik zal jullie missen.

Mijn collega-arts-assistenten in het AMC, OLVG, Flevoziekenhuis en de SNIP: bedankt voor jullie steun en begrip.

De medewerkers van de fotografie- en illustratiedienst en Offpage, dank voor de immer snelle en goede service, ten tijde van dit proefschrift.

&

Alle sponsoren wil ik van harte bedanken voor de financiële ondersteuning voor het drukken van dit proefschrift.

Tenslotte wil ik mijn familie en vrienden bedanken voor hun support along the way.

LIST OF ABBREVIATIONS

AD	Ashy dermatosis
BN	Becker's nevus
Er:YAG	Erbium YAG
FLT	Fractional laser therapy
HeNe laser	Helium-Neon laser
H&E	Haematoxylin and eosin
HQ	Hydroquinone
IPL	Intense Pulsed Light
MASI	Melasma Area and Severity Index
Nd:YAG	Neodymium YAG
PGA	Patients' Global Assessment
PhGA	Physicians' Global Assessment
PIH	Postinflammatory hyperpigmentation
NB-UVB	Narrowband ultraviolet B
SNIP	The Netherlands Institute for Pigment Disorders
SPF	Sun Protection Factor
SPSS	Statistical Package for the Social Sciences
UV	Ultraviolet
VAS	Visual analogue score
VIDA score	Vitiligo Disease Activity score

LIST OF PUBLICATIONS

Wind BS, Kroon MW, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Meesters AA, Bos JD, Wolkerstorfer A. Home versus outpatient narrowband UVB therapy for the treatment of non-segmental vitiligo: a retrospective questionnaire study. Br J Dermatol. 2010;162(5):1142-4.

Wind BS, Meesters AA, Kroon MW, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Punch graft testing in vitiligo; effects of UVA, NB-UVB and 632.8 nm He-Ne laser on the outcome. J Eur Acad Dermatol Venereol. 2010. doi: 10.1111/j.1468-3083.2010.03874.x.

Kroon MW, **Wind BS**, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Non-ablative 1,550 nm fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled pilot study. J Am Acad Dermatol. 2011;64(3):516-23.

Wind BS, Kroon MW, Meesters AA, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Non-ablative fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled split-face study. Lasers Surg Med. 2010;42(7):607-12.

Meesters AA, **Wind BS**, Kroon MW, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Ablative fractional laser therapy as treatment for Becker's nevus; a pilot study. J Am Acad Dermatol. 2010, in press.

Wind BS, Meesters AA, Kroon MW, Beek JF, van der Veen JPW, Van der Wal AC, Bos JD, Wolkerstorfer A. Increased formation of fibrosis after treatment with ablative versus non-ablative fractional laser therapy. Submitted.

Kroon MW, **Wind BS**, Meesters AA, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Non-ablative 1,550 nm fractional laser therapy not effective for erythema dyschromicum perstans and postinflammatory hyperpigmentation; a pilot study. J Dermatol Treatment 2011, in press.

Kroon MW, **Wind BS**, Meesters AA, Wolkerstorfer A, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Beek JF. Laser Doppler Flowmetry as activity parameter in non-segmental vitiligo. Manuscript in preparation.

Kroon MW, **Wind BS**, Joore IKCW, Leloup MAC, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Thyroid disease in adults with non-segmental vitiligo; prevalence and benefit of screening. Submitted.

Kroon MW, **Wind BS**, Joore IKCW, Beek JF, van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Effect of fluticasone proprionate on narrow band UVB phototherapy for the treatment of vitiligo: a randomized observer-blinded controlled trial. Manuscript in preparation.

Van der Veen JPW, **Wind BS**, Taïeb A. Chapter 3.2.1, Topical corticosteroids. In: Picardo M, Taïeb A (eds). Handbook of vitiligo. 1st ed.

REFERENCES

Avram MM, Tope WD, Yu T, Szachowicz E, Nelson JS. Hypertrophic scarring of the neck following ablative fractional carbon dioxide laser resurfacing. Lasers Surg Med 2009;41:185-188.

Becker SW. Concurrent melanosis and hypertrichosis in distribution of nevus unius lateris. Arch Derm Syphilol 1949;60(2):155-160.

Berlin AL, Hussain M, Phelps R, Goldberg DJ. A prospective study of fractional scanned nonsequential carbon dioxide laser resurfacing: a clinical and histopathologic evaluation. Dermatol Surg 2009;35:222-228.

Cameron H, Yule S, Moseley H, Dawe RS, Ferguson J. Taking treatment to the patient: development of a home TL-01 ultraviolet B phototherapy service. Br J Dermatol 2002;147:957-965.

Chan HH, Manstein D, Yu CS, Shek S, Kono T, Wei WI. The prevalence and risk factors of post-inflammatory hyperpigmentation after fractional resurfacing in Asians. Lasers Surg Med 2007;39:381-385.

Chen GY, Hsu MM, Tai HK, Chou TC, Tseng CL, Chang HY, Lan CC, Sheu HM. Narrow-band UVB treatment of vitiligo in Chinese. J Dermatol 2005;32:793-800.

Choi JE, Kim JW, Seo SH, Son SW, Ahn HH, Kye YC. Treatment of Becker's nevi with a long-pulse Alexandrite laser. Dermatol Surg 2009;35:1105-1108.

Dainichi T, Ueda S, Fumimori T, Kiryu H, Hashimoto T. Skin tightening effect using fractional laser treatment II: A pilot animal study on skin remodeling. Dermatol Surg 2010;36:71-75.

Falabella R. Grafting and transplantation of melanocytes for repigmenting vitiligo and other types of leukoderma. Int J Dermatol. 1989;28:363-369.

Falabella R, Arrunategui A, Barona MI, Alzate A. The minigrafting test for vitiligo: detection of stable lesions for melanocyte transplantation. J Am Acad Dermatol. 1995;32:228-232.

Falabella R. Surgical treatment of vitiligo: why, when and how. J Eur Acad Dermatol Venereol. 2003;17:518-520.

Fife DJ, Fitzpatrick RE, Zachary CB. Complications of fractional CO_2 laser resurfacing: four cases. Lasers Surg Med 2009;41:179-184.

Fisher GH, Kim KH, Bernstein LJ, Geronemus RG. Concurrent use of a handheld forced cold air device minimizes patient's discomfort during fractional photothermolysis. Dermatol Surg 2005;31:1242-1243.

Formigon M, Alsina MM, Mascaro JM, Rivera F. Becker's nevus and ipsilateral breast hypoplasia-androgen receptor study in two patients. Arch Dermatol 1992;128:992-993.

Gano SE, Garcia RL. Topical tretinoin, hydroquinone, and betamethasone valerate in the therapy of melasma. Cutis 1979;23:239-241.

Gawkrodger DJ, Ormerod AD, Shaw L, Mauri-Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K. Guideline for the diagnosis and management of vitiligo. Br J Dermatol 2008;159:1051-1076.

Gilchrest B, Fitzpatrick T, Anderson R, Parrish J. Localization of melanin pigmentation in the skin with Wood's lamp. Br J Dermatol 1977;96:245-248.

Glaich AS, Goldberg LH, Dai T, Kunishige JH, Friedman PM. Fractional resurfacing: a new therapeutic modality for Becker's nevus. Arch Dermatol 2007;143:1488-1490.

Goldberg DJ, Berlin AL, Phelps R. Histologic and ultrastructural analysis of melasma after fractional resurfacing. Lasers Surg Med 2008;40:134-138.

Graber EM, Tanzi EL, Alster TS. Side effects and complications of fractional laser photothermolysis: experience with 961 treatments. Dermatol Surg 2008;34:301-305.

Grande SH, Harris R, Hansen CD, Callis Duffin KP, Florell SR, Hadley ML. Androgen receptor expression patterns in Becker's nevi: an immunohistochemical study. J Am Acad Dermatol 2008;59:834-838.

Grimes PE. Melasma. Etiologic and therapeutic considerations. Arch Dermatol 1995;131:1453-1457.

Grimes PE. New insights and new therapies in vitiligo. JAMA 2005;293:730-735.

Gupta AK, Gover MD, Nouri K, Taylor S. Treatment of melasma: a review of clinical trials. J Am Acad Dermatol 2006;55:1048-1065.

Hantash BM, Bedi VP, Kapadia B, Rahman Z, Jiang K, Tanner H, Chan KF, Zachary CB. In vivo histological evaluation of a novel ablative fractional resurfacing device. Lasers Surg Med 2007;39:96-107.

Hantash BM, Bedi VP, Sudireddy V, Struck SK, Herron GS, Chan KF. Laser-induced transepidermal elimination of dermal content by fractional photothermolysis. J Biomed Opt 2006;11:1-9.

Hantash BM, Mahmood MB. Fractional photothermolysis: a novel aesthetic laser surgery modality. Dermatol Surg 2007;33:525-534.

Ingordo V, Gentile C, Iannazzone SS, Cusano F, Naldi L. The 'EpiEnlist' project: a dermo epidemiologic study on a representative sample of young Italian males. Prevalence of selected pigmentary lesions. J Eur Acad Dermatol Venereol 2007;21:1091-1096.

Jordan WP, Jr., Clarke AM, Hale RK. Long-term modified Goeckerman regimen for psoriasis using an ultraviolet B light source in the home. J Am Acad Dermatol 1981;4:584-591.

Karsai S, Raulin C. Fraktionierte photothermolyse, eine neue Option in der Behandlung des Melasma? Hautarzt 2008;59:92-100.

Kim YJ, Han JH, Kang HY, Lee ES, Kim YC. Androgen receptor overexpression in Becker nevus: histopathologic and immunohistochemical analysis. J Cutan Pathol 2008;35:1121-1126.

Koek MB, Buskens E, Steegmans PH, van Weelde H, Bruijnzeel-Koomen CA, Sigurdsson V. UVB phototherapy in an outpatient setting or at home: a pragmatic randomised single-blind trial designed to settle the discussion. The PLUTO study. BMC Med Res Methodol 2006;6:39.

Koek MB, Buskens E, van Weelde H, Steegmans PH, Bruijnzeel-Koomen CA, Sigurdsson V. Home versus outpatient ultraviolet B phototherapy for mild to severe psoriasis: pragmatic multicentre randomised controlled non-inferiority trial (PLUTO study). BMJ 2009;338:b1542.

Kopera D, Hohenleutner U, Landthaler M. Quality-switched ruby laser treatment of solar lentigines and Becker's nevus: a histopathological and immunohistochemical study. Dermatology 1997;194:338-343.

Koster W, Wiskemann A. [Phototherapy with UV-B in vitiligo]. Z Hautkr 1990;65:1022-1024, 1029.

Kroon MW, Wind BS, Beek JF, Van der Veen JPW, Nieuweboer-Krobotová L, Bos JD, Wolkerstorfer A. Non-ablative fractional laser therapy versus triple topical therapy for the treatment of melasma: a randomized controlled pilot study. J Am Acad Dermatol 2010, in press

Lahiri K, Malakar S, Sarma N, Banerjee U. Repigmentation of vitiligo with punch grafting and narrow-band UV-B (311 nm)--a prospective study. Int J Dermatol. 2006;45:649-655.

Lan CC, Wu CS, Chiou MH, Chiang TY, Yu HS. Low-energy helium-neon laser induces melanocyte proliferation via interaction with type IV collagen: visible light as a therapeutic option for vitiligo. Br J Dermatol. 2009;161:273-280.

Larko O, Swanbeck G. Home solarium treatment of psoriasis. Br J Dermatol 1979;101:13-16.

Laubach H, Chan HH, Rius F, Anderson RR, Manstein D. Effects of skin temperature on lesion size in fractional photothermolysis. Lasers Surg Med 2007;39:14-18.

Laubach HJ, Manstein D. Fraktionierte photothermolyse. Hautarzt 2007;58:216-223.

Laubach HJ, Tannous Z, Anderson R, Manstein D. Skin responses to fractional photothermolysis. Lasers Surg Med 2006;38:142-149.

Lee HS, Won CH, Lee DH, An JS, Chang HW, Lee JH, Kim KH, Cho S, Chung JH. Treatment of melasma in Asian skin using a fractional 1,550-nm laser: an open clinical study. Dermatol Surg 2009;35:1499-1504.

Linthorst Homan MW, Spuls PI, de Korte J, Bos JD, Sprangers MA, van der Veen JP. The burden of vitiligo: patient characteristics associated with quality of life. J Am Acad Dermatol. 2009;61:411-420.

Lowe NJ. Home ultraviolet phototherapy. Semin Dermatol 1992;11:284-286.

Manstein D, Herron GS, Sink RK , Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. Lasers Surg Med 2004;34:426-438.

Manuskiatti W, Eimpunth S, Wanitphakdeedecha R. Effect of cold air cooling on the incidence of postinflammatory hyperpigmentation after Q-switched Nd:YAG laser treatment of acquired bilateral nevus of Ota like macules. Arch Dermatol 2007;143:1139-1143.

Matts PJ, Dykes PJ, Marks R. The distribution of melanin in skin determined in vivo. Br J Dermatol 2007;156:620-628.

McLean DI, Gallagher RP. "Sunburn" freckles, cafe-au-lait macules, and other pigmented lesions of schoolchildren: the Vancouver Mole Study. J Am Acad Dermatol 1995;32:565-570.

Milstein HJ, Vonderheid EC, Van Scott EJ, Johnson WC. Home ultraviolet phototherapy of early mycosis fungoides: preliminary observations. J Am Acad Dermatol 1982;6:355-362.

Moreno Arias GA, Ferrando J. Intense pulsed light for melanocytic lesions. Dermatol Surg 2001;27:397-400.

Murphy GM, McCann P, O'Leary A, Rogers S. Guidelines for the use of phototherapy and photochemotherapy in Ireland. Ir J Med Sci 1997;166:92-97.

Naito SK. Fractional photothermolysis treatment for resistant melasma in Chinese females. J Cosmet Laser Ther 2007;9:161-163.

Nirde P, Dereure O, Belon C, Lumbroso S, Guilhou JJ, Sultan C. The association of Becker nevus with hypersensitivity to androgens. Arch Dermatol 1999;135:212-214.

Njoo MD, Westerhof W. Vitiligo. Pathogenesis and treatment. Am J Clin Dermatol 2001;2:167-181.

Njoo MD, Spuls PI, Bos JD, Westerhof W, Bossuyt PM. Nonsurgical repigmentation therapies in vitiligo. Meta analysis of the literature. Arch Dermatol 1998;134:1532-1540.

Pandya A, Berneburg M, Ortonne JP, Picardo M. Guidelines for clinical trials in melasma. Pigmentation Disorders Academy. Br J Dermatol 2006;156(Suppl. 1):S21-28.

Pandya AG, Guevara IL. Disorders of hyperpigmentation. Dermatol Clin 2000;18:91-98, ix.

Paul BS, Stern RS, Parrish JA, Arndt KA. Low-intensity selective UV phototherapy. A clinical trial in outpatient therapy for psoriasis. Arch Dermatol 1983;119:122-124.

Pawaskar MD, Parikh P, Markowski T, McMichael AJ, Feldman SR, Balkrishnan R. Melasma and its impact on health-related quality of life in Hispanic women. J Dermatol Treat 2007;18:5-9.

Percivalle S, Piccino R, Caccialanza M, Forti S. Narrowband UVB phototherapy in vitiligo: evaluation of results in 53 patients. G Ital Dermatol Venereol 2008;143:9-14.

Person JR, Longcope C. Becker's nevus: an androgen-mediated hyperplasia with increased androgen receptors. J Am Acad Dermatol 1984;10(2 Pt 1):235-238.

Picardo M, Carrera M. New and experimental treatments of cloasma and other hypermelanoses. Dermatol Clin 2007;25:353-362.

Rendon M, Berneburg M, Arellano I, Picardo M. Treatment of melasma. J Am Acad Dermatol 2006;54(5 Suppl. 2:S272-281.

Resnik KS, Vonderheid EC. Home UV phototherapy of early mycosis fungoides: long-term follow-up observations in thirty-one patients. J Am Acad Dermatol 1993;29:73-77.

Rokhsar CK, Fitzpatrick RE. The treatment of melasma with fractional photothermolysis: a pilot study. Dermatol Surg 2005;31:1645-1650.

Sanchez N, Pathak M, Sato S. Melasma: a clinical, light microscopic, ultrastructural, and immunofluorescence study. J Am Acad Dermatol 1981;4:698-710.

Sarkany RP, Anstey A, Diffey BL, Jobling R, Langmack K, McGregor JM, Moseley H, Murphy GM, Rhodes LE, Norris PG. Home phototherapy: report on a workshop of the British Photodermatology Group, December 1996. Br J Dermatol 1999;140:195-199.

Tannous ZS, Astner S. Utilizing fractional resurfacing in the treatment of therapy-resistant melasma. J Cosmet Laser Ther 2005;7:39-43.

Taylor SC, Torok H, Jones T, Lowe N, Rich P, Tschen E, Menter A, Baumann L, Wieder JJ, Jarratt M, Pariser D, Martin D, Weiss J, Shavin J, Ramirez N. Efficacy and safety of a new triple-combination agent for the treatment of facial melasma. Cutis 2003;72:67-72.

Trelles MA, Allones I, Moreno-Arias GA, Velez M. Becker's naevus: a comparative study between erbium: YAG and Q-switched neodymium:YAG; clinical and histopathological findings. Br J Dermatol 2005;152:308-313.

Tse Y, Levine VJ, McClain SA, Ashinoff R. The removal of cutaneous pigmented lesions with the Q-switched ruby laser and the Q-switched neodymium: yttrium-aluminum-garnet laser. A comparative study. J Dermatol Surg Oncol 1994;20:795-800.

Tymen R, Forestier JF, Boutet B, Colomb D. [Late Becker's nevus. One hundred cases (author's transl)]. Ann Dermatol Venereol 1981;108:41-46.

Westerhof W, Boersma B. The minigrafting test for vitiligo: detection of stable lesions for melanocyte transplantation. J Am Acad Dermatol. 1995;33:1061-1062.

Whitton ME, Ashcroft DM, Barrett CW, Gonzalez U. Interventions for vitiligo. Cochrane Database Syst Rev 2006:CD003263.

Yu HS, Wu CS, Yu CL, Kao YH, Chiou MH. Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo. J Invest Dermatol. 2003;120:56-64.

Ik wilde dat ik niet in Amsterdam woonde, dan ging ik erheen met vakantie.

(K. Schippers)