Light and Color

Imaging, Outcome Measures and Treatment in Vitiligo

Sanne Elisabeth Uitentuis

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Imaging, Outcome Measures and Treatment in Vitiligo

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1

Introduction

Introduction

Vitiligo is a chronic acquired cutaneous disease associated with white patches caused by destruction of melanocytes.[1] Vitiligo is a common disease with a prevalence of around 0.5-3%, affecting all ethnicities worldwide, irrespective of age, gender or skintype.[1,2]

Vitiligo generally does not cause severe physical complaints, save possible itching and an increased risk for sunburn. However, the condition can significantly impair patients quality of live, especially for patients with darker skin types.[3,4] Many patients deal with stigma and feel stressed or isolated because of their disease.[5] Furthermore patients with vitiligo seem to have an increased risk for other auto-immune conditions like alopecia areata, rheumatoid arthritis, Addison's disease and most frequently described: thyroid disease.[6]

Vitiligo can be subdivided into two subtypes: non-segmental vitiligo (NSV) and segmental vitiligo (SV). NSV is the most common form and characterized by a symmetrical distribution of the lesions all over the body. The disease course of NSV is usually slowly progressive with periods of disease activity interchanged by stable periods. Segmental vitiligo is less common and has a different clinical presentation and course of disease: lesions are distributed unilateral and segmental and SV permanently stabilizes after an initial period of activity.[1]

Pathophysiology

NSV is an autoimmune disease of the skin. It is caused by a complex interaction between different cells and signaling molecules. In 2009 van den Boorn et al. have shown that cytotoxic (CD8+) T cells attack and kill melanocytes causing the skin to depigment.[7] The initial cause what triggers NSV to manifest is not certain but in some patients it is luxated by environmental factors like chemicals or trauma.[8,9] This can trigger cell stress, possibly explaining why melanocytes in vitiligo skin show a greater amount of reactive oxygen species.[10] The melanocyte stress could induce the aberrant activation of the innate immune system which is present in vitiligo skin: a higher activity of natural killer cells and inflammatory dendritic cells.[11,12] These dendritic cells take up melanocyte antigens and migrate to the lymph nodes, where they prime a melanocyte-specific T cell response. These autoreactive CD8+ T cells specifically recognize melanocytes and luxate disease activity through local production of different signaling molecules.[13] Multiple signaling molecules are upregulated in vitiligo skin (produced by e.g. T cells, but also keratinocytes) like the cytokine interferon gamma and increase pro-inflammatory chemokines like CXCL9 and CXCL10 via the intracellular Janus

kinase (JAK) - signal transducer and activator of transcription proteins (STAT) pathway.[13] In addition to cellular immunity, specific antibody responses reactive with melanocytes have been found in the serum of vitiligo patients.[14,15] This indicates that during vitiligo also a CD4+ T cell response and B cell response is taking place. The exact role of these antibody responses in the vitiligo pathogenesis is not fully known. They might enhance uptake of melanocyte antigens by dendritic cells and thereby the activation of the melanocyte-reactive T cell response. Lastly, recent studies show that a subgroup of the CD8+ T-cells does not recirculate but remains in the skin. These so-called resident memory T cells also seem to play an important role in the course of vitiligo, possibly explaining relapses after successful treatment at the same location as previous lesions.[16]

The autoimmune pathogenesis of NSV is further confirmed by genetic studies. Several genetic loci that are associated with NSV have been discovered in multiple large scale genome wide association studies.[17,18] The large majority of these genes encode for proteins related to immunomodulation, while a smaller part is involved in pigment synthesis and melanocytes.[17,18]

For segmental vitiligo CD8+ T-cells also seem involved, one case study found an increase of pro-inflammatory IFN-γ producing CD8+ T-cells in vitiligo lesions compared to normal skin.[19] The underlying cause of segmental vitiligo is assumed to be a genetic mosaicism.[20] This assumption is made because of the specific unilateral distribution of segmental vitiligo, which is comparable to other mosaic diseases involving melanocytes.[21] A genetic mosaicism is the occurrence of a post zygotic mutation of a cell, in this case a melanocyte, during the embryonic phase. Another theory is that neural mechanisms are causing the immune response. This theory is based on the thought that segmental vitiligo is distributed according the dermatomes.[22]

Diagnosis and imaging

Vitiligo can be diagnosed clinically because of its' distinctive clinical presentation with sharply defined depigmentated maculae. With the use of the Wood's lamp, which emits Ultraviolet (UV) A light around 365nm, the distinction between hypopigmentation and depigmentation can be made. The Wood's lamp is recommended to assess vitiligo in selected areas for patients with a light skin type because it increases the contrast between lesional and non lesional skin.[23]

For evaluation of different therapies and for research on vitiligo, photographs are made. When borders of lesions are not clear, it is common to use a Wood's lamp emitting UV light to increase the contrast during photography as well.

Different methods to create UV exposure are used for vitiligo photography, it can be difficult to gain enough light intensity for photographing large areas and an avoid shadowing. There are different techniques for using UV light in photography of vitiligo, however there is no consensus. Four methods for UV exposure in vitiligo photography are: (i) hand-held Wood's lamps (Wood's lamps), these are the most commonly known in the field of dermatology and vitiligo.[23,24] (ii) softboxes with UV lamps without the translucent cloths (softboxes): by reflecting the light towards the patient these can potentially reduce shadow forming. (iii) UV flash attached to the camera (camera flash), this device is potentially easy to use. (iv) separate high output UV flash (high output flash), described in a previous study by van Geel et al.[25] All four methods are shown in Figure 1.

Figure 1. The UV light set-ups: (a) softboxes; (b) camera flash; (c) Wood's lamps; (d) high output flash

These set-ups have specific advantages and disadvantages. The Wood's lamps and softboxes give continuous UV exposure, allowing the photographer to optimize the angle and distance between the UV source and skin. On the other hand, the intensity of the light for both set-ups is low, leading to lack of sharpness and/or

focus hampering large area photography. Another disadvantage of the Wood's lamps is that an assistant is needed to hold the lamps. A benefit of the camera flash is the auto focus assist light. But a disadvantage is its design to fit only one specific camera. The high output flash has the benefit that normal background light does not need to be switched off, due to the high UV exposure.

Furthermore, expenses differ between set-ups. While for Wood's lamps and softboxes costs are quite low (estimate below €200), a camera flash or high output flash are more expensive (estimate around €1000-€2000).

In clinical research, the extent of the vitiligo is often measured with clinical scoring systems (e.g. VES and SA-VES) based on (un)blinded evaluation of images.[26–28] These scoring systems could benefit from large area photography. The UV set-up may influence the outcomes of research urging efforts for optimization and standardization.[25]

Like mentioned above, Wood's light emits UV-A light around 365nm. This light is not visible by the human eye, thereby greatly limiting the observation by the physician to only a small bandwidth of the light present. Conventional cameras are rather insensitive for UV light because they include block-filters for non-visible light such as UV light. UV photography, using a UV camera which passes UV light and filters out visible light, and thus captures all light emitted by the UV lamps could improve imaging of vitiligo. The use of a UV camera in dermatology was first described in 1997 by Fulton.[29] Since then, during the previous decades, UV photography has been increasingly used in the field of dermatology. The technique was mostly used for detecting skin aging, but it has also been used for imaging of skin cancers, melasma, seborrheic dermatitis and other skin diseases.[30]

In the evaluation of repigmentation a difference can be made between target lesions when a specific lesion is being treated and the overall repigmentation of vitiligo. To evaluate repigmentation of targeted therapy repigmentation can be measured by comparing the lesion size before and after treatment. Examples of techniques to measure the size of target lesions in vitiligo are:

- Conventional camera method: A combination of conventional digital camera images with UV light and the subsequent use of digital surface measurement, which calculates surface area digitally.[25,31]

- Transparent sheet method: A combination of drawing target lesions on atransparent sheet and the subsequent use of digital surface measurement.[25,31–34]

- Graph paper method: The surface area can be measured by drawing the target lesionon graph paper and counting the boxes within this area.[35,36]

Recently van Geel et al. published a validation study for the conventional camera method and the transparent sheet method using ImageJ software for digital surface measurement.[25] Possibly, with the use of a UV camera and digital surface measurement the size of target lesions can also be measured accurately.

Outcome measures

There is a need for validated, reliable and interpretable outcome measures in vitiligo research.[37,38] International consensus on the core domains set what should be measured in research on vitiligo has been reached in 2015.[37] This core domain set included the following three domains: repigmentation, side effects and harms and maintenance of gained repigmentation. Other outcomes that were recommended to measure in vitiligo research when relevant were quality of life, cosmetic acceptability of repigmentation, cessation of spreading of vitiligo and tolerability of treatment.[37]

For the evaluation of overall repigmentation of vitiligo, an outcome instrument that assesses the extent of vitiligo is needed. Recently two new outcome instruments were developed to measure the extent of vitiligo with the use of questionnaires: the Vitiligo Extent Score (VES) and the Self-Assessment Vitiligo Extent Score (SA-VES).[39,40] The VES is measured by physicians, while the SA-VES is a patient reported outcome measure. The VES and SA-VES have been validated and are feasible and user friendly outcome measurement instruments for assessing the overall extent of vitiligo.[39,40] However, currently it is not yet known when a change in outcome of the VES and SA-VES signifies a change in the extent of vitiligo that is actually perceived important by patients (SA-VES) or clinicians (VES). To determine this, the minimal important change (MIC) of these instruments has to be assessed - the MIC is the smallest change in score which patients (or clinicians) perceive as important.[41] The MIC is a key element of interpretability and interpretability is an important aspect of outcome measures and is included in the COSMIN (COnsensus-based Standards for the selection of health status Measurement INstruments) taxonomy.[41]

The extent of vitiligo can affect the MIC. A 2% decrease of vitiligo body surface area (BSA) involvement can be very different for a patient with a BSA involvement of 5%

than for a patient with BSA involvement of 50%. Likewise, in the validation studies for the VES and SA-VES, patients with extensive vitiligo (BSA>5%) had a higher smallest detectable change (SDC, change in outcome of a measurement instrument that could be due to measurement error).[39,40] Furthermore the importance of change of the extent of the vitiligo might be different for different locations of vitiligo. Studies showed that quality of life was most significantly impaired with vitiligo located on the hands[42] and other visible areas[43].

Information on how to translate the obtained VES scores into disease severity strata from the physicians' point of view has been described recently while this is still lacking for the patient reported version (SAVES).[40] Stratification can be used to define inclusion criteria for clinical trials, epidemiological research and is important for correct comparison and pooling of trial results. The process to stratify the numeric output of an instrument into categories (mild-moderate-severe) is usually based on an 'anchor question' (e.g. Patient Global Assessment) that is preferably validated in advance.[44,45] A validated Patient Global Assessment for vitiligo is however lacking. A validated Global Assessment instrument could also be used as anchor questions for the interpretation and stratification of other outcome measure in vitiligo ("[e.g. Dermatology Life Quality Index (DLQI), Vitiligo Impact Patient scale (VIPs)].).[46–48]

Treatment

Topical treatment

To date, there is no curative treatment available for vitiligo. For many years now vitiligo therapy consists of topical agents, phototherapy and surgical techniques, which all aim to minimize disease progression and/or stimulate repigmentation.[49]

Topical therapy with corticosteroids or calcineurin inhibitors are suggested as first-line treatment.[50] Potent local corticosteroids are widely used for the treatment of vitiligo and are usually the first therapy of choice. The anti-inflammatory properties can reduce vitiligo activity and support repigmentation. Examples of possible side effects are atrophy of the skin, hypopigmentation and striae, but these rarely occur when patients uses a discontinuation treatment scheme (apply certain consecutive days alternated with days of no use).[49]

Next to corticosteroids, calcineurin inhibitors like tacrolimus and pimecrolimus are also used as topical treatment of vitiligo. Calcineurin inhibitors reduce T-cell activity as well as the production of proinflammatory cytokines. It is often

prescribed for use in the face and body folds for it does not have the possible side effect of skin atrophy. Possible side effects are a burning sensation of the skin in the first days/weeks of use and flushing after the use of alcohol.[49]

Phototherapy

Phototherapy is also a widely used treatment for vitiligo. The use of narrow band ultraviolet B (NB-UVB) phototherapy was first reported in 1997 by Westerhof et al[51] and is now considered to be an important modality for the treatment of generalized NSV.[49] NB-UVB is preferred to other light therapy regimens like psoralen combined with UVA (PUVA) due to its equal or better efficacy while adverse effects are decreased.[52,53] Currently, no consensus exists as to what the optimal treatment regimen and duration is of NB-UVB phototherapy, and practice varies widely.[1] At the Academic Medical Center (AMC), conventional treatment for NSV was NB-UVB alone twice a week, but this has changed in an intensified schedule with NB-UVB thrice a week in combination with topical treatment with fluticasone propionate and/or tacrolimus. This change was implemented in 2011 due to recent studies showing that the combination of topical therapy and NB-UVB is superior to monotherapy.[54,55] However, data on the most effective treatment frequency are lacking. Until now, the NB-UVB treatment regimens have never been compared directly. For the use of NB-UVB expert recommendations have been developed based on current literature by the Vitiligo Working Group Phototherapy Committee.[56] However, little evidence is available to guide clinicians on what is the most effective duration, dosage and treatment frequency of NB-UVB. Two studies have investigated the difference in effectiveness between twice and thrice weekly treatment regimens for vitiligo with the 308-nm excimer laser.[57,58] Both studies demonstrate that the final repigmentation does not depend on the treatment frequency but on the total number of treatments. However, the onset of repigmentation does occur faster with the three times weekly treatment. This could be the same for NB-UVB phototherapy but studies investigating this hypothesis are lacking.

Surgical treatment

In stable depigmented lesions unresponsive to local immune suppressive treatment or phototherapy alone, autologous melanocyte transplantation is the treatment option of choice.[1] Stable vitiligo lesions can be treated with various surgical melanocyte transplantation techniques like split thickness grafting, full surface grafting, blister roof grafting, punch grafting and cellular grafting.[59] Transplantation techniques like cellular grafting, which use an autologous cell suspension offer the advantage of treating a larger recipient area with a smaller donor site, compared to other transplantation techniques.[60] Long term follow-up

studies on autologous cell suspension transplantation (CST), suggest that it is an effective and safe method.[31,61] During CST a cell suspension is produced from a split-thickness skin graft from non-lesional skin, which is subsequently transplanted to a pre-treated depigmented lesion. For segmental vitiligo and piebaldism CST has been shown to be successful (repigmentation area >75%) in approximately 70% to 90% of treatments.[62,63]

Non-segmental vitiligo is a challenging candidate for melanocyte transplantation as hypothetically transplanted melanocytes could be destroyed during the flares of activity. In most studies only patients with no signs of activity for at least 12 to 24 months are included.[34,60,64] In order to induce stability immune suppressive treatments may be given during and after melanocyte transplantation. The standard treatment of non-segmental vitiligo with localized treatment sometimes combined with UVB phototherapy are not satisfactory in all patients, as complete repigmentation is often not achieved. Therefore, the combined treatment of standard of care with CST could be a promising treatment regimen in non-segmental vitiligo patients.

Melanocyte transplantation in stable vitiligo is frequently combined with subsequent phototherapy. Total repigmentation after melanocyte transplantation is uncommon and phototherapy can potentially have a beneficial effect on the outcome.[53] Phototherapy can enhance the repigmentation after melanocyte transplantation by its anti-inflammatory properties and by inducing melanocyte proliferation and migration. However, phototherapy also has side-effects such as increased risk of skin cancer and premature skin aging.[65]

New upcoming treatments

Recent insights on the pathophysiology of vitiligo, like the important role of cytokine interferon gamma and the intracellular JAK-STAT pathway have led to investigations for new treatment modalities in the field of vitiligo. Several new therapies are being developed and investigated.[49,66,67] However, for the clinical implementation of these new treatments it is crucial to know the vitiligo patients' need for novel treatment modalities. What are these novel therapies worth to a patient? In addition, how satisfied are patients with the current treatments? More knowledge of these factors could be of great value to various stakeholders, such as physicians, policy-makers and pharmaceutical companies. Furthermore, disease burden and its relation to the location of the various vitiligo lesions could be correlated to the demand for new therapies.

Aims and outline of this thesis

Summarizing, much is known about vitiligo, about how to image the disease, the importance of validated outcome measures and different treatment options. On the other hand, many questions remain to be investigated. In this thesis, we investigate some of those questions. The overall aim of this thesis is to improve the care of vitiligo by broadening the knowledge about vitiligo imaging using UV *colored light*, outcome measures and treatment, including *light* therapy and surgical techniques where regaining the original skin *color* is the objective of treatment.

In vitiligo imaging using different UV-set ups can influence the quality of evaluation and research. The objective of **chapter 2a** is to compare UV light set-ups for vitiligo photography regarding image quality and ease of use, to determine whether the use of UV photography can improve image quality and contrast and therewith the assessment of vitiligo lesions. This method could be a valuable and easy to use instrument to measure the size of target lesions. The aim of **chapter 2b** is to evaluate image quality of UV photography images in vitiligo and to assess the validity and reliability of UV photography images in combination with digital surface measurement for determining surface area of target lesions in vitiligo.

If we can determine the MIC of the VES and the SA-VES, we will improve the interpretability of these instruments and create the possibility to assign clinical meaning to their scores and change in scores.[41] Since vitiligo extent can possibly affect the MIC it would be interesting to determine the specific MIC (i) for extended disease and (ii) for locations that are important for patients' quality of life, like the hands and face. The primary aim of **chapter 3** is to determine the MIC of the VES and SA-VES. As secondary aims we want to determine the MIC for extended vitiligo (>5% BSA) and for the locations of the face and hands respectively.

A validated Patient Global Assessment for vitiligo is lacking. The aim of **chapter 4** is to assess the content validity, construct validity and intra-rater reliability of a Patient Global Assessment (PtGA) for disease extent, severity and impact. The second aim of this chapter is to determine strata (mild-moderate and severe) for the SAVES based on the validated PtGA for extent, severity and impact.

For future improvement of vitiligo therapy, it is important to assess, compare and evaluate the current treatment regimens, in order to determine what is most effective and satisfactory for the patient. Therefore, the aim of **chapter 5** is to explore whether a combination of NB-UVB three times weekly and topical agents

is superior to NB-UVB alone twice a week for the treatment of non-segmental vitiligo from patients' perspectives.

Next to topical and light therapy, adding melanocytes locally could boost the standard treatment to achieve optimal repigmentation in NSV. The objective of **chapter 6a** is to assess the efficacy and safety of CST combined with standard of care versus standard of care alone in non-segmental vitiligo patients. Additionally we assessed the general outcome reported by the patients and the cellular composition of the graft by flow cytometry and gene expression analysis of lesional skin. The aim of **chapter 6b** is to assess whether the cellular composition of the graft, in terms of cell density, melanocyte or keratinocyte content and cell viability, correlates with repigmentation after CST. In addition, we assessed if a threshold level of (viable) melanocytes required for successful repigmentation after CST can be defined.

To date, there is no consensus on the role of phototherapy in the surgical treatment of vitiligo.[50] The objective of **chapter 7** was to investigate whether phototherapy improves the outcome of melanocyte transplantation in vitiligo. Therefore, we designed a systematic review to summarize and review the evidence of the combination therapy of phototherapy and melanocyte transplantation.

In the last chapter we address the demand for new treatment modalities for NSV. The aim of this **chapter 8** is to assess the patients' perspective on current and novel therapies for vitiligo. Furthermore, we aimed to compare demographic features, such as gender, skin type and location of lesions (facial vs. non-facial) in relation to the primary outcomes on satisfaction of current treatments, disease burden and demand for new therapies.

In this thesis, **light and color**, about imaging, outcome measures and treatment of vitiligo we hope to answer questions on vitiligo care and aim to contribute to future care and research in this field.

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Part I

Imaging

2A

UV light set-ups for vitiligo photography, a comparative study on image quality and ease of use

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Abstract

Background
Ultraviolet (UV) light is an essential tool to assess the extent, spreading and staging of vitiligo. Different UV light set-ups are used for vitiligo photography, including: (i) hand-held Wood's lamps; (ii) softboxes with UV lamps; (iii) UV flash attached to the camera; (iv) high output UV flash.

Objective
Our objective was to compare UV light set-ups for vitiligo photography regarding image quality and ease of use.

Methods
Patients with vitiligo lesions with unclear borders were included. Two images were made with all four UV set-ups per patient, for a large and a small area. Image quality was scored separately by three blinded vitiligo experts on five criteria: overall quality, clearness of borders, contrast, sharpness and for larger areas the shadows. The two professional medical photographers were asked to score the ease of use for each set-up.

Results
A total of 88 photos were assessed from 11 patients. For larger areas the frequency of a 'good' or 'very good' overall quality rating was 12.1%(Wood's), 6.1%(softboxes), 15.2%(camera flash) and 78.8%(high output flash). For smaller areas the score 'good' or 'very good' was given to 54.5%, 3%, 66.6% and 84.8% in the same order. For the shadow criteria each set-up scored below 40% on a 'good' or 'very good' score. The high output flash was scored as most easy to use by the photographers.

Conclusion
When comparing four different UV light set-ups for vitiligo photography we concluded that the UV set-ups strongly influenced the quality scores of the obtained images. The high output flash scored best for both small and large areas and for ease of use. For small areas the Wood's lamp and camera flash were acceptable. All set-ups scored badly for shadows, more research is needed to find the optimal exposure to avoid shadows.

Introduction

Vitiligo is a skin disorders characterized by patchy leukoderma that can have a significant negative effect on the quality of life.[1,2] For evaluation of different therapies and for research on vitiligo, photographs are made. When borders of lesions are not clear, it is common to use a Wood's lamp emitting UV light to increase the contrast. Moreover the Wood's lamp is recommended to assess the extent, spreading and staging of vitiligo in selected areas.[3] Different methods to create UV exposure are used for vitiligo photography, however there is no consensus.

Figure 1. The UV light set-ups: (a) softboxes; (b) camera flash; (c) Wood's lamps; (d) high output flash

Four methods for UV exposure in vitiligo photography are: (i) hand-held Wood's lamps (Wood's lamps), these are the most commonly known in the field of dermatology and vitiligo.[3,4] (ii) softboxes with UV lamps without the translucent cloths (softboxes): by reflecting the light towards the patient these can potentially

reduce shadow forming. (iii) UV flash attached to the camera (camera flash), this device is potentially easy to use. (iv) separate high output UV flash (high output flash), described in a previous study by van Geel et al.[5] All four methods are shown in Figure 1.

These set-ups have specific advantages and disadvantages. The Wood's lamps and softboxes give continuous UV exposure, allowing the photographer to optimize the angle and distance between the UV source and skin. On the other hand the intensity of the light for both set-ups is low, leading to lack of resolution and/or focus hampering large area photography. Another disadvantage of the Wood's lamps is that an assistant is needed to hold the lamps. A benefit of the camera flash is the auto focus assist light. But a disadvantage is its design to fit only one specific camera. The high output flash has the benefit that normal background light does not need to be switched off, due to the high UV exposure.

Expenses differ between set-ups. While for Wood's lamps and softboxes costs are quite low (estimate below €200), a camera flash or high output flash are more expensive (estimate around €1000-€2000).

In clinical research, the extent of the vitiligo is often measured with clinical scoring systems (e.g. vitiligo extent score; the vitiligo area scoring index[6,7]) based on (un) blinded evaluation of images.[8–10] These scoring systems could benefit from large area photography. The UV set-up may influence the outcomes of research urging efforts for optimization and standardization.[5] The objective of our study was to compare UV light set-ups for vitiligo photography regarding image quality and ease of use.

Materials and Methods

This study was performed at the Netherlands Institute for Pigment Disorders in Amsterdam. Inclusion criteria for patients were: (i) diagnosis of vitiligo; (ii) age 18 or older; (iii) vitiligo lesions with (partially) unclear borders on the arms, hands, legs and/or trunk.

All eligible patients visiting the Netherlands Institute for Pigment Disorders in the Amsterdam University Medical Centers in Amsterdam that were send to the medical photographer were consecutively asked to enter the study. If informed consent was given patients were included. This study was not subject to the WMO

(Medical Research Involving Human Subjects Act) and approval was granted by the local Medical Ethical Committee.

We compared four UV set-ups that are commonly used to administer UV light for vitiligo photography:

1. Two hand-held Wood's lamps (1004 Wood's lamp, 22W) placed on both sides of the patient as close as possible without entering the frame of the image (Wood's lamps).

2. Two softboxes with UV light bulbs (Omnilux, 25W) without the translucent cloths placed on both sides of the patient as close as possible without entering the frame of the image (softboxes).

3. A UV flash device with two lamps that can be attached to the camera (Canon MT-24EX Macro Twin Lite Flash)(camera flash).

4. A separate high output UV flash device (Broncolor, Siros 800 S Wi-Fi RFS 2.1 with UV attachment)(high output flash).

All four UV set-ups are shown in Fig 1. Per patient five photographs were taken for both a larger (e.g. the whole limb or trunk) and for a smaller (the lesional) area; one with each UV set-up and a reference photo. Two professional medical photographers took the photographs using a Nikon D750 camera with a 60mm Macro lens. Images were assessed separately by three blinded physicians, not knowing which UV set-up was used for which image. They were asked to score each image on five different criteria: 1) overall quality 2) the clearness of the lesional borders, 3) the contrast between lesional and non-lesional skin, 4) the sharpness of the image and 5) in the case of a larger area for shadows. The five criteria were rated on a 5 point scale: very poor; poor; average; good or very good.

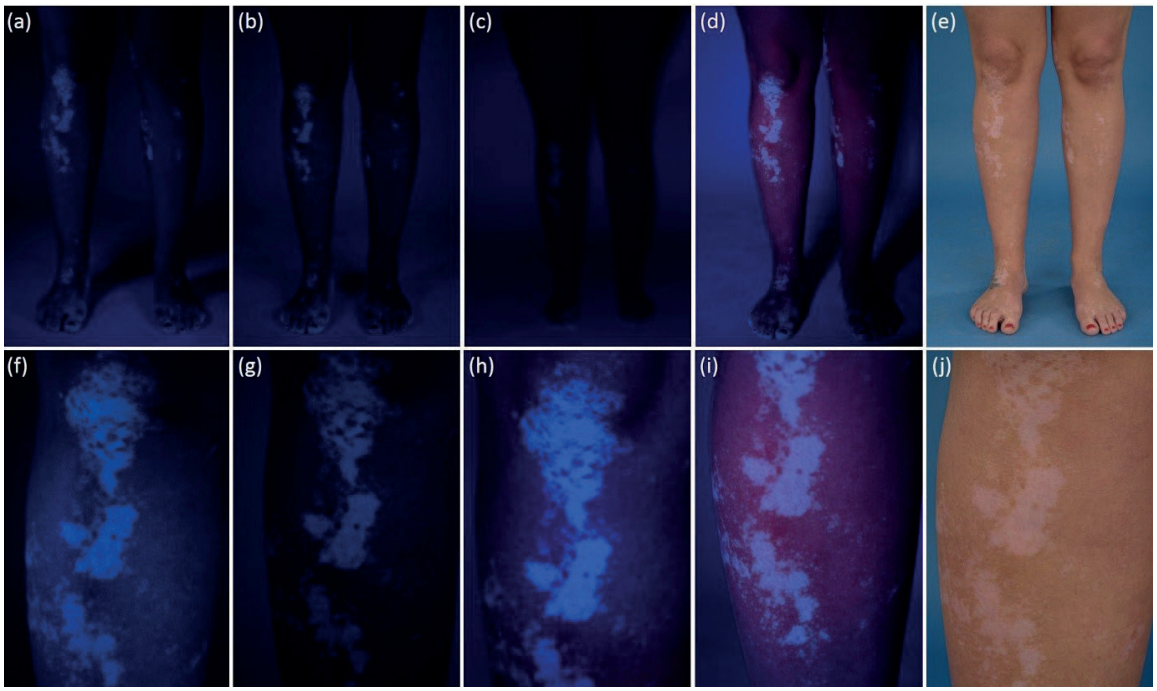
Additionally the photographers were asked separately to score the ease of use, the time needed, and the need of assistance on a 5-point Likert scale ranging from - - (very negative) to ++ (very positive) for each technique.

Scores were collected and frequency of the scores for each criterion was assessed by descriptive statistics. Furthermore, for each criteria Pearson's chi-squared tests were performed to assess the differences in quality scores between the UV set-ups. The tests were two-sided and after Bonferroni-correction for the nine different correlations, the statistical level of significance was set at $P<0.0056$.

Results

A total of 88 images were assessed from eleven patients with non-segmental vitiligo (5 females). The median age was 47 years (range 31-68). An example of all photos taken of one patient is shown in Fig. 2.

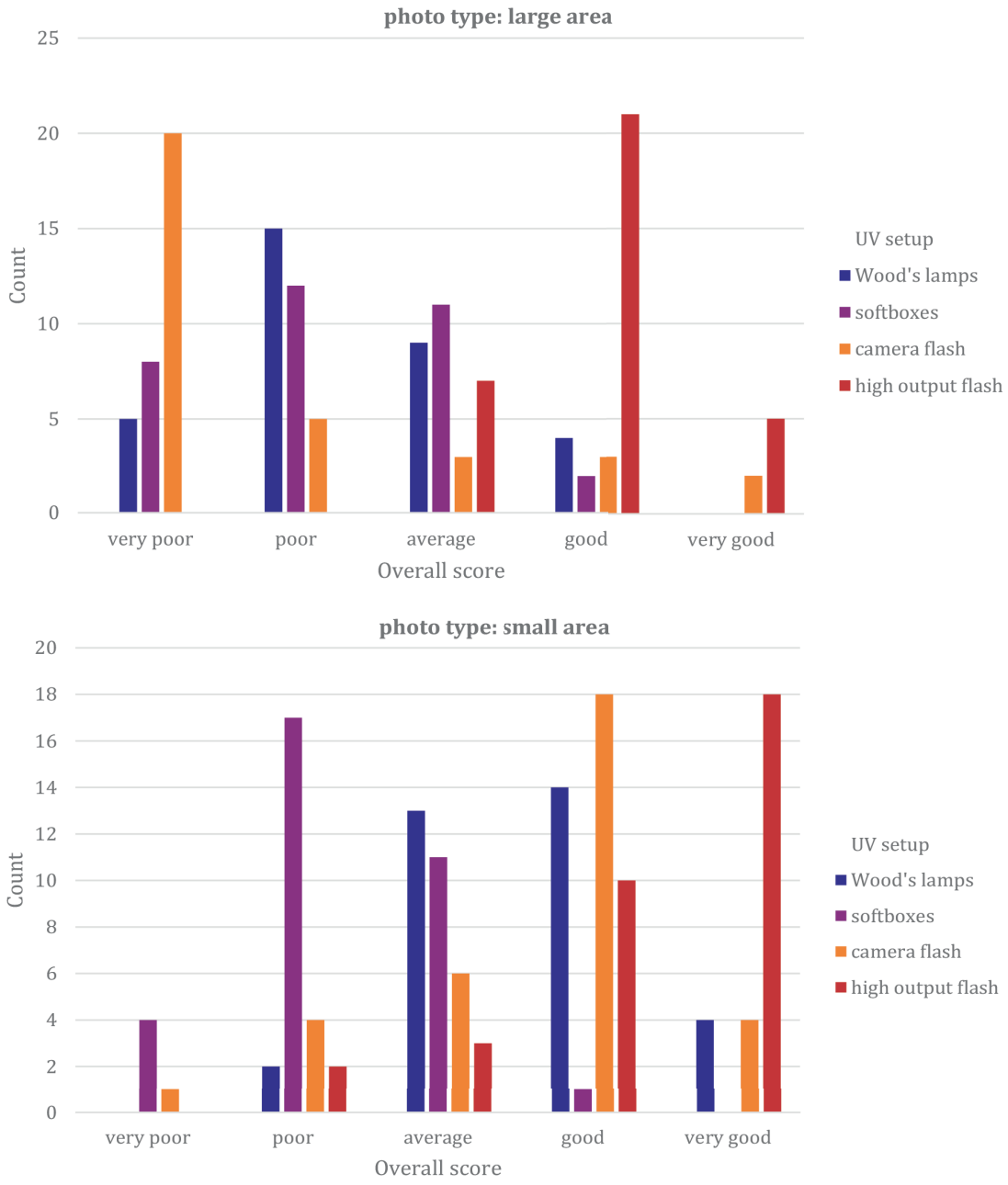
Figure 2. Images of a large area and a smaller area with the different UV light set-ups



Large area: (a) Wood's lamps; (b) softboxes; (c) camera flash; (d) high output fl1ash; (e) normal light
Smaller area: (f) Wood's lamps; (g) softboxes; (h) camera flash; (i) high output flash; (j) normal light

The scores for overall quality of the UV set-ups from the blinded physicians are shown in Fig. 3. The frequency of a 'good' or 'very good' overall quality rating was 12.1% (Wood's lamps), 6.1% (softboxes), 15.2% (camera flash) and 78.8% (high output flash). For smaller areas the score 'good' or 'very good' for overall quality was 54.5% (Wood's lamps), 3%(softboxes), 66.6% (camera flash) and 84.8% (high output flash).

Figure 3. Overall image quality score per UV light set-up



For larger areas the amount of shadows were scored; the better the quality the fewer shadows. The frequency of a 'good' or 'very good' quality score for this item was lower than 40% for all set-ups. Table 1 shows the frequency of a 'good' or 'very good' quality rating per criterion for each UV set-up. For each criterion the differences between the UV-light set-ups were significant with P values below 0.0001.

Table 1 Score 'good' or 'very good' quality by blinded physicians for each UV light set-up

	Wood's lamps	Softboxes	Camera flash	High output flash	P
Larger area					
Overall quality	12.1%	6.1%	15.2%	78.8%	<0.001*
Clearness of the lesional borders	9.1%	3%	15.1%	87.9%	<0.001*
Contrast [a]	12.1%	9.1%	18.2%	90.9%	<0.001*
Sharpness	60.1%	12.1%	9.1%	97%	<0.001*
Shadows [b]	15.2%	21.3%	9.1%	39.4%	<0.001*
Smaller area					
Overall quality	54.5%	3%	66.6%	84.8%	<0.001*
Clearness of the lesional borders	54.6%	3%	57.6%	84.8%	<0.001*
Contrast[a]	60.6%	3%	63.6%	87.9%	<0.001*
Sharpness	48.5%	3%	57.6%	81.8%	<0.001*

Percentages are the frequency that the UV set-up quality was scored 'good' or 'very good' for that criterion.
P: P-value of 2-sided Chi-Square test, * Significant
a) Contrast between lesional and non-lesional skin
b) Shadows were scored 'very good' if the UV set-up did not create shadow in the image

The scores of both photographers working with the different UV light set-ups for the study are shown in table 2.

Table 2 Ease of use scored by two photographers

	Wood's lamps	Softboxes	Camera flash	High output flash
Ease of use				
Photographer 1	--	+	-	+
Photographer 2	-	-	+	+
Time needed				
Photographer 1	--	+/-	+	++
Photographer 2	+/-	-	+	++
Need of assistance				
Photographer 1	--	+	+	++
Photographer 2	--	+	++	++

- - very negative; - negative; +/- average; + positive; ++ very positive

Discussion

We found that the quality scores of the images varied largely between the UV set-ups. The high output flash scored highest for both small and large areas on all criteria. Furthermore this device scored best for ease of use. For small areas the Wood's lamp and camera flash had average scores, while the softboxes scored low on quality for both small and larger areas. The frequency of a 'good' or 'very good' score for the criteria shadows was lower than 40% for each UV set-up.

While the softboxes were scored more easy to use than the Wood's lamps and do not require assistance, the sharpness of the images was scored better for the Wood's lamps. This is probably due to the fact that these lamps can be held very close to the lesional skin. The camera flash seems quite easy to use but the quality scores for large areas were very poor. The quality of the camera flash for smaller areas was scored generally as good, so this set-up could be useful for the photographic assessment of target lesions in vitiligo. The high output flash showed good quality for all criteria except shadows. To create optimal illumination without shadows possibly two high output flash lights could be used, but our estimate is that this would increase the costs to more than a 10-fold of the costs for the Wood's lamps.

Strengths of this study include the consecutive inclusion of patients and the blinded assessment by three different vitiligo experts. On the other hand limitations are the small sample size, the fact that outcomes may depend on specific devices and other brands could differ in UV intensity or technical details.

Comparing four different UV light set-ups for vitiligo photography we concluded that the UV set-ups strongly influenced the quality scores of the obtained images. The high output flash scored best for both small and large areas and for ease of use. For small areas the Wood's lamp and camera flash were acceptable. All set-ups scored badly for shadows. Possibly multiple flash devices could reduce shadows. More research is needed to find the optimal exposure to avoid shadows.

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2b

Ultraviolet photography in vitiligo: image quality, validity and reliability

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Abstract

Background
For clinical care and research in vitiligo, photographs with the use of Ultraviolet (UV) light or Wood's lamp are often made. Conventional cameras are insensitive for UV light. The use of a UV camera (UV photography) might improve image quality and ameliorate the assessment of target lesions in vitiligo.

Objectives
To determine image quality and the validity and reliability of UV photography for the assessment of vitiligo target lesions.

Methods
Images of patients with vitiligo were made with UV photography, a conventional camera and lesions were drawn on graph paper and transparent sheets. Image quality was scored by vitiligo experts and medical interns. The intraclass correlation coefficients (ICCs) of the lesion size determined with UV photography combined with digital surface measurement and the other techniques were hypothesized to be above 0.6. The ICCs between UV images taken by the same physician and between two different physicians were calculated for determining inter- and intra-reliability.

Results
In total 31 lesions of 17 patients were included. Image quality was assessed as good or very good for 100% and 26% for UV photography and the conventional camera respectively. ICCs of UV photography and the conventional camera, drawing the lesions on transparent sheets and graph paper were respectively 0.984, 0.988 and 0.983, confirming our hypotheses. The ICCs of the intra-rater and inter-rater were 0.999 and 0.998 respectively.

Conclusions
The results of this study indicate that the use of UV photography for the assessment of vitiligo lesions improves image quality and is valid and reliable.

Introduction

For the follow-up of vitiligo and for research purposes, photographs are often made with the use of UV light. UV light is not visible by the human eye, thereby greatly limiting the observation by the physician to only a small bandwidth of the light present. Using a UV camera which passes UV light[1–3], and thus captures all light emitted by the UV lamps, could improve image quality and contrast and therefore ameliorate the assessment of vitiligo lesions.

To evaluate repigmentation of targeted therapy, repigmentation can be measured by comparing the lesion size before and after treatment.

Recently van Geel et al. published a validation study for the conventional camera method and the transparent sheet method using ImageJ software for digital surface measurement.[4] No study has been performed on the combination of UV photography images and digital surface measurement software. This method could be a valuable and easy to use instrument to measure the size of target lesions.

The aim of this study is to evaluate image quality of UV photography images in vitiligo and to assess the validity and reliability of UV photography images in combination with digital surface measurement for determining surface area of target lesions in vitiligo.

Materials and methods

This cohort study was executed at the outpatient clinic of the Netherlands Institute for Pigment disorders (SNIP) in the Amsterdam University Medical Centers (Amsterdam UMC). Approval was granted by the local Medical Ethical Committee.

Patients

Patients diagnosed with either segmental or non-segmental vitiligo; aged 16 years or older; with Fitzpatrick skin types 1 to 4 were included. One or two lesions per patient were included. A sample size of at least 30 target lesions was pursued. The vitiligo lesions had to be localized on a flat surface.

Images and measurements

The following four techniques to measure the size of target lesions were used:

1) UV photography images combined with digital surface measurement – the UV photography method.

2) Conventional digital camera images combined with digital surface measurement – the conventional camera method.

3) Lesions drawed on transparant sheets combined with digital surface measurement – the transparant sheet method.

4) Lesions drawed on graph paper containing boxes with a size of 1 mm^2 and counting the number of enclosed boxes – the graph paper method.

UV photography images were obtained using a UV camera: a full spectrum ultraviolet light adapted mirrorless digital camera (Sony α 5000). The UV/NIR filter was removed and the conventional lens was replaced which a UV transparent 50mm focal lens. A 20 nm narrow band filter was used transmitting UV light around 365 nm. The capture of the images was standardized: Two softboxes with UV light bulbs (Omnilux, 25W, 365nm) were placed on both sides of the lesion as close as possible.[5] Conventional camera images (Canon EOS 1100D) were taken with two handheld Wood's lamps (1004 Wood's lamp, 22W). A 0.75 inch size sticker was applied next to the selected lesions as a reference. The lesions were drawn on a transparent sheets and subsequently on semi-transparent graph paper containing boxes with a size of 1 mm^2.

The surface area of the target lesions on the UV photography images, on the conventional camera images and on the transparent sheets were uploaded and measured by the digital image analysis system ImageJ 1.50i (National Institute of Health, USA). The surface area of the target lesions on the graph paper was calculated by counting the number of enclosed boxes.

Image quality

Image quality was assessed independently by two dermatologists with vitiligo expertise and two medical interns. The medical interns knew how to recognize vitiligo but lacked practical experience with the Wood's lamps and were therefore somewhat blinded when looking at the images. They were asked to score each image on overall image quality (considering sharpness, contrast and lesion borders).

Validity

In the absence of consensus on a gold standard for measuring target lesions in vitiligo, validity assessment relies on testing hypotheses.[6] Four hypotheses to test the validity were formulated beforehand:

1) An intraclass correlation coefficient (ICC) of >0.6 for lesions size between the UV photography method and the conventional camera method

2) An ICC of >0.6 for lesions size between the UV photography method and the transparent sheet method.

3) An ICC of >0.6 for lesions size between the UV photography method and the graph paper method.

4) The ICCs of the first two hypotheses will be higher than the ICCs of the conventional camera and graph paper/ transparent sheets.

To confirm the construct validity of using UV photography for target lesion assessment 75% of the hypotheses should be achieved.[7]

Reliability

To test the intra-rater reliability two UV photography images of each lesion were taken by the same physician. To test the inter-rater reliability a UV photography image was taken of each lesion by a second physician.

Statistical analyses

To test the validity of the UV photography method, the ICCs between the different measurement instruments were determined in a two-way random model with absolute agreement and reported as single measures. To test the intra- and inter-rater reliability the ICCs were determined. Calculated P-values were two-sided and given per analysis, with a P-value <0.05 considered significant.

Results

A total of 31 target lesions of 17 consecutively screened patients were included in the study. Baseline characteristics of patients and lesions are reported in Table 1.

Table 1 Baseline characteristics of the patients (n = 17) and the localization of the lesions (n = 31)

Characteristics	
Age (years) mean; median (range)	44; 45 (24-60)
Sex, n (%)	
Male	6 (35.3)
Female	11 (64.7)
Fitzpatrick skin type, n (%)	
I	2 (11.8)
II	9 (52.9)
III	3 (17.6)
IV	3 (17.6)
Localization lesions, n (%)	
Trunk	16 (51.6)
Legs	13 (41.9)
Arms	2 (6.5)

Image quality

Figure 1 shows UV photography and conventional camera images of three vitiligo target lesions. Figure 2 shows the frequency of scores by the vitiligo experts and medical interns on image quality. Overall quality of the UV photography images was mostly scored as 'very good'. Quality for the conventional camera was mainly scored as 'average'.

Validity

All ICCs between the different measurement instruments are shown in Table 2. The ICCs between determining the size of target lesions by using the UV photography method and the three other measurement techniques (conventional camera method, transparent sheet method and graph paper method) were 0.984, 0.988 and 0.983 respectively. Therefore the first three a priori defined hypotheses that these ICCs would be higher than 0.6, are met.

The ICCs comparing UV photography with the transparent sheet method and the graph paper method were higher than the ICCs comparing the conventional camera method with the transparent sheet method and the graph paper method. This confirms our fourth hypothesis.

Figure 1 Vitiligo lesions. UV photography images (**a,c,e**) and conventional camera images (**b,d,f**)

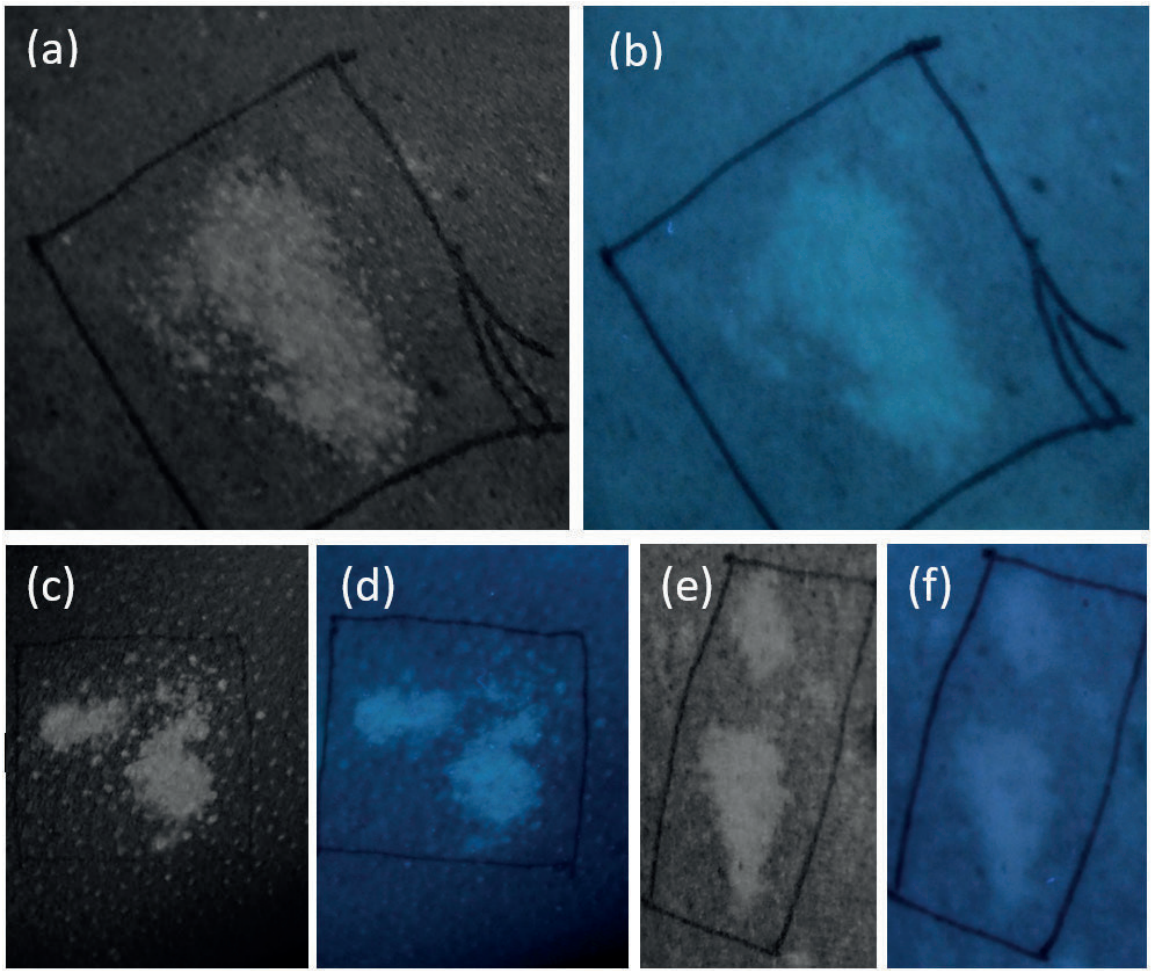
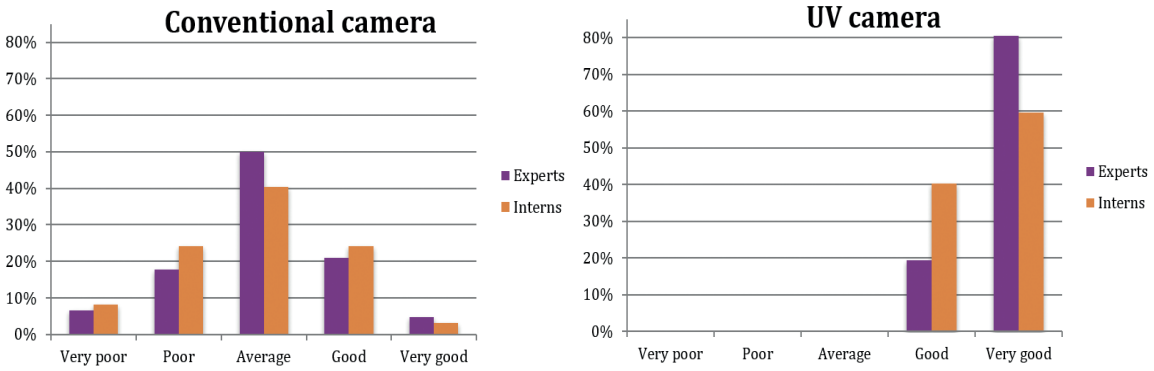


Figure 2 Frequency of quality scores for images of vitiligo lesions made with a conventional camera and UV photography



The scatterplots (supplementary figure 1) show our results of the correlations between the measured surface area of target lesions by the different measurement instruments.

Table 2 Validity: ICCs between the different measurement instruments

	ICC	95% CI
UV photography method – conventional camera method	0.984	0.963 - 0.992
UV photography method – transparent sheet method	0.988	0.974 - 0.994
UV photography method – graph paper method	0.983	0.955 - 0.992
Conventional camera method – transparent sheet method	0.975	0.945 - 0.988
Conventional camera method – graph paper method	0.960	0.869 - 0.984

ICC, intraclass correlation coefficient; CI, confidence interval

Reliability

The ICC for the intra-rater reliability was 0.999 (95% confidence interval (CI) 0.997 - 0.999) and the ICC for the inter-rater reliability was 0.998 (95% CI 0.996 - 0.999). The correlations were closely clustered around the regression lines, both for the intra- and inter-rater reliability (supplementary figure 2).

The mean difference in the intra-rater reliability was 0.04 cm^2 with limits of agreement of 0.35 cm^2 and -0.28 cm^2. The mean difference in the inter-rater reliability was 0.04 cm^2 with limits of agreement of 0.42 cm^2 and -0.33 cm^2 (supplementary figure 3). These limits of only a few millimetres illustrate a high level of agreement for both intra- and inter-rater reliability.

Discussion

UV photography and its promises for dermatology have been described in multiple studies.[1–3] Mustakallio and Korhonen already described the use of UV photography for pigment disorders including vitiligo in 1966.[3] We did not find studies that assess image quality or validation studies for digital UV photography in vitiligo.

We found a high consensus on image quality for the UV photography images, both vitiligo experts and unexperienced medical interns agree separately that the UV photography images were of high or very high quality, and superior to the conventional digital camera images. Furthermore we found very high ICCs when comparing the UV photography method with other measurement instruments and for multiple measurements by the same or different physicians, supporting its validity and reliability. Another benefit of UV photography is the ease of use. A disadvantage is that UV photography cannot be used for assessment of lesions which are localized on curved skin, this could be resolved by using multiple cameras or other 3D image analysis systems.[8]

With a sample size of 31, our study can be considered as of fair quality,[6] but larger studies need to be performed to support our findings. Secondly, our study was performed in one centre and findings should be confirmed in other settings and populations. Thirdly even though the correlations coefficients are high, these correlations do not implicate exact agreement between the different measurement instruments.

Some images showed pinpoint or confetti lesions that were barely visible with UV light. This might imply that UV photography could ameliorate the assessment of vitiligo activity, although this could also be follicular Propionibacterium acnes. Further research is needed to explore this hypothesis.

Conclusion

The digital UV enhanced camera is a very promising tool for assessing vitiligo especially in fair skinned patients in practice as well as in research. The results of this study indicate that the use of UV photography improves image quality and is valid and reliable.

Acknowledgements

We would like to express our gratitude to the volunteering patients who participated in this study.

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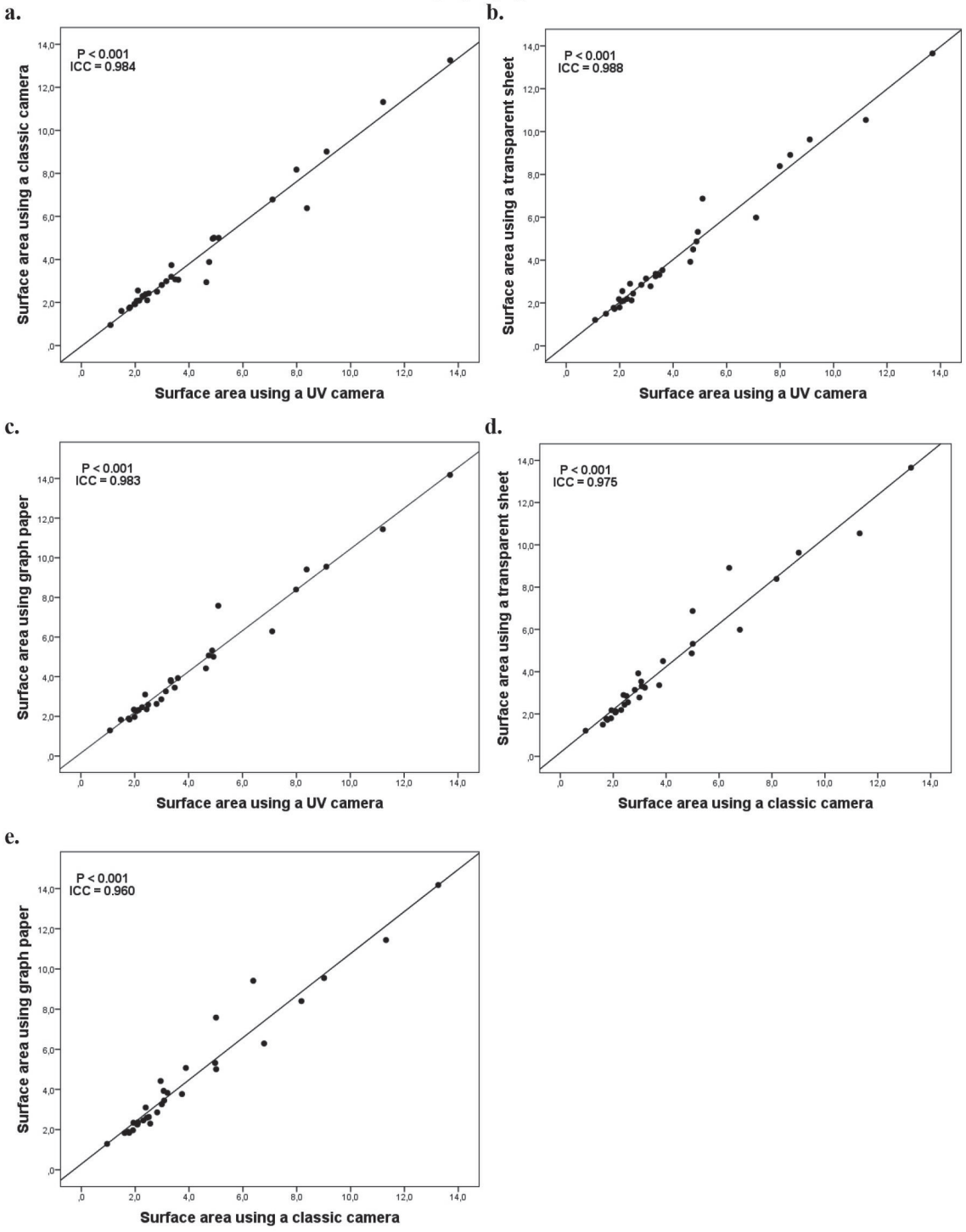
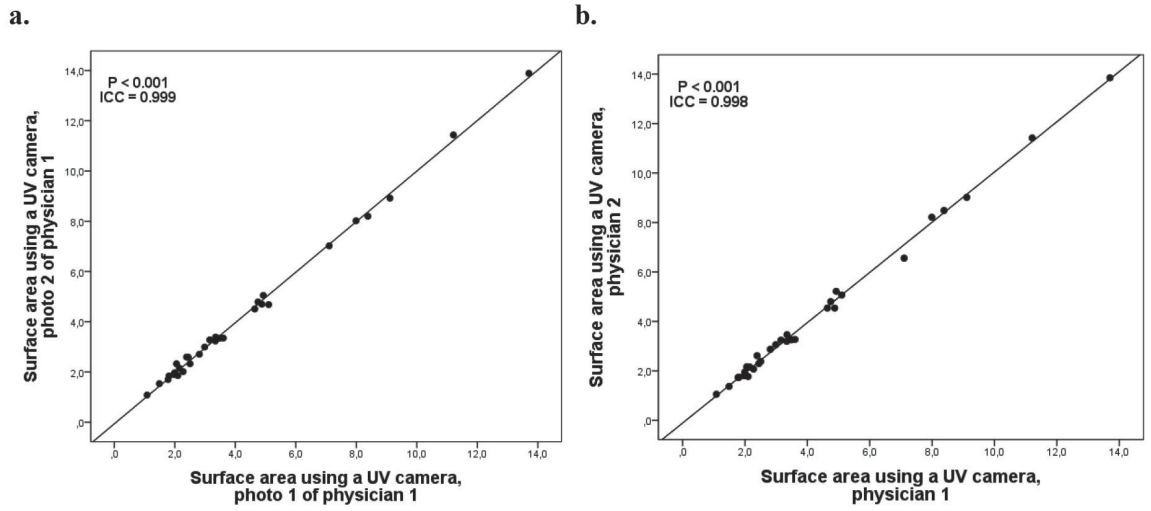
Supplements

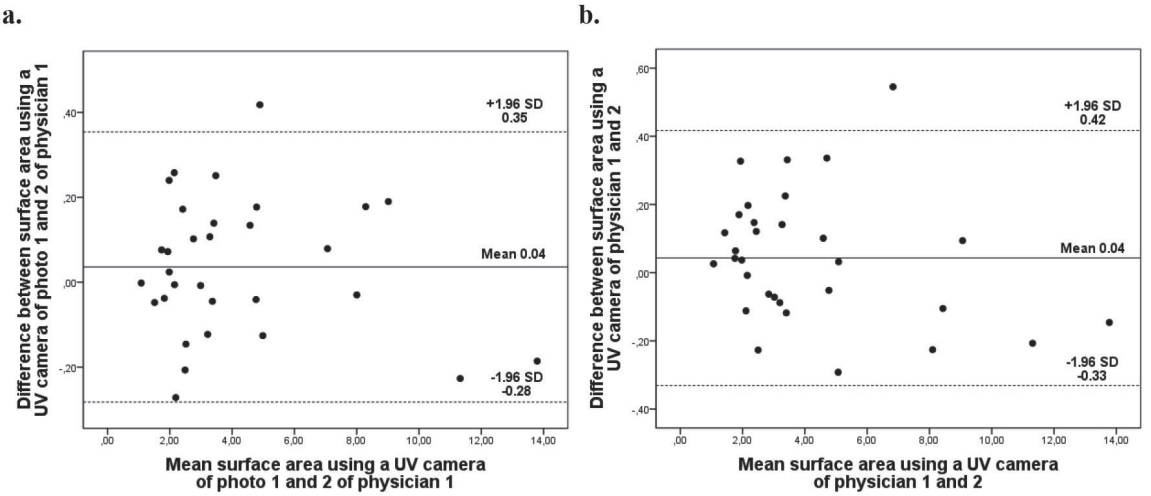
Supplementary figure 1 Correlations between the measured surface area (cm^2) of target lesion assessment by the different measurement instruments (**a**) Correlation UV photography method - conventional camera method (**b**) Correlation UV photography method - transparent sheet method (**c**) Correlation UV photography method - graph paper method (**d**) Correlation conventional camera method - transparent sheet method (**e**) Correlation conventional camera method - graph paper method



Supplementary figure 2 Correlations between the measured surface area (cm^2) of target lesion assessment using UV photography by the same physician and two different physicians (**a**) Intra-rater reliability. (**b**) Inter-rater reliability



Supplementary figure 3 Bland-Altman plots. Differences in the measured surface area (cm^2) of target lesion assessment using the UV photography method (**a**) for the same physician (intra-rater reliability) (**b**) for different physicians (inter-rater reliability)



Part II

Outcome Measures

3

Assessing the minimal important change in the vitiligo extent score and the self-assessment vitiligo extent score

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Abstract

Background
The minimal important change (MIC) of the Vitiligo Extent Score (VES) and the Self-Assessment Vitiligo Extent Score (SA-VES) is not yet known.

Objective
To determine the MIC of the VES and SA-VES.

Methods
Patients with non-segmental vitiligo, starting therapy were included in hospitals in Egypt, Korea, Singapore and Netherlands. The MIC of the VES and SA-VES was determined using the anchor-based method.

Results
Overall 135 patients (58.5% female) completed the study, mean age was 42.3 years. We found MICs of 0.5% and 0.2% for the VES and SA-VES respectively. The MICs we found are smaller than previously reported smallest detectable changes (SDCs).

Limitations
The size of subgroups were too small to make reliable estimates of the MIC for the hands. The MIC was not determined in the same patient population as SDC.

Conclusion
Our results improve the interpretability of these measurement instruments and can be used to determine number of responders in future studies.

Introduction

There is a need for valid, reliable and interpretable outcome measurement instruments in vitiligo research.[1,2] Recently two new instruments were developed to measure the extent of vitiligo with the use of questionnaires: the Vitiligo Extent Score (VES) and the Self-Assessment Vitiligo Extent Score (SA-VES). The VES and SA-VES have been validated and are feasible and user friendly outcome measurement instruments for assessing the overall extent of vitiligo.[3,4] A recent systematic review concludes that the most effective instrument to assess the amount of body surface area (BSA) involvement is the VES.[5] However, currently it is not yet known when a change in outcome of the VES and SA-VES signifies a change in the extent of vitiligo that is actually perceived important by patients (SA-VES) or clinicians (VES). To determine this, the minimal important change (MIC) of these instruments has to be assessed - the MIC is the smallest change in score which patients (or clinicians) perceive as important.[6] The MIC is a key element of interpretability and interpretability is an important aspect of outcome measurement instruments.[6] Furthermore the MIC is an important parameter in clinical studies for determining the number of responders, i.e. the number of patients that have a change at least as large as the MIC. If we can determine the MIC of the VES and the SA-VES, we will improve the interpretability of these instruments and create the possibility to assign clinical meaning to their change scores.[6]

The importance of change of the extent of the vitiligo might be different for different locations of vitiligo. Studies showed that quality of life was most significantly impaired with vitiligo located on the hands[7] and other visible areas[8]. Furthermore, the extent of vitiligo can affect the MIC. A 2% decrease of vitiligo body surface area (BSA) involvement can be very different for a patient with a BSA involvement of 3% than for a patient with BSA involvement of 10%. In the validation studies for the VES and SA-VES, patients with vitiligo with a BSA involvement <5% had a lower smallest detectable change (SDC, change in outcome of a measurement instrument that could be due to measurement error).[3,4] Therefore it would be interesting to determine the MIC specifically for patients with vitiligo locations that are important for patients' quality of life, like the hands and face and (i) for patients with a BSA involvement of <5%.

The primary aim of this study was to determine the MIC of the VES and SA-VES. A secondary aim was to determine the MIC for patients with vitiligo of the face and hands and for patients with limited vitiligo (<5% BSA).

Methods

In this prospective cohort study, patients were included in four different international academic hospitals in Egypt, South Korea, Singapore and the Netherlands. Each center received ethical approval of its Institutional Review Board. Inclusion criteria were (i) 16 years of age or older, (ii) diagnosis of non-segmental vitiligo, (iii) starting any kind of vitiligo therapy except surgical interventions (like epidermal transplantation) and (iv) able to understand and fill in the questionnaires according to the assessment of the physician.

Data collection
Eligible patients visiting the outpatient clinics of the above named centers were consecutively approached and informed about the study. After informed consent had been given, data was be collected at two time points: T0, at inclusion, when the patient visited the physician and was starting therapy. The second time point, T1, was three to six months after starting therapy.

At T0 patients signed a consent form, were photographed and filled in a questionnaire including age, gender, skin type, therapy plan (including location) and the SA-VES. At T1 both patients and physicians filled in questionnaires, including questions about perceived change (called anchor questions) and the VES for physicians and the SA-VES for patients. Physicians used the photographs of both T0 and T1 to answer the VES and anchor questions. We decided to use photographs for practical reasons. Photographs were also used for the validation of the VES and demonstrated similar results compared to live evaluation.[3]

We used an anchor-based method to determine the MIC. With this method a global question about perceived change is used as an external criterion (anchor) to determine whether the extent of vitiligo has actually changed over time. Patients and physicians were asked to answer the following question: “Try to remember the extent of your/the vitiligo, <u>including all vitiligo lesions,</u> before you/the patient started treatment 3 to 6 months ago. How much has your/the vitiligo changed since that moment?” Subsequently, patients and physicians were asked to score the perceived change on the following scale:

1. Completely improved: no vitiligo spots anymore
2. Much improved
3. Slightly improved
4. No change

5. Slightly worsened
6. Much worse

This same question was subsequently asked specifically about the vitiligo of the face and hands. For these questions a separate answer was added: 'I did not have vitiligo on the hands / face'.

Statistical analysis

The MIC was estimated for the follow-up period of 3 to 6 months. The global question about change was used as an external criterion to determine the MIC. Mean change scores were calculated by subtracting the T0 outcomes from the T1 outcomes for both the VES and SA-VES. Mean change scores were calculated across the response categories of the anchor questions. The MIC of the VES and SA-VES was determined as the mean change score in the group of physicians (VES) and patients (SA-VES) that answered the anchor-based question with 'Slightly improved'. Furthermore, we determined the MIC for patients with vitiligo lesions on the hand and face separately using the same method. We also determined the MIC for patients with a BSA smaller than 5%.

Further, we compared the MIC values found for the VES and SA-VES with the smallest detectable change (SDC) as determined in previous studies.[3,4,9] In order to distinguish 'real change' from measurement error in individual patients, the MIC should preferably be larger than the SDC.

Results

A total of 162 patients were included in the study, of which 27 were lost to follow up. The number of included patients and characteristics are reported in Table 1. In total, 135 patients completed the study, with a mean age of 42.3 years; 79 (58.5%) were female. All Fitzpatrick skin types were represented in the study and most patients had skin type III (33.3%) and IV (40.0%). Most patients lost to follow up were from Singapore, due to COVID-19 measures. Age and gender of patients lost to follow up did not differ significantly compared to patients who completed the study. The average VES score at inclusion was 3.5% (median 1.5%) BSA, the mean SA-VES score was 4.3% (median 1.9%) BSA.

Table 1. Included patients from each country

	Total	Netherlands	Egypt	South Korea	Singapore
Included	162	50 (30.9)	42 (25.9)	29 (17.9)	41 (25.3)
Lost to follow up	27 (16.7)	6 (12.0)	5 (11.9)	2 (6.9)	14 (34.1)
Completed	135 (of which 1 only VES and 4 only SA-VES data)	44 (88.0)	37 (88.1)	27 (93.1)	27 (65.9) (of which 1 only VES and 4 only SA-VES data)
General characteristics					
Age	42.3 +/- 15.3	43.6 +/- 11.7	33.7 +/- 16.7	51.2 +/- 14.8	43.0 +/- 13.8
Gender					
Female	79 (58.5)	28 (63.6)	21 (56.8)	16 (59.3)	14 (51.9)
Male	56 (14.5)	16 (36.4)	16 (43.2)	11 (40.7)	13 (48.1)
Skin type					
I	2 (1.5)	2 (4.5)	0	0	0
II	21 (15.6)	21 (47.7)	0	0	0
III	45 (33.3)	8 (18.2)	12 (32.4)	15 (55.6)	10 (37.0)
IV	54 (40.0)	5 (11.4)	25 (67.6)	12 (44.4)	12 (44.4)
V	11 (8.1)	6 (13.6)	0	0	5 (18.5)
VI	2 (1.5)	2 (4.5)	0	0	0
VES score at T0	3.5 +/- 5.3	3.7 +/- 6.3	4.6 +/- 5.4	3.1 +/- 4.9	2.1 +/- 3.4
SA-VES score at T0	4.3 +/- 7.0	5.5 +/- 9.8	4.6 +/- 4.9	3.7 +/- 5.8	2.5 +/- 4.5

Values are n (%) or mean +/- standard deviation, VES and SA-VES scores are percentage body surface area.
VES: vitiligo extent score, SA-VES: self-assessment vitiligo extent score, T0: moment of inclusion.

Patients were divided into 6 subgroups according to the anchor questions. The mean change scores for each subgroup for the VES and SA-VES are shown in Table 2a and 2b respectively. We found a MIC of 0.5% and 0.2% decrease in BSA for the VES and SA-VES respectively. For the face and hands respectively we found a MIC of 0.16% and 0.09% for the VES and 0.22% and 0.01% for the SA-VES. For patients with a BSA smaller than 5% we found a MIC of 0.3% for the VES 0.1% for the SA-VES.

Table 2a. Mean change in % BSA of the VES according to the physician's anchor

Anchor	Mean change VES score in % BSA (SD)							
	n	Total	n	Face	n	Hands	n	BSA<5%
Completely improved	1	-1.13	10	-.29 (.45)	0		1	-1.13
Much improved	31	-1.86 (2.19)	39	-.41 (.39)	4	-.06 (.07)	25	-.85 (.61)
Slightly improved	**74**	**-.53 (1.08)**	**36**	**-.16 (0.22)**	**16**	**-.09 (.09)**	**65**	**-.38 (.49)**
No change	13	.13 (0.99)	12	.00 (.00)	60	-.002 (.04)	10	.33 (1.01)
Slightly worsened	11	1.00 (1.31)	4	.09 (0.15)	12	.06 (.07)	7	.47 (.43)
Much worse	1	1.04	1	.79	0		1	1.03

BSA: body surface area, VES: vitiligo extent score, SA-VES: self-assessment vitiligo extent score, SD: standard deviation

Table 2b. Mean change in % BSA of the SA-VES according to the patient's anchor

Anchor	Mean change SA-VES score in % BSA (SD)							
	n	Total	n	Face	n	Hands	n	BSA<5%
Completely improved	1	-1.13	11	-.39 (.47)	0		1	-1.13
Much improved	48	-1.77 (2.49)	41	-.36 (.48)	16	-.10 (.13)	39	-.94 (.92)
Slightly improved	**56**	**-.20 (2.31)**	**35**	**-.22 (.39)**	**24**	**-.01 (.13)**	**44**	**-.23 (1.28)**
No change	14	-1.44 (5.06)	16	-.05 (.30)	38	-.04 (.18)	9	.33 (.85)
Slightly worsened	12	-.25 (1.79)	4	.23 (.32)	14	.08 (.15)	10	-.05 (.74)
Much worse	3	1.48 (2.32)	0		4	.00 (.29)	3	1.48 (2.32)

BSA: body surface area, VES: vitiligo extent score, SA-VES: self-assessment vitiligo extent score, SD: standard deviation

Discussion

This is the first study to assess the minimal important change (MIC) in a large group of vitiligo patients from different countries and cultures. We found an anchor-based MIC of 0.5% BSA decrease for the VES, which is somewhat lower compared to previously found SDCs (between 0.86% and 4.7%).[3,9] For the SA-VES we found an even smaller MIC of 0.2% BSA, compared to a previously found SDC of 3.23% reported only in 1 study so far including a small group of 50 patients.[4]

This means that a decrease in body surface area measured by the VES/SA-VES as great as the MIC might be considered as important for physicians/patients, but this change cannot be distinguished from the measurement error with 95% confidence.[10] The same goes for the MIC we found for patients with a BSA <5%. For lesions on the face we found a MIC of 0.16% BSA for the VES and 0.22 for the SA-VES. For the hands the subgroups were very small and our preliminary estimation of the MIC is 0.09% for the VES and 0.01% for the SA-VES. We could not find any previously reported SDCs for patients with vitiligo of the face and hands separately.

In an ideal situation the SDC is smaller than the MIC (although this is often not the case) so that any change in outcome as large as the MIC can be interpreted as being clinically relevant. Another measurement instrument for measuring the extent of vitiligo is the Vitiligo Area Scoring Index, but SDCs of this outcome measure are higher than the SDCs of the VES and SA-VES.[3,11] Future studies could try to reduce the SDC to become smaller than the MIC, e.g. by increasing the number of items in a patient reported outcome measure, more standardization of measurements, or using the average of repeated measurements. For this aspect it could be valuable to investigate the use of the VESplus more in depth.[9] The VESplus is a variant of the VES that includes more items (e.g. perifollicular scale can be added) and was introduced to score more in detail. So far, its use has only been investigated in a limited patient population (n=57). An important value of our findings in this study is that it can be used to determine number of responders (i.e. number of people having a change as large as the MIC) in studies using the VES and SA-VES as outcome measures.

The MICs we found are very small, especially for patients. This could be due to the fact that when patients start therapy they might have actively spreading vitiligo (not measured in this study) and stabilization of the vitiligo might already feel like an improvement. The value of cessation of spread of vitiligo for patients has been previously reported.[12] Furthermore, we should keep in mind that the MIC in this study was assessed in another patient population compared to the so far rather limited populations used to assess the SDC in the past. Therefore, caution should be exercised by its interpretation and future studies could preferably aim to include both evaluations within the same patient population.

To the best of our knowledge, there is no previous data on the MIC for the VES and SA-VES. The international, cross-cultural approach is a strength of the study. On the other hand, the heterogeneity of the study population can be seen as a limitation, as we do not know whether the MIC differs between different cultures

or different skin types. The sample size of the subgroups was not large enough to make comparisons of the MIC between countries or skin types. Another limitation is that Singapore had a relatively large rate of patients lost to follow-up, which was because many patients were included in the end of 2019 and were not able to perform the follow-up visits due to COVID-19 measures. We do not believe this could have biased the results. Thirdly, even though 135 patients completed the study, the numbers of patients in the subgroups, especially for patients with lesions on the hands were too small to make reliable statements about the MIC. The small numbers in other response categories (no change, worsened) also inhibited us to use a receiver operating characteristic (ROC) curve as an alternative method for calculating the MIC. A previous study on variation between studies determining the MIC concludes that the MIC cannot be trusted when based on only one study.[13] We therefore recommend future studies to also determine the MIC by using a modified 'anchor question' to consolidate our findings.

Future studies could assess whether the MIC varies among different countries, cultures and skin types as the MIC could differ for different ethnicities. Furthermore, as the SDC and MIC depend on the affected BSA, future studies could assess the SDCs and MICs for patients categorized by different recently determined severity strata.[14]

We conclude that patients already seem to perceive very small changes in BSA involvement as important change and that location and disease extent are important to take into account when interpreting changes in VES and SA-VES. We found a MIC for the VES and SA-VES both smaller than the previous reported SDCs but caution should be exercised by its interpretation. Our results improve the interpretability of these measurement instruments and can be used to determine number of responders in future studies using the VES and SA-VES as outcome instruments.

Acknowledgements

We would like to express our gratitude to the volunteering patients who participated in this study.

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4

Validation of a Patient Global Assessment for extent, severity, and impact to define the severity strata for the Self Assessment Vitiligo Extent Score (SA-VES)

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Abstract

Background

The Self Assessment Vitiligo Extent Score (SA-VES) is a validated, patient reported outcome measure to assess the body surface area affected with vitiligo. Information on how to translate the obtained score into extent, severity and impact strata (mild-moderate-severe) is still lacking. Stratification is helpful to define inclusion criteria for trials, enables comparison and pooling of trial results and can be used for epidemiological research.

Objectives

The aim was to develop extent, severity and impact strata for the SA-VES based on validated anchor based questions.

Methods

In total, 315 patients with vitiligo (non-segmental; age ≥16) recruited at the Ghent University Hospital (Belgium) completed a questionnaire that was conducted in cooperation with the Dutch Society for Vitiligo patients to ensure content validity. First 3 anchor questions included in the questionnaire [Patient Global Assessment (PtGA) for vitiligo extent, severity and impact] were assessed for content validity, construct validity and intrarater reliability. Subsequently, the PtGAs were used to stratify the SA-VES based on ROC analysis.

Results

For all PtGAs (PtGA extent, PtGA severity, PtGA impact) at least 75% of hypotheses evaluated for construct validity were confirmed. Intrarater reliability of all PtGAs were good to excellent (ICCs PtGA extent: 0.623; PtGA severity: 0.828; PtGA impact: 0.851).-The optimal cut-off values of the SAVES between the 3 global categories (mild/limited - moderate - severe/extensive) were 1.05% and 6.45% based on PtGA extent, 2.07% and 4.8% based on PtGA severity and 2% and 3.35% based on PtGA impact.

Conclusion

This study provides the first guide for the interpretation of the numerical output obtained by the SA-VES (vitiligo extent) and enables the translation into a global vitiligo grading for extent, severity and impact. As patients' interpretation of vitiligo extent, severity and impact may vary amongst patients worldwide, future international studies will be required.

Background

The Self Assessment Vitiligo Extent Score (SA-VES) is a validated, patient reported outcome measure for monitoring vitiligo extent. It is based on an assessment of the affected Body Surface Area (BSA) which is considered to be a relevant outcome in the assessment of vitiligo severity. It is a patient reported version of the Vitiligo Extent Score (VES) for physicians.[1] Information on how to translate the obtained VES scores into disease severity strata from the physicians' point of view has been described recently while this is still lacking for the patient reported version (SAVES).[2] Stratification can be used to define inclusion criteria for clinical trials, epidemiological research and is important for correct comparison and pooling of trial results. The process to stratify the numeric output of an instrument into categories (mild-moderate-severe) is usually based on an 'anchor question' (e.g. Patient Global Assessment) that is preferably validated in advance.[3,4] A validated Patient Global Assessment for vitiligo is however lacking. A validated Global Assessment instrument could also be used as anchor questions for the interpretation and stratification of other outcome measure in vitiligo ("[e.g. Dermatology Life Quality Index (DLQI), Vitiligo Impact Patient scale (VIPs)].).[5–7]

The first aim of this study was to assess the content validity, construct validity and intrarater reliability of a Patient Global Assessment (PtGA) for disease extent, severity and impact. The second aim was to determine strata (mild-moderate and severe) for the SAVES based on the validated PtGA for extent, severity and impact.

Materials and Methods

Study design and ethics

In this observational study patients with vitiligo (non-segmental) age ≥16 were consecutively recruited at the Ghent University Hospital (Belgium) (October 2017-september 2019) and were asked to complete a Dutch questionnaire including the SAVES as well as a 5-point global assessment scale for extent (PtGA extent), severity (PtGA severity) and impact (PtGA impact) (Fig 1).

In the preparative stage of this study the PtGA instruments were evaluated and modified by several members of the Dutch Society for Vitiligo patients (vitiligo.nl) to ensure the content validity. The patients' global assessment instruments were subsequently evaluated by asking the relevance, completeness and comprehensibility of all items included. The questions used to assess the Patients

Reported Global Assessment were pilot tested at the department of dermatology in Ghent. The COSMIN checklist was used as a guidance for designing and reporting our study.[8,9] This study was approved at the local ethics committee (reference number: B670201421409) and written informed consent was obtained from all patients who completed the questionnaire. The cooperation with the Dutch Society for vitiligo patients (Vitiligo.nl) was reported at the Amsterdam Medical Center (W17_355#17.413).

Validity and reliability

Construct validity of the PtGA instruments was evaluated by testing at least 4 predefined hypotheses by Spearman correlations with other PROMs (e.g. SAVES, DLQI, impact score 0-10 and the PtGA scores included in this study) (supplementary file 1).[10] As an impact score from 0 to 10 was also included to evaluate construct validity of the PtGA score, this impact score 0-10 was also validated for use (supplementary file 1). To evaluate the intrarater (test-retest) reliability of the PtGA instruments and Impact score 0-10, a subgroup of patients was asked to complete the questionnaire twice with an interval of 2 weeks. To increase the usability of the PtGA in an international setting all related questions were translated (including 2 forward and 2 backward translations) in English (Fig 1) by a professional translation institute (ElaN Languages, Heusden-Zolder; Belgium) following the instructions for translations of measurement instruments as a guidance.[11] All questions (PtGA and impact score 0-10; including translated version) used for this study are available on request at the corresponding author.

Stratification of affected self-assessed body surface area (SAVES)

In the second part of this study the validated PtGA for extent, severity and impact (Fig 1) were used as anchor questions to stratify the SAVES. By this anchor-based approach, the SAVES outcomes were compared (anchored) to the results of Patient Global Assessments collected at the same time. By defining the thresholds based on ROC analyses the SAVES could be stratified into global strata (mild-moderate-severe).

Statistics/Data analysis

Statistical analyses were performed using SPSS 25.0 (SPSS Science, Chicago, IL, USA). The intrarater agreement of the PtGA scores was calculated by ICC and reported as single measures [two way mixed, absolute agreement]. The following guidelines for the interpretation of the ICC were used: below 0.4 considered as poor, between 0.4-0.59 as fair, between 0.6-0.74 as good, and ≥ 0.75 as excellent. A cut off point of a Spearman's correlation of at least 0.4 was used in all hypotheses used to test construct validity (supplementary file 1). Paired analysis was performed

using Wilcoxon signed-rank test. Global strata (cut off points) for the SAVES were based on Youden's index assessed by ROC analyses using MedCalc 19.1 software (Medcalc, Mariakerke, Belgium). MedCalc was also used to assess the 95% CI for the median of the SAVES scores per severity strata for each PtGA (extent, severity and impact).

To check the degree of agreement between the obtained severity strata (ranges of cut off points) and the PtGAs the intraclass correlation coefficient (ICCs) were subsequently assessed. Missing values were excluded from the final analysis. For the DLQI score, if some questions were left unanswered this was scored 0. However, the questionnaire was not scored in case the questionnaire was considered not to be finalized by the patient (e.g. half of all questions left unanswered). In all cases, significance level was set at $p < 0.05$.

Results

Preparative stage (content validity PtGAs)

A draft version of the PtGA questions was thoroughly checked and modified multiple times according to the patients' preferences (7 members of Dutch society for vitiligo patients), based on completeness, comprehensibility and relevance. Based on the patient's comments the question that introduced the rating of the patients' global assessment of severity (PtGA severity) was modified to a more detailed and comprehensible version for patients in order to rate their vitiligo severity without further explanation (Fig 1). This detailed version illustrated more the different dimension of the PtGA severity compared to the PtGA for impact and extent. The question included in the global impact score was clarified by adding the word 'influence'.

Reliability and construct validity of PtGA scores

In total, 315 patients (age ≥16) were included (male/female 43.5%/56.5%; mean age (± sd) at inclusion was 40 ± 14 years, range 16 – 73 years), photo skin type: I (1.4%, 4/289*)*, II (36%, 104/289*)*, III (48.1%, 139/289), IV (12.1%, 35/289), V (2.4%, 7/289), median (mean) BSA (SAVES) score was 1.65% (4.27%) (range 0.04 – 73.88%), median DLQI score was 2 (range 0 – 21).

Figure 1

Translations Patient Global Assessments (PtGA) from original Dutch version

1. Patient Global Assessments for severity

The following question concerns how you would describe the <u>overall severity</u> of your vitiligo. By this, we mean your perception of your vitiligo when considering all factors such as the extent of your vitiligo, your skin type (skin colour), the influence of vitiligo on your daily life, the location of the spots, and the vitiligo's activity/stability.

a. **How do you experience the <u>severity</u> of your vitiligo at this moment?** *Tick the appropriate box*

☐ very severe ☐ severe ☐ moderate ☐ mild ☐ not severe at all

2. Patient Global Assessments (PtGA) for extent

How <u>extensive</u> do you consider your vitiligo to be at this moment? *(By extensive, we mean the **amount of the skin's surface affected** by vitiligo). Tick the appropriate box*

☐very extensive ☐extensive ☐moderate extent ☐limited extent ☐no vitiligo any more

3. ImpactScore 0-10 (a) and Patient Global Assessments (PtGA) for impact (b)

What <u>influence</u> (impact) does vitiligo currently have on your daily life? *Please complete both (a) and (b)*

a. Pick a number on a scale from 0 to 10, where 0 = no influence and 10 = a lot of influence: …….

b. ☐very severe influence ☐severe influence ☐moderate influence ☐little influence ☐no influence at all

Remark related to PtGA 1: the wording "skin colour" was added at the translational stage but was not included in the original Dutch version. Within the Dutch version we considered this to be clear as an additional question related to different skin types (I-VI) was included in the same questionnaire in the majority of cases. In a minority of cases skin type was assessed by the physician for instance if the self assessment question related to skin type was not included in the questionnaire."

Table 1: Correlations including those used for the construct validity testing of PtGAs and Impact score 0-10

Spearman's correlations (rho) Included in the construct validity test	PtGA extent	PtGA severity	PtGA impact	DLQI	SAVES	Impact score 0-10
PtGA extent	NA	0.586*	0.479*	0.411*	0.633*	0.455
PtGA severity	0.586*	NA	0.729*	0.489*	0.442*	0.712
PtGA impact	0.479*	0.729*	NA	0.598*	0.359*	0.876
Impact score 0-10	0.455*	0.712*	0.876*	0.601*	0.350*	NA

*significant at 0.01 level; boxes in light grey: correlations not included in the hypotheses used for construct validity testing. Cut of level for rho's correlation of 0.4 not reached for 2 correlations. Number of patients included for the correlation test ranged between 199 and 291.

Supporting evidence for construct validity was provided for PtGA extent, PtGA severity, PtGA impact and Impact score 0-10 as at least 75% of hypotheses (supplementary file 1) per instrument were confirmed (see table 1). All Spearman correlations included in the hypotheses were significant, although 2 did not reach the cut off level of 0.4. Highest correlation coefficients for the global assessment of severity (PtGAs) were observed with PtGA impact (r=0.729). The different dimension of the PtGA severity compared to the PtGA impact was however confirmed by a paired analysis showing a highly significant difference ($p < 0.001$). In total 111/287 (38.7%) patients rated impact and severity differently with most patients reporting a higher severity compared to impact (75/111, 67.6%). Not surprisingly, the correlation between DLQI was strongest for PtGA impact (r = 0.598), followed by PtGA severity (r = 0.489) and PtGA-extent (r = 0.411). For extent (PtGA extent) highest correlation coefficients were observed with the SAVES (r=0.633) followed by PtGA severity (r=0.586).

Fifty patients were included for the test-retest study. Test-retest results of the PtGA extent [n=50; ICC = 0.623 (95% CI: 0.418-0.768)], PtGA severity [n= 50; ICC = 0.828 (95% CI: 0.716-0.898)], PtGA impact [n=48; ICC = 0.851 (95% CI: 0.749-0.914)] and Impact score 0-10 [n=34; ICC = 0.872 (95% CI: 0.760-0.934)] demonstrated all good to excellent test-retest intraclass correlation coefficients.

Stratification of SA-VES

Median estimation of the PtGA severity was scored as 'moderate'. Most patients (mode) reported 'limited extent' of their vitiligo (58.3%, 169/290). About 207 patients (72.1%, 207/287) reported a mild to moderate impact of vitiligo, 53 (18.5%, 53/287) reported severe to very severe influence of vitiligo on their quality of life. Table 2 shows the optimal cut of points and confidence interval of the SAVES per 3 categories based on the respective anchor questions (PtGA extent, PtGA severity, PtGA impact). The optimal cut-off values of the SAVES between the 3 global categories (1. mild/limited 2. moderate 3. severe/extensive) were 1.05% and 6.45% based on PtGA extent, 2.07% and 4.8% based on PtGA severity and 2% and 3.35% based on PtGA impact. ICCs between obtained cut off point ranges and PtGAs for extent, severity and impact are included in table 2.

Box plots for PtGA for extent, severity and impact representing 3 categories (mild -moderate- severe) based on SAVES are presented in figure 2a-c.

The distribution, mean, median, mode and IQR range of the SAVES per category of the PtGA extent, PtGA severity and PtGA impact are presented in table 3.

Table 2: Cut off points/severity strata for the SAVES into 3 categories.

	Affected body surface area % (SAVES)		
PR-GA extent	**No more vitiligo - limited**	**moderate**	**Extensive – very extensive**
Range	≤1.05%	>1.05%-6.45%	>6.45%
Cut-off ICC= 0.642	1.05% (0.91 – 2.78)	6.45% (3.13 – 10.83)	
PR-GA severity	**Not at all severe - mild**	**moderate**	**Severe – very severe**
Range	≤2.07%	>2.07%-4.8%	>4.8%
Cut-off ICC= 0.386	2.07 (1.32 – 4.47)	4.8 (2.5 – 10.3)	
PR-GA impact	**No impact – mild impact**	**Moderate impact**	**High- very high impact**
Range	≤2%	>2%-3.35%	>3.35%
Cut-off ICC = 0.311	2% (0.61 – 3.99)	3.35% (0.25 – 6.15)	

*ICC, intraclass correlation between obtained ranges per strata and PtGAs; PtGA, Patients global assessment; SA-VES, Self Assessment Vitiligo Extent Score. *in the questionnaire the wording was "no influence at all - little influence". **in the questionnaire the wording was "moderate influence".***in the questionnaire the wording was "severe influence - very severe influence".*

Table 3: Median SAVES scores for each PtGA for 3 and 4 categories of extent, severity and impact**.**

PtGA categories	N	Median SAVES (%)	IQR	95% CI for the median*
PR-GA extent				
No vitiligo-limited	163	0.78	0.315-1.915	0.678 – 1.032
Limited	159	0.790	0.335 – 1.915	0.692 – 1.040
Moderate	72	2.740	1.3938 – 5.3838	2.033 – 3.580
Extensive	28	9.370	4.210– 16.3225	5.185 – 13.631
Very extensive	9	20.235# (mean 33.745)	11.3475– 58.250	10.911 – 63.771
Extensive & very extensive	37	11.935	5.465– 19.6475	7.752– 16.808
PtGA severity				
Not severe- mild	113	0.790	0.315-1.8875	0.587-1.141
Mild	85	0.985	0.3775 – 2.0325	0.700 – 1.305
Moderate	96	2.2325	0.821 – 4.740	1.518 – 3.128
Severe	52	4.675	1.581 – 10.7225	2.425 – 7.057
Very severe	11	1.580# (mean 14.8477)	0.655 – 15.00	0.641 – 21.402
Severe & very severe	63	4.475	1.220 – 10.780	2.140 – 6.454

Table 3: Median SAVES scores for each PtGA for 3 and 4 categories of extent, severity and impact. **(continued)**

PtGA categories	N	Median SAVES (%)	IQR	95% CI for the median*
PtGA impact (influence)				
No impact-little	143	1.035	0.370-2.425	0.780-1.486
Little	118	1.2875	0.385 – 2.486	0.892 – 1.657
Moderate	74	2.2425	0.9313 – 5.4163	1.430 – 3.349
Severe	41	5.060	1.86 – 10.5025	3.472– 7.707
Very severe	10	1.175# (mean 6.073)	0.530 – 4.5887	0.480 – 7.288
Severe & very severe	51	4.225	1.225 – 10.350	2.136 – 6.455

IQR: interquartile range; CI: confidence interval; # Median based on very low numbers of cases within this category; *95% confidence interval assessed by MedCalc

Figure 2a Box plots for SAVES representing 3 categories (mild – moderate – severe) based on PtGA extent.

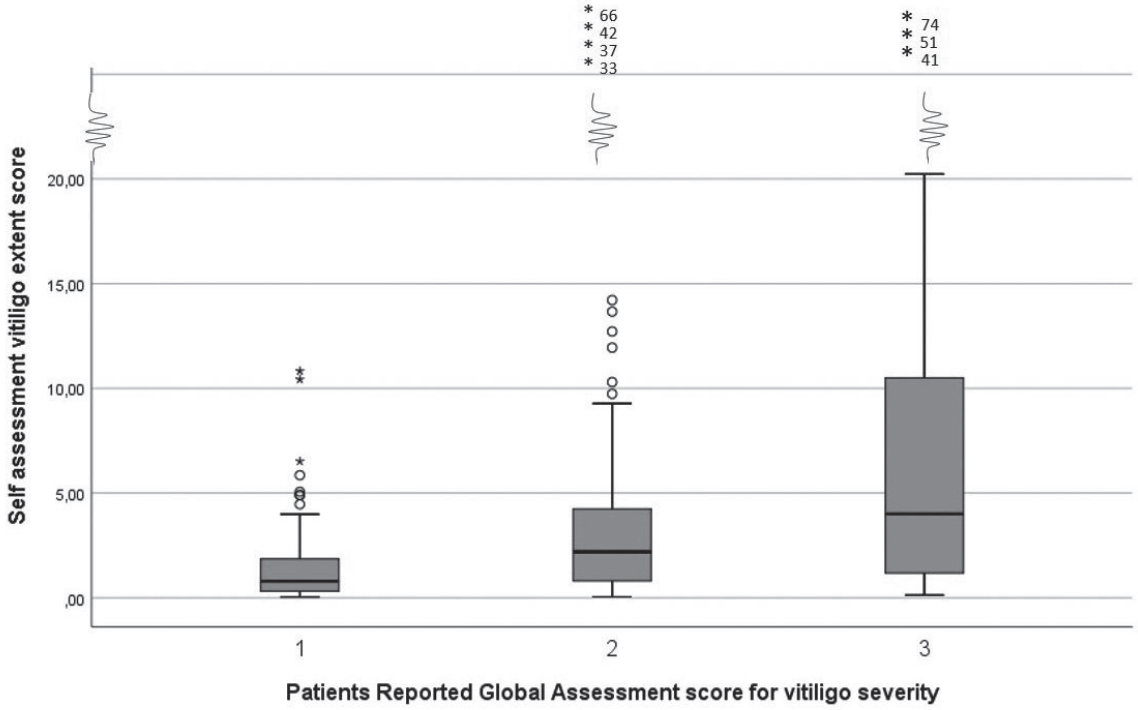


Figure 2b Box plots for SAVES representing 3 categories (mild– moderate– severe) based on PtGA severity.

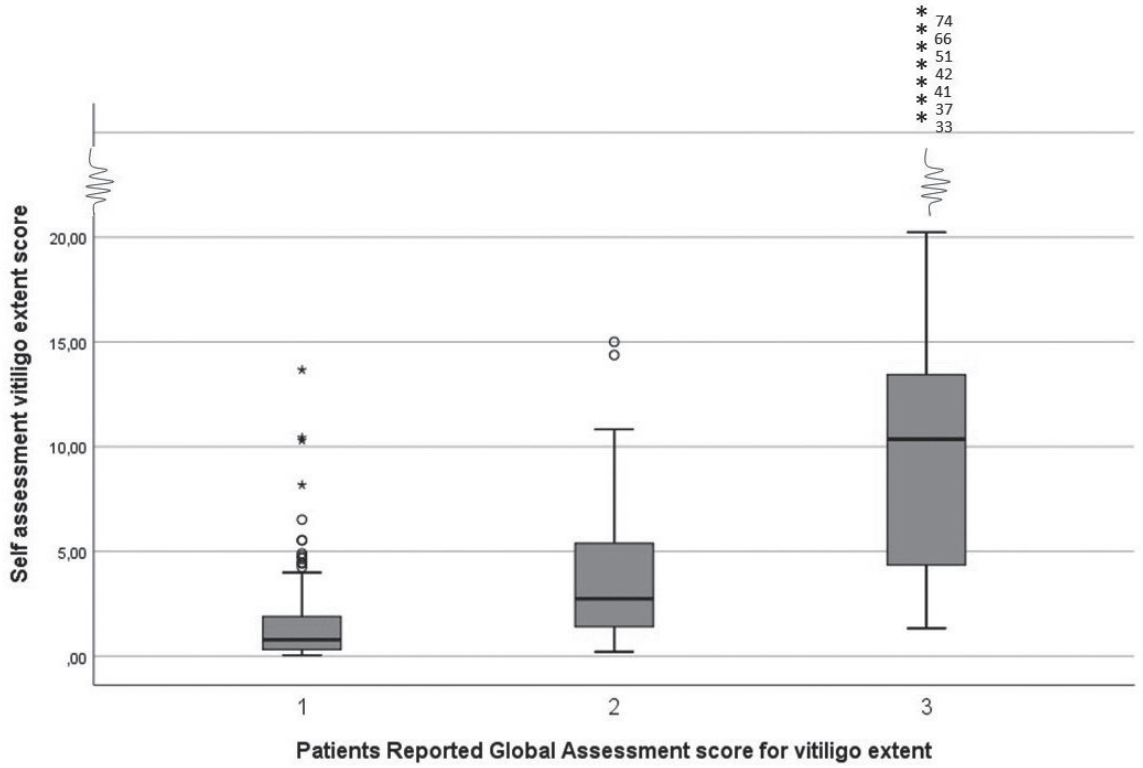
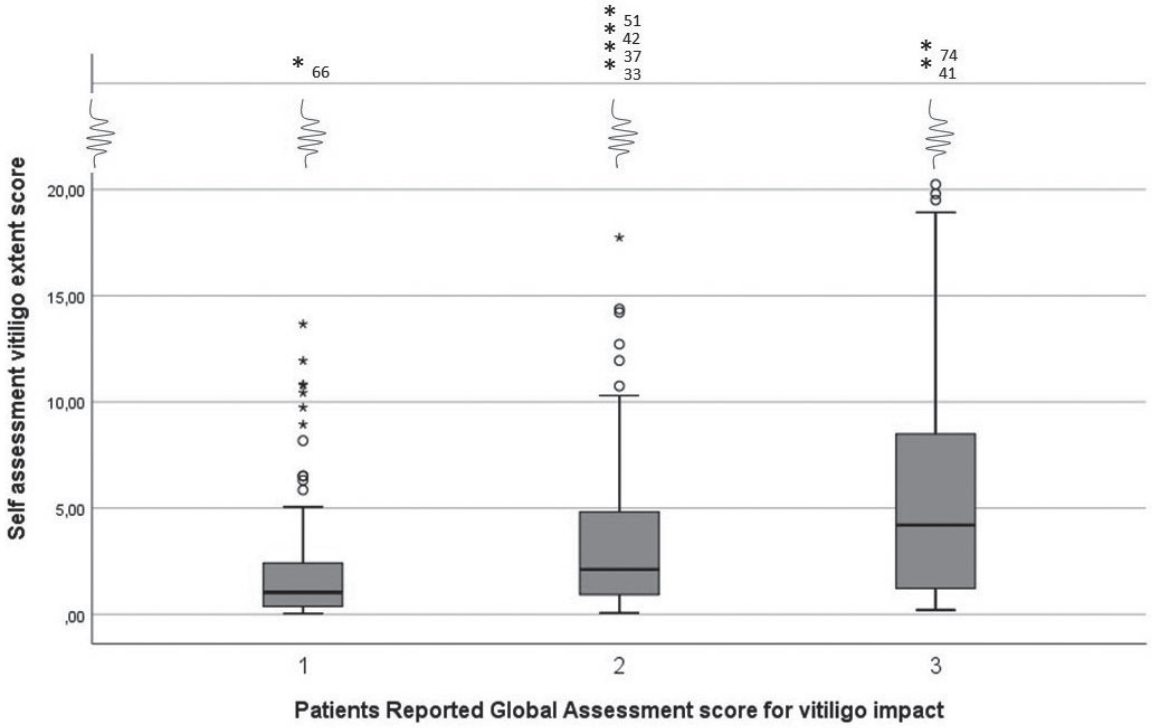


Figure 2c Box plots for SAVES representing 3 categories (mild – moderate – severe) based on PtGA impact.



Discussion

In this study, we validated a PtGA for extent, severity and impact using a simple scoring system based on a global assessment question. The PtGA is an intuitive and simple measure that is often used in clinical trials. The PtGAs were used to stratify the numeric score obtained by the SA-VES. Stratification of outcome measures is crucial to interpreted the obtained scores and to perform research on homogenous study populations. Here, we confirm the intrarater reliability and construct validity of 3 PtGAs based on hypotheses testing. Using the validated PtGA-anchor questions the possible strata per category could be assessed for the SAVES score. Based on the cut off values, an affected body surface area (SAVES) of more than 1.05% and 6.45% was already considered as moderate extensive and extensive, respectively. The median SAVES score for moderate extensive and extensive were 2.740 and 9.370 respectively. Cut-off values for PtGA severity were different compared to PtGA extent, emphasizing the importance of separating these 2 aspects in future trials.

Severity assessment requires an additional dimension from patient's point of view including additional influencing factors such as location of the lesions or patients photo skin type especially for a pigmentary disorder like vitiligo. Insight into patients' experiences is crucial to properly define severity in a way that is both useful to the physician and reflective of the patient's status. A thorough investigation within a large vitiligo patient population may offer additional criteria to better define vitiligo severity.

Impact assessment involves a different dimension as it measures the individual experience of the influence of the disease on daily life. This may be a crucial factor in the therapeutic choice during a vitiligo consultation. Interestingly >1/3 patients rated impact and severity differently indicating that patients recognize that the more objective concept of severity may result in a different subjective impact from one patient to another. Stratification based on PtGA for impact indicated that even a low affected BSA (SAVES) of 3.35% can have a high to very high impact on the quality of life. Conversely, a very high SAVES can have a moderate impact. This is also clearly illustrated by the distribution of outliers of BSA>32 in the boxplots, in which most variation within the 3 categories is present for 'impact' (PtGA impact), followed by 'severity' (PtGA severity), while all highest outliers are included in the highest category for 'extent' (PtGA extent). This points again the importance to differentiate these 3 domains. An added value of the validated PtGA scores is that they may be used to stratify outcomes of other scores. In addition they can be helpful in the clinic or trials to provide information 'at a

glance'. However, for studies investigating the impact of the disease more in-depth, a detailed questionnaire is recommended [e.g. VIPs, DLQI, vitiligo-specific quality-of-life instrument (VitiQoL)] so ensure the required information.[7,12] To check the relevance of outliers, a sensitivity analysis was performed. Correlations were performed including and excluding the 7 outliers which provided similar results.

A strength of this study was that patients were involved in the construction of the questions used to assess the PtGA for extent, severity and extent. However, for future studies, a diversity (e.g. different ethnic backgrounds) in patients population participating in the construction of the PtGA questions should be pursued to ensure generalization of its use.

Another important limitation of this study was the single-center setting including most often Caucasian photo skin types with rather limited BSA involvement. This study should therefore be repeated within different centers including patients of darker skin types (IV-VI) and more variation in extent. Future studies are required to confirm other measurement properties such as responsiveness and cross cultural validity. Moreover, it could be interesting to compare the physicians' point of view with the patients' point of view related to the global assessments of extent and severity and related to the stratification into categories (by using VES) within the same population in future studies.

In conclusion, this study confirmed the content validity, construct validity and intrarater reliability of the PtGA for extent, severity and impact. These tools can be used for the interpretation and stratification of scores obtained with other Patient-Reported Outcome Measures (PROMs) and can guide treatment decisions in vitiligo management.

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Supplementary file 1

Hypotheses for construct validity

1. Validation severity 5-point scale

1. We expect a positive correlation of at least 0.4 between the score on the severity 5-point scale and the score on the extent 5-point scale over the entire population. (Extent)

2. We expect a positive correlation of at least 0.4 between the score on the severity 5-point scale and the score on the impact 5-point scale over the entire population. (Impact5 point scale)

3. We expect a positive correlation of at least 0.4 between the score on the severity 5-point scale and the score for the impact on 10 over the entire population. (Impact(scale: 0-10))

4. We expect a positive correlation of at least 0.4 between the score on the severity 5-point scale and the score on the DLQI over the entire population. (DLQI)

5. We expect a positive correlation of at least 0.4 between the score on the severity 5-point scale and the SA-VES score over the entire population. (SA-VES)

2. Validation extent-5-point scale

1. We expect a positive correlation of at least 0.4 between the score on the extent 5-point scale and the score on the severity 5-point scale over the entire population. (Severity)

2. We expect a positive correlation of at least 0.4 between the score on the extent 5-point scale and the score on the impact 5-point scale over the entire population. (ImpactLikert)

3. We expect a positive correlation of at least 0.4 between the score on the extent 5-point scale and the score for the impact o 10 for the entire population. (Impact(scale: 0-10))

4. We expect a positive correlation of at least 0.5 between the score on the extent 5-point scale and the SA-VES score over the entire population. (SA-VES)

3. Validation Impact 5-point scale

1. We expect a positive correlation of at least 0.4 between the score on the impact 5-point scale and the score on the severity 5-point scale over the entire population. (Severity5 point scale)

2. We expect a positive correlation of at least 0.4 between the score on the impact 5-point scale and the score on the DLQI over the entire population. (DLQI)

3. We expect a positive correlation of at least 0.5 between the score on the impact 5-point scale and the score for the impact on 10 over the entire population. (Impact(scale: 0-10))

4. We expect a positive correlation of at least 0.4 between the score on the impact 5-point scale and the SA-VES score over the entire population. (SA-VES)

4. Validation Impact scale: 0-10

1. We expect a positive correlation of at least 0.4 between the score for the impact on 10 and the score on the severity 5-point scale over the entire population. (Severity)

2. We expect a positive correlation of at least 0.4 between the score for the impact on 10 and the score on the DLQI for the entire population. (DLQI)

3. We expect a positive correlation of at least 0.5 between the score for the impact on 10 and the score on the impact 5-point scale over the entire population. (Impact 5-point scale)

4. We expect a positive correlation of at least 0.4 between the score on the impact on 10 and the SA-VES score over the entire population. (SA-VES)

Part III

Treatment

5

Patient Reported Outcomes for Intensified versus Conventional NB-UVB Treatment in Non-Segmental Vitiligo

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Abstract

Introduction

Treatment of non-segmental vitiligo (NSV) remains a challenge. Efficacy of NB-UVB treatment may increase with more frequent use or in combination with topical agents. Currently, data on the most effective treatment regimen lacking. Our objective is to retrospectively compare NB-UVB treatment regimens for non-segmental vitiligo.

Methods

Patients with NSV treated with NB-UVB therapy were included in two time periods. Group I received NB-UVB therapy twice a week (conventional treatment) and group II received NB-UVB thrice a week, combined with topical agents (intensified treatment). Patients completed a questionnaire regarding the degree and onset of repigmentation, satisfaction and side effects.

Results

Repigmentation scores did not differ significantly between the two groups. Onset of repigmentation in the first three months seemed higher in group II, but this difference was not significant (23.4% vs 51.1%; p=0.11). In both groups the majority of the patients were moderately to very satisfied (group I: 70.2% group II: 73.3%). The occurrence of adverse effects was comparable.

Conclusion

This study indicates that conventional and intensified treatment for NSV seem to be comparable. The intensified treatment might be more effective to speed up the onset of repigmentation, but larger prospective studies are needed to objectify these findings.

Introduction

Non-segmental vitiligo (NSV) is an acquired chronic pigment disorder of the skin characterized by the development of patchy leukoderma, with a significant negative effect on the quality of life.[1–3] NSV is the most common depigmenting disorder affecting approximately 1% of the world's population, regardless of gender, ethnicity or skin type.[4] Current treatments include topical agents, phototherapy and surgical techniques which aim to improve melanocyte proliferation and stimulate repigmentation.[5] Topical therapy with corticosteroids or calcineurin inhibitors are suggested as first-line treatment.[6] The use of narrow band ultraviolet B (NB-UVB) phototherapy was first reported in 1997 by Westerhof et al.[7] and is now considered to be a growing modality for the treatment of generalized NSV.[5] NB-UVB is superior to psoralen and UVA (PUVA, another commonly used phototherapy regimen in vitiligo) due to its increased efficacy and decreased adverse effects.[8,9]

Currently, no consensus exists as to what the optimal treatment regimen and duration is of NB-UVB phototherapy, and practice varies widely.[10] At the Academic Medical Center (AMC), conventional treatment for NSV was NB-UVB alone twice a week, but this has changed in an intensified schedule with NB-UVB thrice a week in combination with topical treatment with fluticasone propionate and/or tacrolimus. This change was implemented in 2011 due to recent studies showing that the combination of topical therapy and NB-UVB is superior to monotherapy.[11,12] However, data on the most effective treatment frequency are lacking. Until now, the NB-UVB treatment regimens have never been compared directly. For the use of NB-UVB expert recommendations have been developed based on current literature by the Vitiligo Working Group Phototherapy Committee.[13] However, little evidence is available to guide clinicians on what is the most effective duration, dosage and treatment frequency of NB-UVB. Two studies have investigated the difference in effectiveness between twice and thrice weekly treatment regimens for vitiligo with the 308-nm excimer laser.[14,15] Both studies demonstrate that the final repigmentation does not depend on the treatment frequency but on the total number of treatments. However, the onset of repigmentation does occur faster with the three times weekly treatment. This could be the same for NB-UVB phototherapy but studies investigating this hypothesis are lacking.

For future improvement of vitiligo therapy it is important to assess, compare and evaluate the current treatment regimens, in order to determine what is most effective and satisfactory for the patient. Therefore, the aim of this study is to explore whether a combination of NB-UVB three times weekly and topical agents

is superior to NB-UVB alone twice a week for the treatment of non-segmental vitiligo from patients' perspectives.

Materials and methods

Patients

This retrospective cohort study was performed at the Netherlands Institute for Pigment Disorders in Amsterdam. Inclusion criteria were: (i) diagnosis of non-segmental vitiligo; (ii) age 16 or older; (iii) received total body NB-UVB therapy for at least six months.

Patients were included in two different time periods: Group I finished their NB-UVB therapy between March 2008 and January 2009. Group II finished their therapy between February 2017 and October 2017. Questionnaires were sent to the patients within four months after the end of the NB-UVB therapy. In between these time periods the standard of treatment had changed (see treatment). Inclusion criteria were patients aged 16 years or older, diagnosed with NSV who received total body NB-UVB therapy at home prescribed for one year.

Patients were sent the questionnaire with a consent form and a return envelope. If a patient did not respond, he or she was reminded by phone with a maximum of three follow-up calls. In case a patient was not reachable by phone a second questionnaire was sent by post. This study was not subject to the WMO (Medical Research Involving Human Subjects Act) and approval was granted by the Medical Ethical Committee of the AMC.

Treatment

In both groups NB-UVB treatment was given at home. The equipment comprised of semi-circular Waldmann UV-100 units with TL-01 tubes. These tubes contain ultraviolet B lamps that irradiate light with a wavelength of 311 nanometer. Standardized cumulative doses were calculated by using the intensity measurements together with the treatment charts of patients. Nursing staff cared for guidance and explanation of the NB-UVB equipment and treatment.

In group I irradiation took place twice a week on non-consecutive days. The patients were not advised to use an additional topical treatment except emollients. In group II irradiation was given thrice a week on non-consecutive days with topical therapy on the non-irradiation days, consisting of a calcineurin inhibitor (tacrolimus or pimecrolimus) for lesions on the face and skinfolds and a corticosteroid such as

fluticasone propionate 0.005% for lesions on the rest of the body. Patients were advised to increase the intensity by 50 mJ/cm2 if no suberythema of the skin occurred directly after the NB-UVB therapy. When suberythema occurred, an equal dose was recommended for the next irradiation and when painful erythema or blisters were observed patients were instructed to omit the next treatment.

Questionnaire

A questionnaire comprising 21 questions was constructed (Supplementary Appendix I). Questions included patient characteristics, disease duration, The Vitiligo Disease Activity (VIDA) score for disease activity and previous treatments. Patients were requested to note the repigmentation percentage per treatment area (face, neck, trunk, arms, hands, legs and feet) and to state the onset of repigmentation. Additionally, patients were asked to rank their level of satisfaction regarding the result of the therapy and the appearance of the repigmentation. Seven questions were included about the NB-UVB regimen such as average times of irradiation per week and total amount of months of the treatment. Additionally, patients were asked whether they had used topical therapy and if so, which topical agent was used and the average application of the cream per week. Finally, questions were asked about adverse effects; temporary occurrence of suberythema, painful erythema and a burnt skin.

Statistical analysis

All statistical analyses were conducted using SPSS software version 24.0 for Windows (Armonk, NY, USA). Mean scores between both groups were compared using the T-test and Mann-Whitney-U test for normally and not-normally distributed numerical variables, respectively.

For categorical variables Chi-square tests were performed to analyze differences between the groups. The Spearman's rank correlation test was used to define the association between the onset of repigmentation and patient satisfaction within the groups. Statistical tests were two-sided and the statistical level of significance was set at $P<0.05$.

Results

Patient and treatment characteristics

A total of 109 eligible patients with non-segmental vitiligo aged 16 years or older were sent the questionnaire. The response rate was 47 (87%) in group I and 45 (82%) in group II. The patient characteristics are presented in Table 1. The two

groups were comparable regarding age, duration of disease, previous treatments and skin type. The Vitiligo Disease Activity (VIDA) scores showed no significant differences in disease activity between the two groups (p=0.46).

Table 1. Patient Characteristics.

	Group I (conventional) N=47	**Group II (intensified) N=45**
Male : female ratio	1 : 1.8	1 : 1.1
Mean age ± SD (min-max)	43 ± 13 (17-68)	46 ± 14 (21 – 74)
Disease duration > 5 years	66%	56%
Skin type		
I	2%	4%
II	55%	64%
III	34%	11%
IV	4%	7%
V	4%	13%
VI	0%	0%

SD: standard deviation

Treatment characteristics and results are shown in Table 2. Patients in group II received significantly more irradiation per week. Of this group 96% had used additional topical agents during the irradiation period: 88% applied tacrolimus 0.1% for the face with a mean number of 3.3 times per week and 79% applied fluticasone propionate 0.005% with a mean application of 3.2 times per week. Other creams such as Pimecrolimus and Betamethasone were used by 9% of the patients.

Table 2. Characteristics and results of treatment.

	Group I (conventional)	**Group II (intensified)**	**P-value**
Mean number of treatments per week	2.3	3.0	<0.001*
Time investment per week in minutes median – [IQR]	20 – [10-40]	25 – [12-40]	0.72
Missed – more than 10 treatments	13%	27%	0.38
Suberythema – more than 20 times	40%	42%	0.27
Painful erythema occurrence – more than 10 times	8.5%	4.4%	0.79
Blistering	0%	4.4%	0.14

*significant

The majority of patients in both groups were treated more than nine months with the NB-UVB therapy (group I 68%, group II 83%). The median cumulative doses in group I and II were 62.3 (N=47) and 63.8 J/cm2 (N=22), respectively. In the intensified group half of the cumulative doses (23 patients) were unknown due to incomplete treatment charts of the patients.

Repigmentation and satisfaction

The median repigmentation within the two groups differed widely for different body sites. The median repigmentation for the face and neck were reported slightly higher in group II, this difference was not significant. In both groups, patients reported that acral areas tended to repigment less than the face and neck (Table 3). The onset of repigmentation in the first three months seems higher in group II, but this difference is not significant (Group I 23.4%, group II 51.1%; p=0.11) (Fig. 1). The start of repigmentation in both groups was predominantly reported in the first six months (66% of the group I and 80% group II).

Table 3. Median repigmentation (%) at different body sites at the end of therapy.

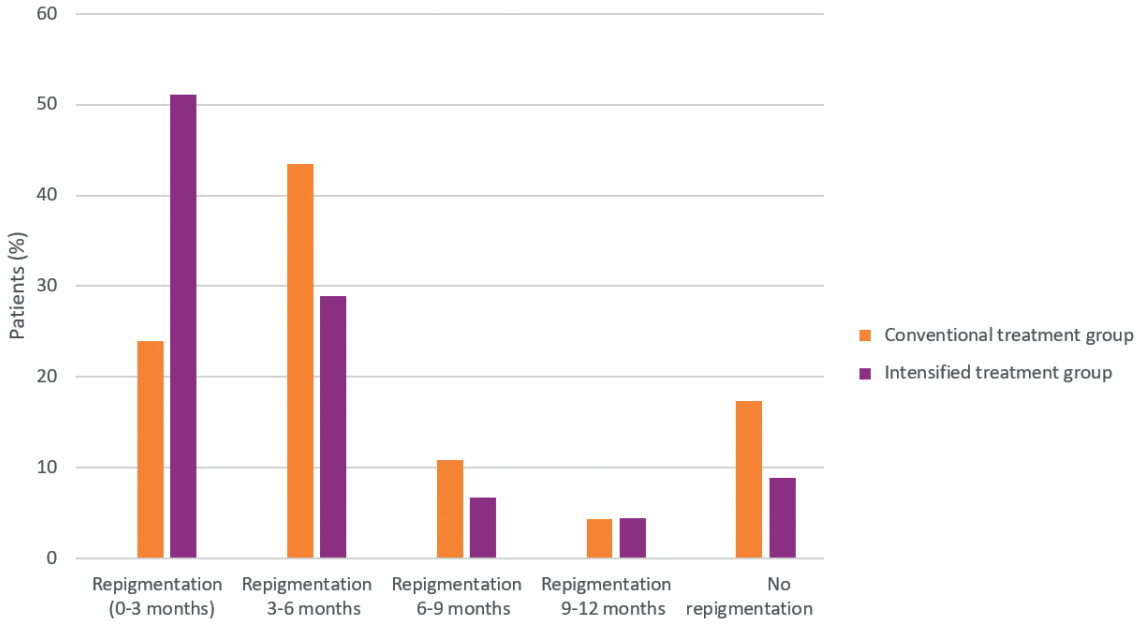
	Group 1 (conventional) Median [IQR]	N	Group II (intensified) Median [IQR]	N	P-value
Face	60 [6 - 80]	28	75 [11 - 90]	40	0.20
Neck	40 [30 - 70]	19	50 [9 - 80]	25	0.79
Trunk	30 [10 - 55]	30	20 [6 - 50]	33	0.50
Arms	40 [10 - 60]	29	25 [6 - 60]	31	0.49
Hands	10 [0 - 30]	31	3 [0 - 30]	32	0.37
Legs	35 [6 - 58]	24	40 [6 - 70]	33	0.78
Feet	0 [0 - 15]	17	0 [0 - 5]	25	0.60

IQR: interquartile range

Additionally, a significant correlation between the onset of repigmentation and satisfaction of the result was found in group II (intensified); Spearman's rho 0.44, p=0.003. In group I (conventional) this correlation was not significant (Spearman's rho 0.28; p=0.064). The majority of the patients were moderately to very satisfied in both groups (conventional group 70.2% versus intensified group 73.3%, p=0.91) (Fig. 2).

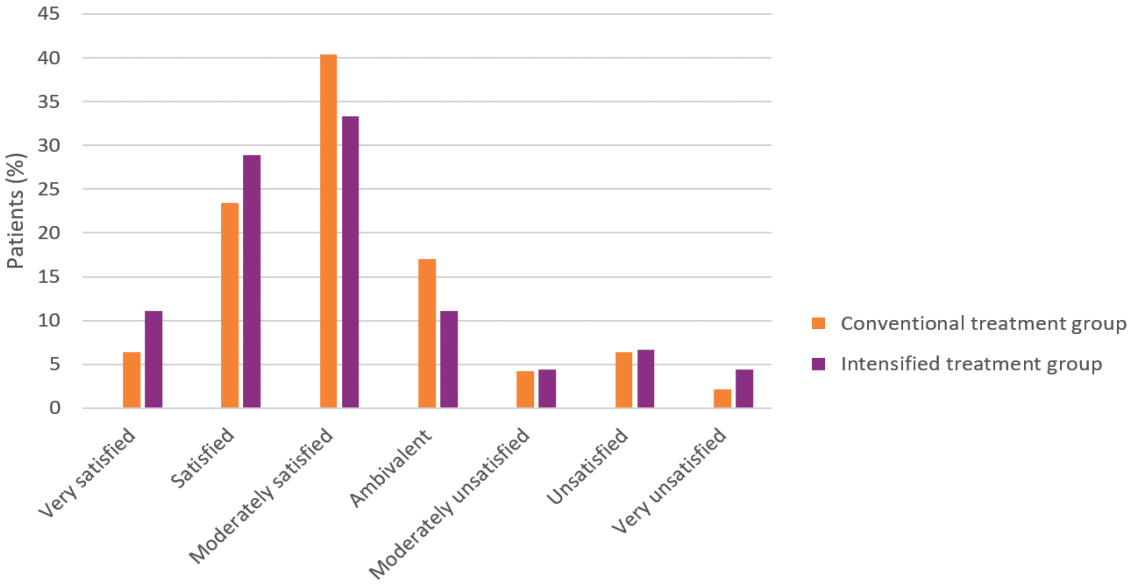
Furthermore, most of the patients would recommend their treatment to others (98% of the conventional group, 89% of the intensified group; p=0.08).

Figure 1. The onset of repigmentation per patient group.



No significant differences were found between groups (Chi square test, p=0.11).

Figure 2. Satisfaction with result after one year NB-UVB therapy per patient group.



No significant differences were found between groups (Chi square test, p=0.91).

Discussion

This questionnaire study examined patient reported outcomes regarding repigmentation and satisfaction in non-segmental vitiligo patients after completing NB-UVB therapy. According to this retrospective study, the intensified treatment

regimen with NB-UVB thrice a week in combination with topical agents does not seem to be superior to the conventional treatment regimen with NB-UVB twice a week.

Our NB-UVB protocol changed from twice to thrice a week (in combination with topical therapy), however no reports are available demonstrating the superiority of one above the other. Previous studies indicate that repigmentation is dependent on the total number of light treatments.[14,15] Earlier onset of repigmentation has been seen in the thrice weekly regimen, which seems to be comparable to our study. Similar results have been observed in a study comparing twice and thrice weekly NB-UVB therapy for psoriatic patients.[16] Furthermore, systematic review studies comparing combination therapy (NB-UVB and topical calcineurin inhibitors) with monotherapy (NB-UBV) showed improved repigmentation for the combination therapy, especially in the face and neck.[17,18] However, we cannot confirm this in our study.

To our knowledge, our retrospective study is the first to compare different treatment regimens with each other from patients' perspectives. A strength of our study was that it concerns real life data from clinical practice. Another strength is that it had equal and comparable treatment groups regarding baseline patient and treatment characteristics, which was essential to make proper comparisons between the two different treatment regimens. In addition, the surveys were only sent to patients that had recently completed their NB-UVB therapy, which kept the recall bias as limited as possible.

However, there are some limitations to this study that need to be addressed. Firstly the study was performed in two different time periods with a 10 year interval, patients expectations might have changed over time. Since the prognosis and treatment regimens for vitiligo have not changed much in this period we expect this difference to be small. Furthermore this study was based on daily practice, additional therapy (such as topical agents and skin transplantation) during NB-UVB therapy were allowed in the conventional group. This could have had an influence on the outcomes and makes a proper comparison between our treatment regimens more difficult. Moreover, not all cumulative doses were known of the patients in the intensified group due to incomplete treatment charts. The observation that the final repigmentation per body site was comparable for both groups may be due to the similar cumulative doses in both groups. However this cannot be objectified due to the fact that not all cumulative doses could be determined. Another important limitation is the lack of validated outcome measures. However, it is remarkable that the percentage of repigmentation in the various body locations

is compatible with the expected response pattern in both groups. Finally, disease extent and comorbidity were not assessed, although these factors might influence the patients' perspective on the therapeutic outcomes.

The design of our study was constructed in such a way that two and three times weekly NB-UVB therapy could not directly be compared with each other; we also added the topical treatment to our standard of care. A direct comparison between twice weekly and thrice weekly NB-UVB in vitiligo patients is still needed to determine what the optimal frequency is. It is possible that the additional topical treatment is responsible for the earlier repigmentation in the intensified group. Thus, prospective studies are required, since it is difficult to critically investigate these regimens in retrospective study designs due to the possible variations in dosimetry, irradiation time and other outcome measures. A twice-weekly regimen might be more convenient regarding time and effort for patients. However, this could be accompanied by psychological distress experienced by patients due to slow responses, leading to reduced therapy compliance.[13]

In conclusion, this study shows that the twice-weekly and thrice-weekly regimen in combination with topical agents seem to be comparable regarding patient reported repigmentation, satisfaction and occurrence of short term adverse effects. Though, patients seem to notice an earlier onset of repigmentation in the intensified group, this difference is not significant. Finally, the start of repigmentation in the intensified group significantly correlates with the satisfaction after completing the NB-UVB therapy. The intensified treatment might be more effective to speed up the onset of repigmentation, but costs and burden should be taken into account. Larger prospective studies are needed to objectify these findings.

Acknowledgements

We would like to express our gratitude to the volunteering patients who participated in this study.

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Supplementary Appendix I – The questionnaire (in Dutch)

Vragenlijst voor patiënten met vitiligo die een UVB-kuur hebben gehad

Hieronder vindt u een aantal vragen over uw vitiligo en de behandeling met UVB.
Wilt u bij elke vraag het hokje aankruisen dat het meest op u van toepassing is?
Het is belangrijk dat u alle vragen beantwoordt en geen vragen overslaat.
Het invullen van deze vragenlijst kost ongeveer 5 minuten.

Voorletter(s) + achternaam: .....
Geboortedatum :...../...../.....
Geslacht : M / V

Voorgeschiedenis

1) Hoe lang heeft u vitiligo?

- ☐ Minder dan 1 jaar
- ☐ 1-5 jaar
- ☐ Langer dan 5 jaar

2) Heeft u voor deze laatste lichtbehandeling al eens eerder een behandeling voor vitiligo gehad? Zo ja, welke?
(Omcirkel uw antwoord, meerdere antwoorden mogelijk)

UVB-lichttherapie		ja	nee	weet niet
Zo ja,	Thuis	ja	nee	
	Ziekenhuis	ja	nee	
UVA (zonnebank)		ja	nee	
PUVA lichttherapie		ja	nee	weet niet
Hormoonzalf/crème		ja	nee	weet niet
Huidtransplantatie		ja	nee	weet niet
Anders,	namelijk:	.....		

3) Hoeveel jaar heeft u, de laatste UVB-kuur meegerekend, in totaal UVB-lichttherapie gehad?

- ☐ Minder dan 1 jaar
- ☐ 1 jaar
- ☐ 2 jaar
- ☐ 3 jaar
- ☐ Meer dan 3 jaar
- ☐ Anders:.....

4) Hoe gedroeg de vitiligo zich voordat u begon met de lichtbehandeling?

- ☐ Het breidde zich uit de laatste 6 weken voor start lichtbehandeling
- ☐ Het breidde zich uit de laatste 3 maanden voor lichtbehandeling
- ☐ Het breidde zich uit de laatste 6 maanden voor lichtbehandeling
- ☐ Het breidde zich uit de laatste 12 maanden voor lichtbehandeling
- ☐ Geen uitbreiding laatste 12 maanden voor lichtbehandeling
- ☐ Geen uitbreiding laatste 12 maanden en mijn huidskleur kwam spontaan terug in de witte plekken
- ☐ Ik kan het me niet meer herinneren

Vragen over uw laatste UVB-kuur

Wilt u bij elke vraag het hokje aankruisen dat het meest op u van toepassing is?

5) Waar vond de laatste lichtbehandeling plaats?

- ☐ Thuis
- ☐ SNIP

6) Hoe vaak belichtte u gemiddeld per week?

- ☐ 1x per week
- ☐ 2x per week
- ☐ 3x per week
- ☐ 4x per week
- ☐ 5x per week

7) Hoe lang heeft u belicht?

- ☐ Minder dan 3 maanden
- ☐ 3 tot 9 maanden
- ☐ 9-12 maanden
- ☐ Meer dan 12 maanden

8) Hoe vaak heeft u door omstandigheden een behandeling moeten missen?

- ☐ Nooit
- ☐ 1-5 keer
- ☐ 5-10 keer
- ☐ Meer dan 10 keer

9) Na hoeveel maanden merkte u dat de bruine kleur in de witte plekken terugkwam?

- ☐ Ongeveer 0-3 maanden
- ☐ Ongeveer 3-6 maanden
- ☐ Ongeveer 6-9 maanden
- ☐ Ongeveer 9-12 maanden
- ☐ Niet

10) Hoe vaak merkte u dat direct na de belichting de plekken tijdelijk (korter dan 12 uur) roze werden?

- ☐ Minder dan 10 keer
- ☐ 10-20 keer
- ☐ 20-40 keer
- ☐ Meer dan 40 keer

11) Bent u verbrand met pijnlijke roodheid tijdens de laatste periode lichtbehandeling? (Indien 'ja', geef aan hoe vaak)

- ☐ nee
- ☐ ja:
- ☐ ☐ minder dan 5 keer
- ☐ ☐ 5-10 keer
- ☐ ☐ meer dan 10 keer

12) Bent u verbrand tot blaren toe tijdens de laatste UVB-kuur?
(Indien 'ja', geef aan hoe vaak)

- ☐ Nee
- ☐ Ja

- ☐ ☐ minder dan 5 keer
- ☐ ☐ 5-10 keer
- ☐ ☐ meer dan 10 keer

13) Als de plekken direct na de vorige belichting roze (minder dan 12 uur) werden, ...
- ☐ Sloeg ik hierna de volgende belichting over
- ☐ Ging ik bij de volgende belichting een stap terug
- ☐ Bleef ik gelijk bij de volgende belichting
- ☐ Ging ik bij de volgende belichting een stap hoger
- ☐ Niet van toepassing: de plekken werden nooit roze

14) Als de plekken na de vorige belichting rood en pijnlijk waren, ...
- ☐ Sloeg ik hierna de volgende belichting over
- ☐ Ging ik bij de volgende belichting een stap terug
- ☐ Bleef ik gelijk bij de volgende belichting
- ☐ Ging ik bij de volgende belichting een stap hoger
- ☐ Niet van toepassing: de plekken werden nooit rood en pijnlijk

Vragen over gebruik van crèmes tijdens UVB-kuur

15) Heeft u gedurende de periode van de UVB-kuur ook crèmes gesmeerd voor de behandeling van vitiligo:
(Indien 'ja', geef aan welke (meerdere opties mogelijk) en hoe vaak)
- ☐ Nee
- ☐ Ja
  - ☐ Protopic
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden
  - ☐ Cutivate
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden
  - ☐ Overig, namelijk: .....
    - ☐ Gemiddeld 4 maal per week
    - ☐ Gemiddeld 3 maal per week
    - ☐ Gemiddeld 2 maal per week
    - ☐ Gemiddeld 1 maal per week
    - ☐ Zelden

Resultaten

16) Hoe tevreden bent u over het uiterlijk van de gerepigmenteerde huid na de behandeling?
- ☐ zeer tevreden
- ☐ tevreden
- ☐ tamelijk tevreden
- ☐ noch tevreden - noch ontevreden

- ☐ tamelijk ontevreden
- ☐ ontevreden
- ☐ zeer ontevreden

17) Bent u voldoende voorgelicht over de lichtbehandeling en het belichtingsschema?

- ☐ ja
- ☐ nee

Indien 'nee'; wat hebt u gemist?

……

18) Hoe tevreden bent u over het resultaat van de behandeling?

- ☐ zeer tevreden
- ☐ tevreden
- ☐ tamelijk tevreden
- ☐ noch tevreden - noch ontevreden
- ☐ tamelijk ontevreden
- ☐ ontevreden
- ☐ zeer ontevreden

19) Hoe reageerde uw vitiligo op de laatste UVB kuur?
U kunt hier aangeven op welke plekken uw vitiligo aanwezig was en hoeveel bruine kleur in elke witte plek is teruggekomen (meerdere antwoorden mogelijk)

	Plaats	% verbetering
☐	Gelaat	…….%
☐	Hals	…….%
☐	Romp	…….%
☐	Armen	…….%
☐	Handen	…….%
☐	Benen	…….%
☐	Voeten	…….%
	_____	
☐	Totaal	…….%

20) Zou u anderen de behandeling aanraden?

- ☐ Ja
- ☐ Nee

Waarom (niet)?

……

22) Hoeveel tijd was u in totaal per week kwijt met lichtbehandeling?

…… minuten
…… uren

Overige opmerkingen die u kwijt wil:

……

6a

Addition of cell suspension transplantation to standard of care in non-segmental vitiligo: a randomized controlled study

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Submitted

Abstract

Background
Autologous cell suspension transplantation (CST) is an effective surgical treatment method in segmental vitiligo and piebaldism. However, the efficacy in non-segmental vitiligo is not yet sufficiently validated in randomized controlled trials. Stability of vitiligo for 12 to 24 months is generally regarded essential for this therapy, which does not occur in all patients.

Objectives
The objective of this study was to assess the efficacy and safety of CST in patients with non-segmental vitiligo receiving concurrent standard of care immunosuppressive therapy (UVB + topical anti-inflammatory).

Methods
A prospective, randomized, observer blinded, within-patient controlled trial was performed in patients with non-segmental vitiligo receiving standard of care (311 nm UVB therapy and topical anti-inflammatory therapy) for at least 6 months. To indicate disease stability, a punch grafting test was performed and evaluated after 3 months. Subsequently, two comparable depigmented regions per patient were randomized to receive CST or no grafting, while standard of care was continued. Cellular composition of the graft was analysed by flow cytometry. Outcomes were assessed after 3 and 6 months.

Results
The median repigmentation in 17 patients of treated and control areas were 17.3% and 9% after 3 months and 5.3% and 8.3% after six months (p=0.11), respectively. Repigmentation failure could not be attributed to graft composition. Twelve patients had progressive disease before starting UVB and topical therapy. Two patients showed repigmentation of more than 75%. The repigmentation of the treated lesion was scored as good to very good by three of the patients versus one patient for the control lesion. The study was discontinued due to ineffectiveness in the evaluated patient population.

Conclusions
Our findings indicate that non-segmental vitiligo lesions that are unresponsive to prolonged standard of care will generally not benefit by adding CST and stability of vitiligo without therapy might be necessary for successful CST.

Introduction

Vitiligo is an acquired cutaneous condition associated with white patches caused by destruction of melanocytes, and it has a significant negative effect on the quality of life.[1,2] Non-segmental vitiligo is the most common subtype and is usually slowly progressive with periods of activity interchanged by stable periods.[1] In stable depigmented lesions unresponsive to local immune suppressive treatment or phototherapy alone, autologous melanocyte transplantation is the treatment option of choice.[1]

Autologous cell suspension transplantation techniques offer the advantage of treating a larger acceptor site with a smaller donor site, compared to other transplantation techniques.[3] Long term follow-up studies on autologous cell suspension transplantation (CST), suggest that it is an effective and safe method in other depigmenting skin conditions.[4,5]

Non-segmental vitiligo is a challenging candidate for melanocyte transplantation as hypothetically transplanted melanocytes could be destroyed during flares of activity. In most studies only patients with no signs of activity for at least 12 to 24 months are included.[3,6,7] In order to induce stability, immune suppressive treatments may be given during and after melanocyte transplantation. Data from a previous systematic review suggests that phototherapy as an addition to melanocyte transplantation improves the repigmentation outcome.[8] The standard treatment of non-segmental vitiligo consists of topical treatment with immunomodulators which is often combined with UVB phototherapy. However, these interventions are not satisfactory in all patients as complete repigmentation is often not achieved. Therefore, the combined treatment of standard of care with CST could be a promising treatment regimen in non-segmental vitiligo patients. Adding melanocytes locally could boost the standard treatment to achieve optimal repigmentation.

The objective of this study was to assess the efficacy and safety of CST in patients with non-segmental vitiligo receiving concurrent standard of care immunosuppressive therapy (UVB + topical anti-inflammatory), irrespective of vitiligo activity before starting standard of care. Additionally, we assessed the general outcome reported by the patients and the cellular composition of the graft by flow cytometry.

Materials and methods

This prospective, randomized, observer blinded, within-patient controlled trial was conducted from December 2016 until May 2019 at the Netherlands Institute for Pigment Disorders (NIPD) in the Amsterdam University Medical Centre, was approved by its Medical Ethical Committee (NL 57683.018.16) and registered at clinicaltrials.gov (NCT03022019). We consecutively selected patients with non-segmental vitiligo, skin type II-VI, aged ≥18 years, with 2 comparable depigmented lesions of at least 10cm^2 each or one large lesion (>30cm^2) on the trunk, extremities or face. All patients had to be receiving standard of care involving topical corticosteroids (fluticasone propionate 0.05% cream for body) and/or topical calcineurin inhibitors (tacrolimus 0.1% ointment for face and body folds) and 311 nm narrow band ultraviolet B (NB-UVB) phototherapy for at least 6 months. Exclusion criteria were disease activity in the past 6 months during standard of care treatment, recurrent herpes simplex virus infections, hypertrophic scars, keloids, cardiac insufficiency, hypersensitivity to (UVB or UVA) light, allergy to local anaesthesia or clarithromycin, pregnancy, breast-feeding, a history of melanoma, non-melanoma skin cancer or atypical nevi.

Punch grafting test

At inclusion, patients first underwent a punch grafting test to evaluate stability in the treatment regions. Four 1.5 mm pigmented punch grafts were taken from the hip area and placed in two selected treatment regions. Subsequently, standard of care was continued and after 3 months pigmentation of the punch grafts was assessed. The patients proceeded to randomization only if their punch graft showed perigraft repigmentation.

Randomization

Randomisation was performed using digital randomization software (Graph Pad Software Inc., La Jolla, CA, U.S.A.). Sealed opaque envelopes indicating the treatment and control lesion were opened after demarcation of the selected lesions. The independent observer responsible for the measurements and the global assessment was blinded to treatment allocation. The patient and the treating physician were not blinded as this was practically impossible.

Cell suspension transplantation and flow cytometry

We performed non-cultured autologous CST using a cell harvesting device

(ReNovaCell; Avita Medical, London, U.K.). All of the steps in harvesting skin, processing the cell suspension and preparing the acceptor region were performed

as described in two previously published studies from our centre.[9,10] Before transplantation lesions were pre-treated with full surface CO2 laser ablation (Ultrapulse, Lumenis ActiveFx hand piece, Santa Clara, CA); with 150 mJ and a single pass (estimated depth 209µm), with settings 60W, density 3. A small amount of the suspension was used for analysis of the graft composition. The number of cells was microscopically assessed using a counting chamber within one hour after extraction. Cells were fluorescently labelled for flow cytometry analysis. Melanocytes were identified using mouse anti-human Melan-A (clone A103, Dako), polyclonal biotinylated goat anti-mouse IgG antibody antibody (both Dako, Agilent Technologies Netherlands B.V., Amstelveen, the Netherlands) and APC-conjugated Streptavidin (Biolegend, San Diego, CA, US). Fibroblasts were identified using biotinylated goat anti-human Collagen-I (Southern Biotec, Birmingham, AL, US) and APC-conjugated Streptavidin (Biolegend). Keratinocytes were identified using PE-conjugated mouse anti-pan Cytokeratin (clone C11, Abcam, Cambridge, UK). Finally, total leukocytes were identified using APC-Cy7-conjugated mouse anti-human CD45 (clone 2D1, BD Biosciences, San Jose, CA, US) and myeloid cells, including skin dendritic cells using mouse anti-human CD11c (clone B-ly6, BD Biosciences). Additionally, to determine live and dead cells, cells were stained using the LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (ThermoFisher Scientific, Naarden, the Netherlands). Samples were acquired on a FACS Canto II A (BD Biosciences, San Jose, CA, USA) and data was analysed using FlowJo software (BD Biosciences).

Three months after the intervention, NB-UVB was discontinued (patients had then received 12 months of phototherapy in total), topical treatment remained continued.

Outcomes

Our primary outcome was repigmentation at three and six months after CST. This was calculated with the use of transparent sheets in combination with digital image analysis (Image J software).[11]

Secondary outcomes were colour match (hyperpigmentation or hypopigmentation), side effects (e.g. erythema and scarring) at donor and treatment sites assessed on a categorical scale (absent, mild, moderate or severe), patient assessment of general outcome (measured as: bad, average, good, very good) and the cosmetic acceptability per treatment area measured on a 5-point Likert scale (very dissatisfied to very satisfied). Furthermore, the following cellular characteristics of cell suspension were analysed and correlated to repigmentation rate: total cell number, melanocytes, keratinocytes, fibroblasts, leukocytes, myeloid cells, including skin dendritic cell numbers and viability.

Sample size
We expected to find a mean repigmentation after 6 months in lesions treated with standard of care alone of 33.36% with a standard deviation of 32.56%, based on a previous study on NB-UVB therapy in non-segmental vitiligo patients.[29] We used Mann-Whiney U test in nQuery Advisor 7.0 software to calculate the sample size. With test significance level set at α=0.05 and a power of 80%, 18 patients were needed. Taking possible dropouts into account we aimed to randomize 20 patients.

Statistical analysis
Assuming a non-normal distribution in paired observations, Wilcoxon signed-ranks tests were used to compare the repigmentation rate of the different treatment regions. Spearman's rho correlation tests were performed to assess the correlation between cell counts and repigmentation rate.

Results

A total of 23 patients were included for punch graft testing. Five patients were not randomized: three months after punch grafting, three patients had depigmentation of the grafts or no perigraft repigmentation, and the other two patients were satisfied with the amount of repigmentation after 9 months standard of care and chose not to undergo the CST procedure. In total 17 patients were randomized, with a mean (median) age of 39 (40) years, of which 9 females, 11 patients had selected lesions on the extremities and 6 on the trunk. Basic characteristics of all randomized patients are presented in Table 1. The Vitiligo Disease Activity Score (VIDA) at inclusion was +1 (active in past year, but stable for at least 6 months) for 12 patients and 0 (stable for at least one year, no spontaneous repigmentation) for 5 patients. No patients were lost to follow-up.

Twelve patients had progressive disease before starting UVB and topical therapy. The median (IQR) repigmentation for the treated and control area were 17.3% (2.7%-37.8%) and 9% (1.9%-22.3%) after 3 months; this difference was not significant (p=0.09, paired analysis, Fig. 1A). The median (IQR) repigmentation for the treated and control area after 6 months were 5.3% (1.5%-33.6%) and 8.3% (1.7%-14.2%) after six months; this difference was also not significant (p=0.11, paired analysis, Fig. 1B). Two patients (11.8%) showed repigmentation of more than 75% of the treated area. The repigmentation of the treated lesion was scored as good to very good by 3 (17.7%) patients versus 1 (5.9%) patient for the control lesion. Three (17.7%) patients were satisfied to very satisfied with the cosmetic acceptability of the treated area.

Table 1 basic characteristics at inclusion and repigmentation rates

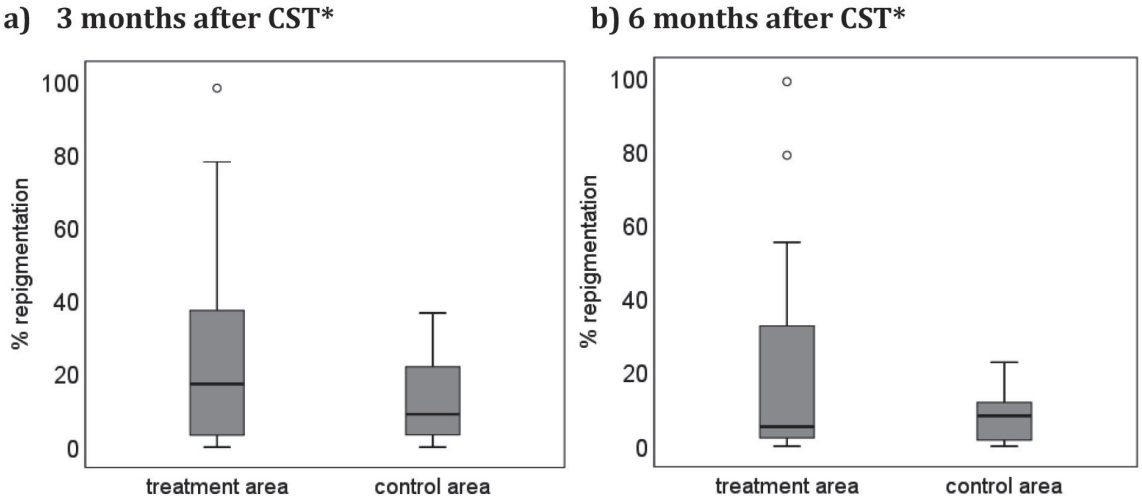
Age	Gender	Fitzpatrick skin type	Location	VIDA score*	Repigmentation treatment area**	Repigmentation control area***
29	male	3	hip	1	99%	20%
41	male	2	abdomen	1	79%	0%
47	female	2	leg	1	55%	10%
43	male	3	legs	0	34%	23%
40	male	2	wrists	1	33%	8%
47	male	2	wrists	0	27%	16%
46	female	2	back	1	15%	5%
41	female	5	hands	1	12%	11%
39	female	2	hands	1	5%	2%
33	female	2	hip	0	5%	2%
35	male	2	wrists	1	5%	12%
51	male	5	wrists	1	3%	10%
26	female	2	wrists	0	2%	4%
25	male	2	abdomen	1	1%	2%
29	female	2	legs	1	1%	5%
43	female	3	wrists	0	0%	0%
40	female	2	hip	1	0%	18%

* VIDA Score (measured at inclusion, after 6 months of standard of care treatment):
0: stable for at least one year, no spontaneous repigmentation
+1: active in past year, but stable for at least 6 months
** Repigmentation of treatment area 6 months after cell suspension transplantation
*** Repigmentation of control area 6 months after cell suspension transplantation

The repigmentation rate after six months, absolute numbers of total cells, melanocytes, total cells, their viability and proportion in the cell suspension per patient are shown in Figs 2-3. The number of transplanted melanocytes or viable melanocytes per mm^2 did not correlate significantly with repigmentation, nor did the absolute number and the viable number of total cells, keratinocytes, fibroblasts, leukocytes or myeloid cells, including skin dendritic cells.

No adverse events related to the study or long-term side effects occurred. After an interim assessment of the results of the first 12 patients by three independent experts not affiliated with our centre, the study was discontinued due to futility. Patients that were already treated at the time of discontinuation (patient 13-17) were followed to completion and included in all assessments according to protocol.

Figure 1 Repigmentation of treatment (CST*) and control area



*CST, cell suspension transplantation.

Figure 2 Overall graft composition and repigmentation

Rho: Spearman's rho correlation coefficient. Significant as indicated by the P value.

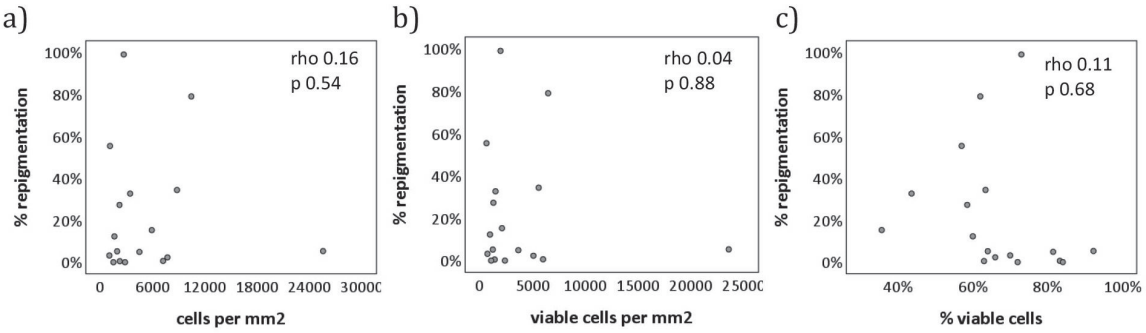


Figure 3 Graft composition specific cell types and repigmentation

Rho: Spearman's rho correlation coefficient. Significant as indicated by the P value.

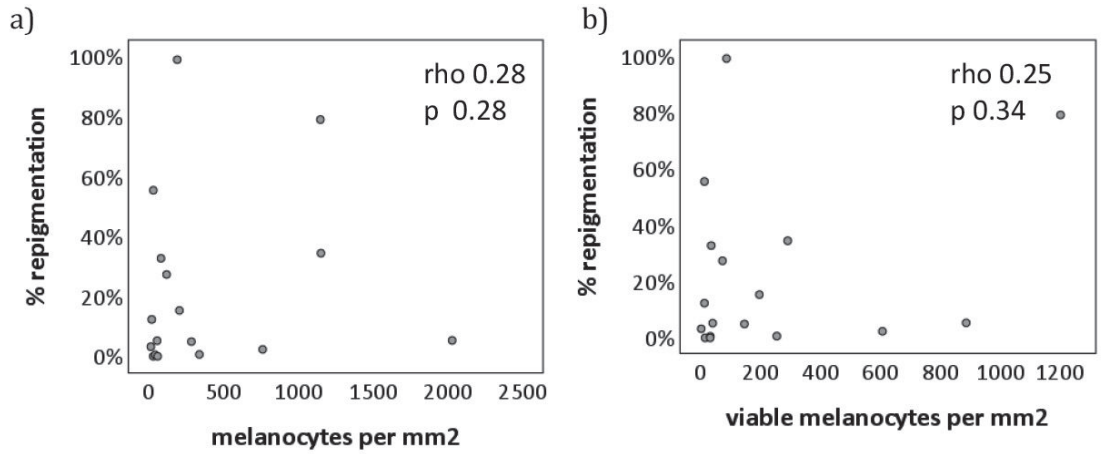
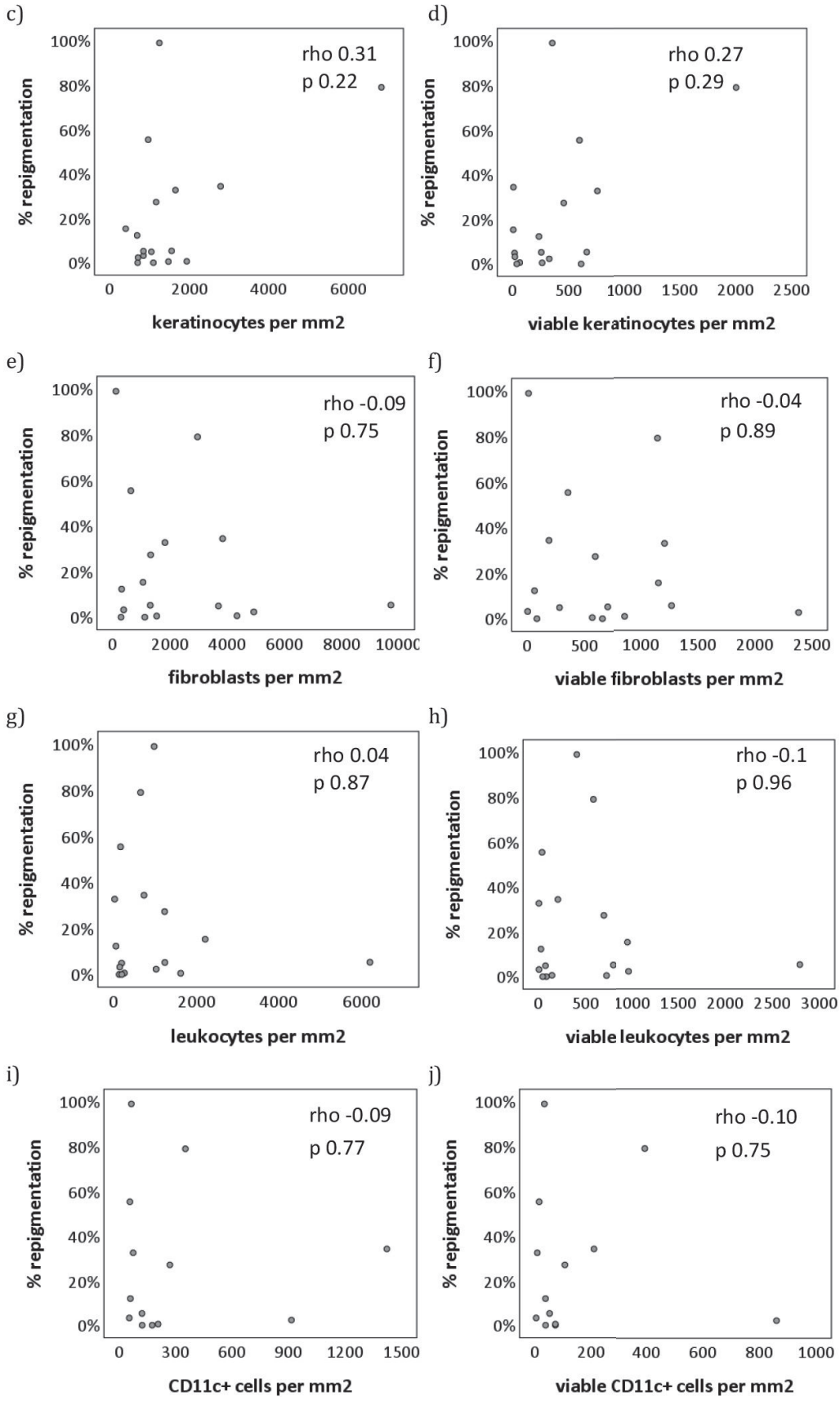


Figure 3 Graft composition specific cell types and repigmentation (continued)

Rho: Spearman's rho correlation coefficient. Significant as indicated by the P value.



Discussion

In this study, no significant benefit was found for the addition of CST treatment in patients with non-segmental vitiligo who had lesions unresponsive to standard of care (UVB, topical corticosteroids). Only two patients showed successful repigmentation (>75% repigmentation).

Our results are contradictory to a number of publications in this field, which show successful repigmentation varying from 52% to 89%.[12–14] In these studies only patients with stable vitiligo were included. These studies defined disease stability as follows: Verma et al: no increase in the size of the lesions for 1 year, Kachhawa et al.: no expansion of existing lesions and no appearance of new lesions in the past year, Budania et al.: no spreading or Koebnerization for at least 1 year. In contrast, we included patients during standard of care, mostly having an active vitiligo before the start of NB-UVB combined with topical therapy. CST was performed 9 months after the start of NB-UVB therapy and we expected to have induced stability due to immunosuppressive treatment, allowing the transplanted cells to successfully induce repigmentation. This generally did not occur, with only two patients achieving successful repigmentation of the treatment area. Stability induced by standard of care (UVB phototherapy and topical therapy) may be misleading: our findings suggest that stability of vitiligo without therapy is essential for successful CST and that standard of care treatment does not contribute enough to enable successful repigmentation upon CST. In line with our findings, one study on punch grafting observed that a group of patients only showed repigmentation during UVB therapy, after the UVB therapy, the punch grafts depigmented.[15] We agree with their recommendation to select only those patients for CST who have a stable vitiligo for 1 to 2 years without therapy, because NB-UVB therapy given before transplantation may lead to incorrect patient selection.[15]

Another reason for poor results may be due to the design of the study. We selected lesions that were still depigmented after 9 months of standard of care and therefore mostly unresponsive to treatment. These lesions might have an intrinsically low potential of repigmentation to any kind of treatment, including melanocyte transplantation. Yet, the guidelines for vitiligo mention that melanocyte transplantation should be considered in patients unresponsive to conventional treatment.[1]

The locations of the treated lesions could also have influenced our results. Many of our patients were treated on the wrists (n=6) or hands (n=2). The hands are known to respond inferior to transplantation compared to other locations.[16,17] Separate results

for wrists are often not mentioned in studies, but other joints like elbows and knees are also known to be difficult to repigment upon transplantation.[16,17] Lastly, each individual patient and treatment can differ; maybe some patient or treatment characteristics that we are currently unaware of could also have influenced the outcomes.

We used punch grafting to assess stability and predict outcomes of CST. In other studies punch grafting has been proposed as an indicator to determine disease stability and predict successful repigmentation after surgical therapies.[18,19] In contrast to their findings our results suggest that punch grafting is not a good indicator to assess possible efficacy of CST in patients with stability recently induced by standard of care therapy. A possible explanation could be that with laser preparation of the acceptor site, the vitiligo was activated due to koebnerization. Another possible explanation could be the lack of tissue architecture of skin cell suspensions during transplantation. Melanocytes might benefit from intercellular contact with other epidermal cells for cell survival and growth.[22] We recommend to use the punch graft test only to predict results for tissue grafts but not for cellular grafting techniques.

We did not find a significant relationship between cell suspension cell/melanocyte density or viability and repigmentation. Characterization of the cellular suspension indicated viable cells and melanocytes in all patient samples, thus, the quality of the suspension does not explain the poor repigmentation rates.

A limitation of this study could be the short-term follow-up. But as we found repigmentation to decrease between 3 and 6 months it is unlikely that a longer follow up would have contributed to better results. Another limitation is that some parts of the transplantation procedure (harvesting of the skin sample, separation of the epidermal cells and suspension application) are performed manually which might vary per procedure.

In this randomized controlled trial, no significant benefit was found for additional CST in patients with non-segmental vitiligo receiving concurrent standard of care immunosuppressive therapy (UVB + topical anti-inflammatory). We mainly included patients with initially progressive vitiligo and lesions unresponsive to 9 months of standard of care. Our findings indicate that non-segmental vitiligo lesions unresponsive to prolonged standard of care will generally not benefit by adding CST.

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We would like to express our gratitude to the volunteering patients who participated in this study.

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6b

Impact of graft cell density and viability on repigmentation upon non-cultured autologous cell suspension transplantation in vitiligo and piebaldism

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Abstract

Non-cultured autologous cell suspension transplantation (CST) is a treatment method for vitiligo. Our objective was to determine whether the amount of transplanted (viable) melanocytes and overall cell numbers correlates with repigmentation after CST.

Twenty patients with segmental vitiligo or piebaldism were treated with suspension grafts from split-skin grafts using a cell-harvesting device. Correlations between repigmentation six months after transplantation and melanocytes, viable melanocytes, or total transplanted cells numbers were determined.

Twenty patients had a median repigmentation of 78%. No significant correlations were found between the absolute number of (viable) melanocytes or (viable) total cells and repigmentation. Successful repigmentation was induced by both low and high numbers of melanocytes transplanted per mm^2. The proportion of viable cells during CST correlated significantly with repigmentation rate ($p=0.015$).

Our findings suggest that repigmentation is determined predominantly by the proportion of viable cells in the cell suspension, rather than melanocyte content or absolute cell number.

Dear editor, an upcoming surgical treatment method for segmental vitiligo and piebaldism is non-cultured autologous cell suspension transplantation (CST). Currently the criteria for successful transplantation using this methodology is not fully understood. Repigmentation might be determined by the number and/or viability of melanocytes or total cell numbers transplanted per square millimeter (mm^2). The aim of this study is to assess whether graft cell density, melanocyte or keratinocyte content and cell viability, correlates with repigmentation after CST. In addition, we assessed if a threshold level of (viable) melanocytes required for successful repigmentation after CST can be defined.

Table 1: Analyses of CST graft composition and correlation with repigmentation after 6 months

Variable	N	Median	IQR	Spearman's rho	*P* value
Melanocytes[a]	20	81	15–396	-0.075	0.753
Viable melanocytes[a]	19	76	11–202	0.084	0.733
Keratinocytes[a]	20	633	137-2720	-0.171	0.472
Viable keratinocytes[a]	19	25	6-63	-0.186	0.446
Total cells[a]	20	4479	1358–7761	0.134	0.573
Total viable cells[a]	19	2844	446–4268	0.305	0.204
Proportion total viable cells in suspension	19	56%	42%-63%	0.551	0.015*

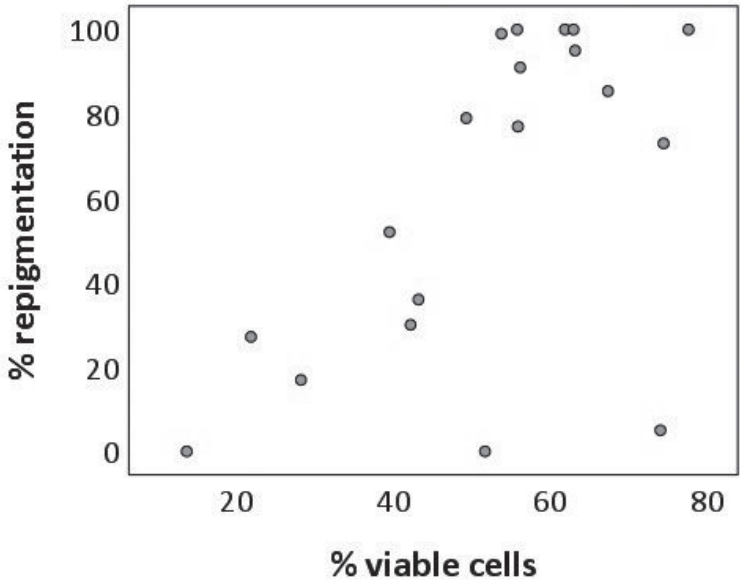
IQR: Interquartile range
[a] Absolute number per mm^2 treatment area
**Correlation is significant at the 0.05 level (2-tailed)*

Data was collected from twenty patients, comprising 12 piebaldism and 8 stable segmental vitiligo patients, mean age 32 years, 50% male, of two previously published, randomized, observer blinded, within-patient controlled trials in our centre.[1,2] Inclusion criteria and methods were performed as described in these two studies.[1,2] No patients were lost to follow-up. Median repigmentation, assessed six months after treatment was 78% (IQR: 27.9%-99.8%). The suspension was analyzed for the cellular graft composition. Total number was assessed using a counting chamber, melanocyte and keratinocyte numbers and viability were determined by flow cytometry using melanA-specific antibody (clone A103) (Dako, Agilent Technologies) or pancytokeratin antibody (Abcam), polyclonal biotin-labelled goat anti-mouse IgG antibody (Dako, Agilent Technologies) and stained (Live/DeadTM Fixable Violet Cell Stain Kit; Thermo Fisher Scientific, Invitrogen) for 405 nm excitation (Invitrogen). In one patient with piebaldism the Live/Dead cell analysis was not evaluable due to technical issues.

Figure 1: Scatterplot proportion total viable cells in the suspension and repigmentation

Y-axis: repigmentation in percentage after 6 months.

X-axis: Percentage viable cell in the cell suspension graft.

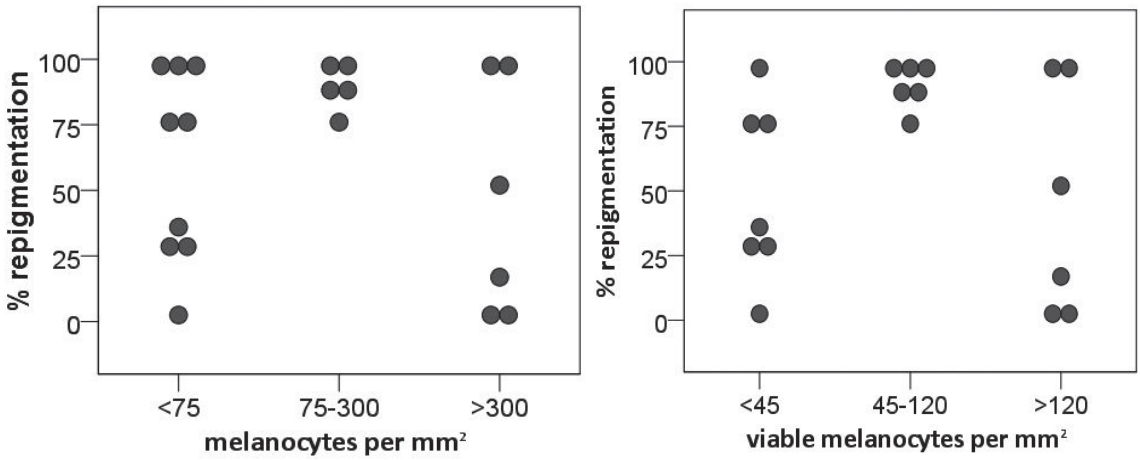


The median absolute numbers of melanocytes, keratinocytes, total cells, their viability and proportion in the graft are shown in table 1. We only found a significant correlation between the proportion of total viable cells in the cell suspension and repigmentation (Table 1 and Fig. 1, Spearman's rho 0.55, p=0.015).

Figure 2: Categories of melanocyte numbers and repigmentation

Y-axis: repigmentation in percentage after 6 months.

X-axis: Absolute number of melanocytes and viable melanocytes per mm² treatment area



To determine a potential threshold value for optimal repigmentation, melanocytes and viable melanocytes were divided into three categories with at least 5 patients, based on the number of melanocytes transplanted per mm^2 (figure 2). We found successful repigmentation (>75%) in all categories (figure 2), most frequently in the medium category.

Our results are in contrast with two previous studies, where the minimum number of melanocytes needed for visible repigmentation was estimated at 190 and 210-250mm^2.[3,4] The differences between theirs and our findings show that repigmentation might also be determined by other factors than the number of transplanted cells per mm^2, e.g., the quality of the cell suspension preparation and wound bed preparation. Moreover, patients' characteristics like vitiligo disease activity, age or skin type could play a role. Our results emphasize that further research is needed to find out which other factors could influence repigmentation after cell suspension transplantation.

A limitation of this study is the small sample size (N=20). To investigate a broader spectrum of potential factors as predictors of successful repigmentation after CST, will require larger patient groups.

Our findings suggest that the proportion of viable cells in the cell suspension is a more important factor than the absolute number of cells or melanocytes. Factors other than graft composition seem to be responsible for the clinical outcomes.

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We would like to express our gratitude to the volunteering patients who participated in this study.

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7

The role of phototherapy in the surgical treatment of vitiligo: a systematic review.

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Abstract

Vitiligo is frequently treated with the combination of phototherapy and melanocyte transplantation. However, the additional benefit of phototherapy is unclear. Moreover, the optimal type and regimen of phototherapy are unknown. The objective of this systematic review was to identify whether phototherapy improves the outcome of melanocyte transplantation in vitiligo. We searched and screened for eligible studies in the databases of MEDLINE, EMBASE and CENTRAL. We included all clinical studies investigating melanocyte transplantation combined with phototherapy. After screening and selection of abstracts and full-texts, we found 39 eligible clinical studies with 1624 patients. The eligible studies investigated several phototherapy modalities, such as NBUVB (n = 9), PUVA (n = 19), UVA (n = 1), MEL (n = 4) and active sunlight exposure (n = 9). Four studies directly compared phototherapy versus no phototherapy and two studies confirmed the benefit of phototherapy for melanocyte transplantation. We found no significant differences in repigmentation in studies directly comparing phototherapy modalities. The overall quality of the studies was moderate to poor and high heterogeneity between studies was found. We found limited evidence that phototherapy improves the outcome of melanocyte transplantation in vitiligo. There is insufficient evidence to recommend a specific type or regimen of phototherapy. More studies should be performed investigating the additional benefit of different phototherapies and the preferred moment of phototherapy.

Introduction

Vitiligo is a common depigmenting skin disorder with a worldwide prevalence of 0.5-1%.[1] Stable vitiligo lesions are treated with various surgical melanocyte transplantation techniques.[2] Melanocyte transplantation in stable vitiligo is frequently combined with subsequent phototherapy. Total repigmentation after melanocyte transplantation is uncommon and phototherapy can potentially have a beneficial effect on the outcome.[3] Phototherapy can enhance the repigmentation after melanocyte transplantation by its anti-inflammatory properties and by inducing melanocyte proliferation and migration. However, phototherapy also has side-effects such as increased risk of skin cancer and premature skin aging.[4] To date, there is no consensus on the role of phototherapy in the surgical treatment of vitiligo.[5]

The objective of our study was to identify whether phototherapy improves the outcome of melanocyte transplantation in vitiligo. Therefore, we designed a systematic review to summarize and review the evidence of the combination therapy of phototherapy and melanocyte transplantation.

Materials and methods

Search strategy and selection

We designed our systematic review with the use of the PRISMA Guideline and registered our study in the PROSPERO database under #53500.[6] Our search strategy comprised main keywords on vitiligo and melanocyte transplantation (Supplements S1). We performed a literature search for relevant studies from inception up to June 2016 of MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL). Subsequently, screening for other relevant articles in the reference lists of the identified studies was performed. We used Endnote (version X7.7.1) for reference management and duplicate finding. We conducted a first selection based on title and abstract. This was followed by screening for eligibility of the full text copies of selected studies. The selection procedure and the details for exclusion per study were systematically documented in a PRISMA flow diagram.[7]

Eligibility criteria

Studies were found eligible when they met the following pre-specified inclusion criteria: ≥10 vitiligo patients, intervention study with melanocyte transplantation and phototherapy, quantitative repigmentation as outcome measure and English

language. Phototherapy was defined as the administration of any type of light before or after melanocyte transplantation. Quantitative repigmentation included mean or median repigmentation, pigment spread and ordinal repigmentation measurement instruments. Furthermore, studies were only included when the results per phototherapy modality were provided separately. Eligible transplantation techniques included punchgrafting (PG), suction blister grafting (SBG), non-cultured autologous melanocyte transplantation (NCAMT), cultured autologous melanocyte transplantation (CAMT), split-thickness grafting (STG) and hair follicle transplantation (HFT).

Quality assessment

We assessed the quality of the clinical trials with the Risk of Bias assessment tool of the Cochrane Collaboration.[8] We evaluated per randomized controlled trial (RCT) or (non-randomized) clinical trial the risk of bias (low, unclear, high) of the random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and selective outcome reporting. The quality assessment of the eligible case series was performed with the Quality Appraisal Tool for Case Series. [9] This tool comprises 18 questions on quality appraisal and we added specific assessment criteria to 6 questions in order to adapt the checklist to our specific research question (Supplement S2).

Data extraction and analyses

We included from each study pre-specified data (author, year of publication, study design, patient characteristics, type of surgical treatment, follow-up duration, quantitative repigmentation measures and outcome). Furthermore, from each study we extracted per phototherapy modality the moment of initiation, frequency, duration, initial dose, dose adjustment and cumulative dose of phototherapy. Two assessors (JL and SU) performed the selection, quality assessment and data extraction independently. Disagreements were resolved by discussion with these two assessors and a third assessor (AW).

Results

Literature search

The search strategy generated a total of 1815 unique hits of which 39 articles met the inclusion criteria. The PRISMA flow diagram with the screening and selection procedure with reasons for exclusion is presented in Fig. 1. From these 39 studies, 7 studies compared one phototherapy with another or with no phototherapy as

an addition to the surgical treatment in vitiligo; these studies were marked as 'comparative'.[10-16] Furthermore, in the other 32 studies the results of only one phototherapy were presented; these studies were marked as 'non-comparative'.[17-48] In all studies, a total of 1624 patients with vitiligo were evaluated. The study characteristics of the eligible studies are presented in Table 1. In the eligible studies the following phototherapy modalities were studied: narrowband ultraviolet B (NBUVB, n=9), psoralen combined with ultraviolet A (PUVA, n=19), ultraviolet A (UVA, n=1), monochromatic excimer light or laser (MELi and MELa, n=4) and active sunlight exposure (n=9). The specific characteristics of the phototherapy per study can be found in the supplements.

Figure 1 Screening and selection procedure. This PRISMA flowdiagram shows the screening and selection procedure; n, number of studies

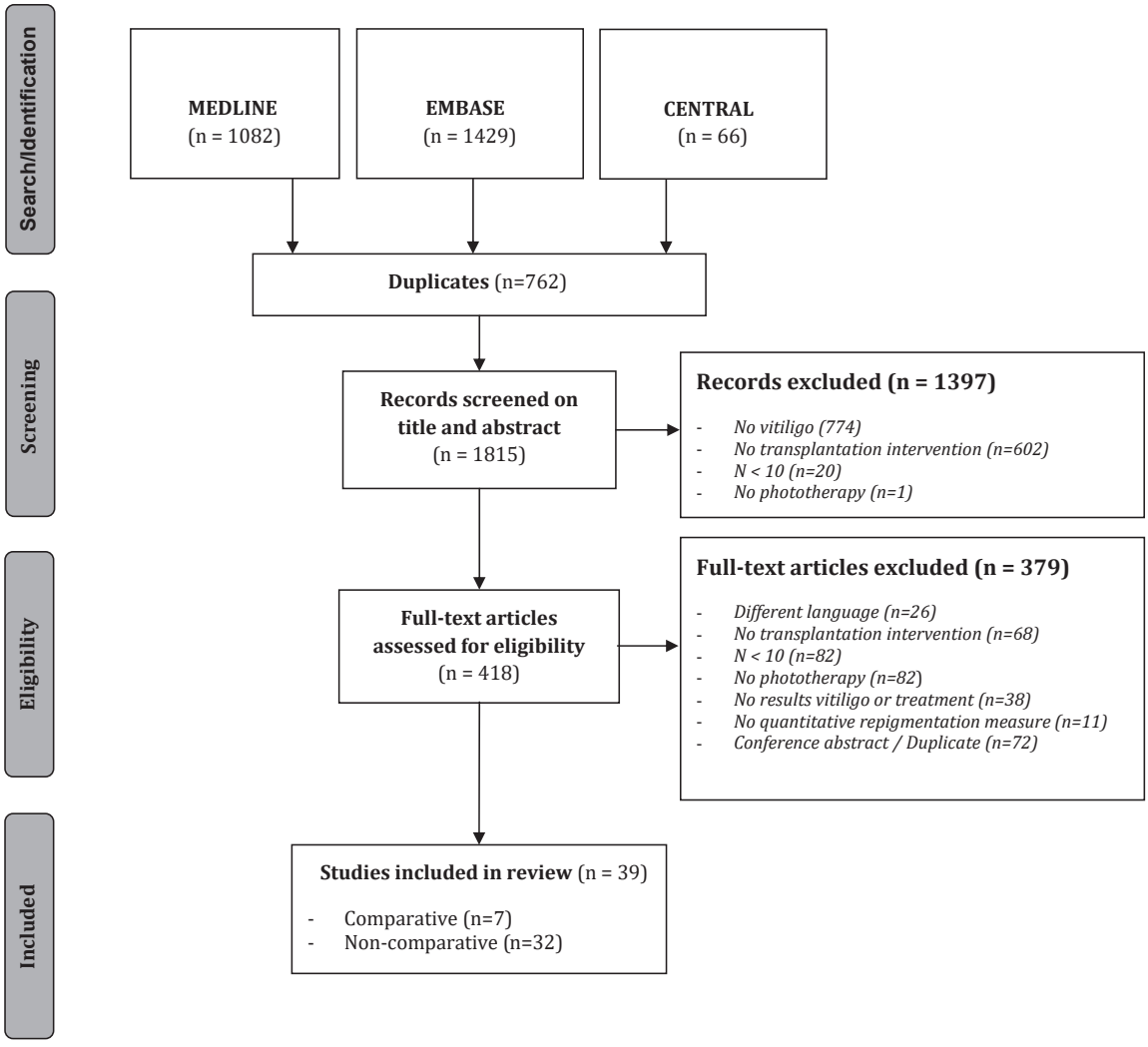

Table 1 – Study characteristics.

Author, year	N	Study design	Age in years (range)	Patient characteristics I. Vitiligo type (% of patients) II. Stability duration	Phototherapy	Surgical treatment
Comparative studies						
Barman, 2004	42	RCT	13-60	I. SV (59.5%);NSV (40.5%) II. ≥ 6 mo	sPUVA	PG
Ebadi, 2015	10	CT	21-48	I. NSV (100%) II. ≥ 1 yr	MELa	NCAMT
Lim, 1999	25	CS	14-64	I. NSV (52%), SV (32%), FV (16%) II. > 3 yr	PUVA	SBG
Linthorst Homan, 2012	14	RCT	18-69	I. NSV (100%) II. ≥ 6 mo	MELa NBUVB	PG
Sheth, 2012	10	RCT	21-74	I. Unknown II. ≥ 6mo	MELi NBUVB	PG
Tsuchiyama, 2016	13	CS	7-16	I. SV (100%) II. Unknown	MELa NBUVB	PG
Zhang, 2014	473	RCT	5-55	I. NSV (72%); SV (18%), uk (10%) II. ≥ 6mo	NBUVB	CAMT
Non-comparative studies						
Awad, 2008	20	CS	5-44	I. NSV (60%), SV (20%), halo nevi (20%) II. ≥ 3 months	K-UVA	SBG
Babu, 2008	18	RCT	11-65	I. NSV (5.6%), SV (50%), focal (44.4%) II. ≥ 1yr	PUVASOL	PG SBG
Boersma, 1995	19	CS	15-55	I. NSV (100%) II. ≥ 6 mo	UVA	PG
Budania, 2012	41	RCT	12-40	I. NSV (36.6%), SV (39%), FV (24.4%) II. ≥ 1yr	Sunlight	NCAMT SBG
Chen, 2000	25	CS	13-72	I. SV (100%) II. ≥ 1yr	Sunlight	CAMT
Czajkowski, 2007	40	RCT	14-59	I. Unknown II. ≥ 6 mo	sPUVA	SBG CAMT
De Leeuw, 2011	19	RCT	25-68	I. Unknown II. ≥ 1 yr	K-NBUVB	SBG

Table 1 – Study characteristics. (continued)

Author, year	N	Study design	Age in years (range)	Patient characteristics I. Vitiligo type (% of patients) II. Stability duration	Phototherapy	Surgical treatment
El-Zawahry, 2011	25	CS	8-45	I. NSV (84%), SV (8%), FV (8%) II. ≥ 1 yr	PUVA	NCAMT
Ghorpade, 2004	10	CS	18-65	I. Unknown III. ≥ 1 yr	PUVASOL	PG
Gupta, 2002	10	CS	11-19	I. NSV (70%), SV (20%), FV (10%) II. ≥ 1 yr	sPUVA	SBG
Hallaji, 2003	20	CS	13-40	I. NSV (40%), SV (20%), FV (40%) II. ≥ 6 mo	tPUVA	PG
Holla, 2014	31	CS	10-38	I. NSV (38.7%), SV (51.6%), FV (9.7%) II. unknown	Sunlight	NCAMT
Holla, 2013	36	CS	16-47	I. NSV (91.7%), FV (8.3%) II. ≥ 1 yr	Sunlight	NCAMT
Khandpur, 2005	64	RCT	10-42	I. NSV (50%), SV (26.6%), FV (23.4%) II. ≥ 6 mo	PUVASOL	PG STG
Krishnan, 2012	26	CS	15-28	I. Unknown II. ≥ 1 yr	PUVASOL	NCAMT
Lahiri, 2006	66	CS	21-48	I. NSV(39.4%), SV(27.3%), FV(21.2%), other(12.1%) II. ≥ 6 mo	NBUVB	PG
Majid, 2012	40	CS	uk	I. Unknown II. ≥ 1 yr	NBUVB	STG
Malakar, 2004	108	CS	uk	I. NSV (53.7%), other (46.3%) II. ≥ 6 mo	PUVASOL	PG
Mapar, 2014	25	RCT	20-47	I. NSV (100%) II. ≥ 1 yr	NBUVB	PG HFT
Matsuzaki, 2013	27	CS	9-48	I. NSV (25.9%), SV(74.1%) III. unknown	Sunlight	CAMT
Olsson, 1997	19	CS	12-62	I. NSV (94.7%), SV (5.3%) II. unknown	Sunlight	STG

Table 1 – Study characteristics. (continued)

Author, year	N	Study design	Age in years (range)	Patient characteristics I. Vitiligo type (% of patients) II. Stability duration	Phototherapy	Surgical treatment
Pai, 2002	29	CS	14-60	I. NSV (10.3%), localized (89.7%) II. Unknown	PUVA	PG SBG
Pianigiani, 2005	93	CS	14-62	I. NSV (19.4%), SV (28.0%), FV (52.7%) II. ≥ 2 yr	NBUVB	CAMT
Sachdev, 2000	13	CS	19-58	I. NSV (23.1%), FV (7.7%), u.k. (69.2%) II. Unknown	PUVA	PG
Sahni, 2011	13	CS	8-17	I. NSV (46.2%), SV (46.2%), FV (7.7%) II. ≥ 1 yr	Sunlight	NCAMT
Sahni, 2011	25	RCT	14-55	I. NSV (72%), SV (12%), FV (16%) II. Mean ± SD: 3.0 ± 3.5 and 3.3 ± 4.2	Sunlight	NCAMT
Sarkar, 2001	15	CS	16-42	I. SV (100%) II. Unknown	PUVASOL	PG
Singh, 1995	32	CS	11-60	I. NSV (6.3%), SV (37.5%), FV (56.3%) II. ≥ 1 yr	PUVASOL	PG
Singh, 1997	55	CS	10-30	I. SV, FV (% percentage unknown) II. Unknown	PUVASOL	PG
Suga, 1996	28	CS	12-60	I. NSV (28.6%), SV (35.7%), FV (35.7%) II. Unknown	tPUVA	SBG
Tegta, 2006	20	RCT	10-54	I. NSV (55%), SV (20%), FV (25%) II. ≥ 1 yr	Sunlight	NCAMT
Verma, 2014	25	RCT	u.k.	I. NSV (72%), SV (8%), FV (12%), other (8%) II. ≥ 1 yr	PUVASOL	NCAMT CAMT

N, number of patients; CS, case series; RCT, randomized controlled trial; CT, clinical trial; NSV, non-segmental vitiligo; SV, segmental vitiligo; FV, focal vitiligo; SBG, suction blister grafting; K-UVA, khelline application with ultraviolet-A phototherapy; PUVASOL, psoralen combined with sunlight exposure; sPUVA, systemic psoralen combined with ultraviolet-A phototherapy; UVA, ultraviolet-A phototherapy; K-NBUVB, khelline application with narrowband ultraviolet-B phototherapy; MELa, monochromatic excimer laser; PUVA, psoralen combined with ultraviolet-A phototherapy; tPUVA, topical psoralen combined with ultraviolet-A phototherapy; NBUVB, narrowband ultraviolet-B phototherapy; MELi, monochromatic excimer light; PG, punchgrafting; NCAMT, non-cultured autologous melanocyte transplantation; CAMT, cultured autologous melanocyte transplantation; STG, split-thickness grafting; HFT, hair follicle transplantation.

Quality assessment

The risk of bias assessment of the RCTs and quality assessment of the case series are summarized in Table 2. We used the risk of bias tool in 13 RCTs and 1 clinical trial. We identified 25 case series which we critically appraised with the Quality Assessment Checklist of case series (Supplement S3).

Table 2 - Risk of bias assessment of clinical trials.

Type of bias / Author, year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data addressed	Selective outcome reporting
Babu, 2008	Unclear	Unclear	High	Unclear	Low	Unclear
Barman, 2004	Unclear	Unclear	High	Unclear	Low	Unclear
Budania, 2012	Unclear	Unclear	High	Unclear	Low	Unclear
Czajkowski, 2007	Unclear	Unclear	High	Unclear	Unclear	High
De Leeuw, 2011	Unclear	Unclear	High	Low	Unclear	Unclear
Ebadi, 2015	High	High	High	Unclear	Low	High
Khandpur, 2005	Unclear	Unclear	High	Unclear	Low	High
Linthorst, 2012	Low	Unclear	High	Unclear	Low	Low
Mapar, 2014	Low	Unclear	High	Unclear	Low	Unclear
Sahni, 2011	Low	Unclear	High	Unclear	Unclear	Unclear
Sheth, 2012	Low	Unclear	High	Low	Low	Low
Tegta, 2006	Unclear	Unclear	High	Unclear	Low	High
Verma, 2014	Unclear	High	High	High	Low	Unclear
Zhang, 2014	Low	Unclear	High	Unclear	Unclear	Unclear

Comparative studies

These studies investigated the effect of different phototherapy regimens on the outcome of PG (n=4), SBG (n=1), CAMT (n=1) and NCAMT (n=1). The results of the comparative studies are presented in Table 3.

Comparison between phototherapy and no phototherapy

Ebadi et al. compared repigmentation in 39 lesions within 10 patients after NCAMT with or without MELa treatment. Median repigmentation was higher for the combination NCAMT and MELa than for NCAMT alone (41.9% vs. 15.9%, p=0.01). More than 65% repigmentation in lesions treated with NCAMT and MELa or NCAMT alone was reached in 40% and 11.1% of lesions, respectively.

Table 3 – Repigmentation results of comparative studies.

Author, year	FU	Outcomes	N	Intervention	Results
Barman, 2004	6 mo	Mean pigment spread	17	PG + sPUVA	6.38
			25	PG + topical CS	6.94
Ebadi, 2015	u.k.	I. Median % repigmentation II. 95-100% repigmentation III. 65-94% repigmentation IV. 25-64% repigmentation V. 0-24% repigmentation	9*	NCAMT	I. 15.9% II. 0% III. 11.1% IV. 33.3% V. 55.6%
			10*	NCAMT + MELa	I. 41.9% II. 20% III. 20% IV. 40% V. 20%
			10*	Only MELa	I. 4.7% II. 0% III. 0% IV. 10% V. 90%
			10*	No treatment	I. 0.1% II. 0% III. 0% IV. 0% V. 100%
Lim, 1999	2 mo	I. 100% repigmentation II. 0% repigmentation	65*	Pre-and post PUVA + SBG	I. 87.7%** II. 4.6%**
			26*	Pre PUVA + SBG	I. 53.8%** II. 11.5%**
			4*	Post PUVA + SBG	I. 50%** II. 50%**
			10*	SBG	I. 80%** II. 10%**
Linthorst Homan, 2012	4w	I. >75% repigmentation II. Mean repigmentation	14	PG + MELa	I. 14% II. 31%
				PG + NBUVB	I. 21% II. 38%
Sheth, 2012	12w	I. Median repigmentation II. Mean pigment spread *(times original size)*	10	PG + MELi	I. 9% II. 2 times
				PG + NBUVB	I. 13% II. 4.5 times
Tsuchiyama, 2016	6-32 mo	Mean repigmentation	11	PG + MELa	80.3%
			2	PG + NBUVB	89%

Table 3 – Repigmentation results of comparative studies. (continued)

Author, year	FU	Outcomes	N	Intervention	Results
Zhang, 2014	6 mo	I. Mean repigmentation II. > 90% repigmentation III. 50-89% repigmentation IV. 20-49% repigmentation V. 0-19% repigmentation	108	CAMT + *pre*NBUVB	I. 83.5% II. 68.5% III. 24.1% IV. 1.8% V. 5.6%
			113	CAMT + *post*NBUVB	I. 79.9% II. 61.9% III. 27.4% IV. 8.0% V. 2.7%
			116	CAMT + *pre*NBUVB + *post*NBUVB	I. 87.7% II. 81.3% III. 13.8% IV. 4.3% V. 0.9%
			110	CAMT + No PT	I. 68.1% II. 43.6% III. 29.1% IV. 19.1% V. 8.2%

*FU, follow-up duration after treatment; N, number of patients; mo, months; u.k., unknown; w, weeks; PG, punchgrafting; sPUVA, systemic psoralen combined with ultraviolet-A phototherapy; CS, corticosteroids; NCAMT, non-cultured autologous melanocyte transplantation; MELa, monochromatic excimer laser; PUVA, psoralen combined with ultraviolet-A phototherapy; SBG, suction blister grafting; NBUVB, narrowband ultraviolet-B phototherapy; MELi, monochromatic excimer light; CAMT, cultured autologous melanocyte transplantation; PT, phototherapy; * number of treated lesions or grafts; ** repigmentation per graft*

Barman et al. compared repigmentation in 22 patients treated with PG and systemic PUVA (sPUVA) versus 28 patients treated with PG and topical fluocinolone acetonide 0.1% cream once daily.[16] Six months post-grafting, no significant difference in pigmentspread was found between sPUVA and topical corticosteroids groups (6.38mm vs 6.94mm respectively, p>0.05).

In the case series of Lim et al., 105 SBG-grafts in 25 patients were treated with either pre-and post-PUVA (n=65 grafts), pre-PUVA (n=26 grafts), post-PUVA (n=4 grafts) or without phototherapy (n=10 grafts). In 50-87.7% of the grafts 100% repigmentation was seen. No differences in percentage of SBG-grafts treated with or without PUVA that reached 100% repigmentation were seen.

Zhang et al. assessed repigmentation after CAMT in 437 patients randomly allocated to four different study groups. Group 1 received NBUVB before CAMT, group 2 NBUVB after CAMT, group 3 NBUVB before and after CAMT, and group 4 received CAMT only.

Six months after transplantation the mean repigmentation was higher in the groups treated with CAMT and NBUVB (79.9-87.7%) than with CAMT only (68.1%). The differences between the four groups were statistically significant ($p < 0.001$).[10]

Comparison between different phototherapies

Linthorst Homan et al. compared repigmentation in 14 patients with non-segmental vitiligo after punchgrafting with subsequent MELa or NBUVB. In this intra-patient comparison, mean repigmentation 4 weeks post punchgrafting was 31% after MELa and 38% after NBUVB. Although >75% repigmentation was more frequently observed in the lesions treated with NBUVB (21%) than with MELa (14%), no significant differences were found ($p>0.05$).

Sheth et al. compared repigmentation in 10 patients treated with punchgrafting combined with either MELi or NBUVB in an intra-patient comparison. Median repigmentation of 13% in the NBUVB-lesions versus 9% in the MELi-lesions were found ($p>0.05$). The mean pigmentspread was higher ($p>0.05$) in the group with NBUVB (4.5 times original size) than in the MELi lesions (2 times original size).

In the case series of Tsuchiyama et al., 11 patients were treated with MELa after PG and 2 patients with NBUVB after PG. The mean repigmentation between the MELa and NBUVB post grafting was similar (80.3% vs. 89%, respectively).

In the study of Zhang et al., mean repigmentation was not significantly different between patients treated with NBUVB before (83.5%), NBUVB after (79.9%) or NBUVB before and after (87.7%) CAMT. More patients showed >90% repigmentation after grafting in the group treated with pre- and post NBUVB (81.3%) than in the groups treated with pre-NBUVB (68.5%) or post NBUVB (61.9%).

Non-comparative studies

We identified 23 studies investigating only one transplantation technique (PG=9, SBG=3, NCAMT=5, CAMT=3, STG=2) and 10 studies comparing different transplantation techniques within or between patients. In the supplements a table with the results per non-comparative study can be found (Supplement S5). No eligible non-comparative studies investigating MELi or MELa were found.

PUVA

We identified 17 studies assessing the repigmentation after melanocyte transplantation in combination with PUVA (systemic PUVA n=2, topical PUVA n=3, PUVASOL n=9, unknown type n=3). In 3 studies, >75% repigmentation was found in 44-69% of patients treated with PG, in 0-80% of patients treated with SBG, and

in 83% of patients treated with STG combined with PUVA.[38,41,43] In 5 studies, 100% repigmentation was found in 25-99% of patients treated with SBG, in 66%-100% of patients treated with PG, and in 50% of patients treated with CAMT combined with PUVA.[33,35,44,46,48] In 6 studies, >90% repigmentation was found in 23%, 39%, 65%, 72%, 80% and 100% of treated patients. [33,36,39,40,42,45] Mean pigment spread was assessed in 2 studies and ranged from 3.9-5.4 mm.[36,44]

NBUVB

We identified 5 studies which examined NBUVB. One study found a mean repigmentation of 65.3% after SBG combined with NBUVB and khelline application.[31] In 1 case study, 100% repigmentation was found in 60% of patients treated with CAMT and NBUVB. [27] In the case study of Majid et al., >90% repigmentation was found in 83% of patients treated with STG and subsequent NBUVB. Mean pigment spread was addressed in 2 studies and ranged from 5.0-6.5mm.[28,30]

UVA

We identified only one study that addressed the repigmentation after melanocyte transplantation and UVA.[26] In this case series, >80% repigmentation was found in 38.9% of all lesions treated with PG and UVA.

Sunlight exposure

In 9 studies the repigmentation after melanocyte transplantation and active sunlight exposure was assessed. Most studies (n=8) were performed in sunny areas (India n=6, Taiwan n=1, Japan n=1), whereas one study was performed in Sweden. Six studies found >75% repigmentation in 0-93% of patients and in 45-100% of lesions treated with NCAMT and in 85% of patients treated with SBG combined with sunlight exposure.[17-19,22,23,25] Olsson et al. found 100% repigmentation in 49% of lesions after STG followed by sunlight exposure. Five studies found >90% repigmentation in 29-79% of lesions and in 44-89% of patients treated with NCAMT, in 52% of patients treated with CAMT, and in 27% of patients treated with SBG combined all combined with sunlight exposure.[18,19,21,22,25]

Discussion

In this study, we reviewed 39 studies investigating 1624 patients with vitiligo. The results of our systematic review suggest that phototherapy improves the outcome of melanocyte transplantation in vitiligo.

We found 2 RCTs, 1 clinical trial and 1 case series comparing phototherapy (MEL, PUVA, UVB) with no phototherapy after melanocyte transplantation.[10,14-16] These studies confirm the benefit of phototherapy for melanocyte transplantation. Recent guidelines and a recent systematic review also have recommended combination therapy in the treatment of vitiligo and state that combination therapies are associated with more repigmentation than monotherapies.[3,5,49,50] Four studies directly compared phototherapy modalities. These studies found no significant differences in repigmentation between phototherapy modalities.

The eligible studies showed high heterogeneity in terms of outcome measures, transplantation techniques, stability of vitiligo, phototherapy regimen, quality and follow-up duration. Most studies (n=25) included different subtypes of vitiligo, such as non-segmental and segmental vitiligo, but in only 14 of 39 studies[13-15,18,21,24,26,28,33,36,37,42,43,45] the results per subtype were provided. Basically, melanocyte transplantation is more effective in patients without a persisting auto-immunity against melanocytes, such as in segmental vitiligo. Due to the high heterogeneity and unavailable data per subtype, we were not able to pool the data, compare the results between modalities and perform a sub-analysis per vitiligo subtype. We also found a high variation in measurement instruments, which further impedes the comparison between studies. Therefore, consensus on a core set of measurement instruments is essential. Only 13 studies reported the duration of the phototherapy (< 6 months = 10 studies, 6 months = 3 studies, unknown = 26 studies). The timing of the phototherapy was mainly post-grafting (n=37 studies), although 2 studies also showed a beneficial effect of NBUVB pre-grafting. Hypothetically, pre-treatment with phototherapy induces more immunosuppression on the acceptor site and melanocyte stimulation on the donor site, leading to more repigmentation after grafting. In the eligible studies, PUVA (n=19 studies) was more frequently used as phototherapy than NBUVB (n=9 studies). In 1997, there was a shift of paradigm in the phototherapy of vitiligo (i.e. from PUVA to NBUVB) as Westerhof et al. were the first to show that NBUVB was as effective as PUVA.[51] Only 3 out of 19 eligible studies investigating PUVA dated before this publication in 1997, although recent international guidelines recommend NBUVB as standard monotherapy in vitiligo due to the favorable safety profile.[5]

A limitation of our review is that we were not able to pool the results per phototherapy modality and/or transplantation technique due to the high heterogeneity between studies. Another limitation is that we included all techniques of melanocytes transplantation even though repigmentation is known to depend on the transplantation technique. We were not able to pool the results

per transplantation technique as the procedures of similar transplantation techniques were different between studies. Lastly, we included both prospective and retrospective clinical studies, which led to low quality of the evidence and unknown biases.

We recommend NBUVB as the standard phototherapy after melanocyte transplantation. The immunosuppressive effects and safety profile of NBUVB make this modality the most preferred phototherapy option, especially in non-segmental vitiligo. A recent systematic review and meta-analysis also shows that monotherapy with NBUVB is even as and in some studies more effective than PUVA in vitiligo.[52] The other available phototherapy modalities also have more potential limitations than NBUVB. PUVA for example has more phototoxic effects and a higher potential risk on skin cancer than NBUVB whereas MEL is only appropriate for small areas and only provides immunosuppression locally.[52] The frequency, duration, initial dose, dose adjustments, timing of phototherapy and cumulative dose of the phototherapies varied significantly between studies and no recommendation can be made on which treatment scheme should be used.

In conclusion, the results of our review suggest that phototherapy improves the outcome of melanocyte transplantation in vitiligo. We recommend NBUVB as standard phototherapy after melanocyte transplantation. More prospective randomized controlled studies are needed to investigate the additional benefit of the different phototherapy modalities and the preferred moment of phototherapy for non-segmental and segmental vitiligo separately.

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Supplements S1 - Search

Search strategy per database

MEDLINE
(vitiligo OR "Vitiligo"[Mesh]) AND (surgical OR surgery OR graft* OR transplantation OR transfer OR "Melanocytes/transplantation"[Mesh] OR "Keratinocytes/transplantation"[Mesh] OR "Skin Transplantation"[Mesh] OR "Transplantation, Autologous"[Mesh])

EMBASE:
(vitiligo) AND (surgical OR surgery OR graft* OR transplantation OR transfer)

CENTRAL:
(vitiligo) AND (surgical OR surgery OR graft* OR transplantation OR transfer)

Terms for search

Terms for vitiligo
Non-MESH: Vitiligo; Depigmentation; Hypopigmentation
MESH: Vitiligo (MESH); Vitiligo/surgery (MESH); Vitiligo/therapy (MESH)

Terms for melanocyte transplantation
Non-MESH: Surgery; Surgical treatment; Surgical therapy; Graft*; Transplantation; Skin transplantation; Cell suspension; Tissue transplantation; Tissue graft*; Cell* transplantation; Cell* graft*; Cultured melanocyte*; Non-cultured melanocyte*; Melanocyte* transplantation; Melanocyte-keratinocyte transplantation; Melanocyte-keratinocyte suspension; Epidermal cell transplantation; Blister roof transplantation; Epidermal blister graft*; Thiersch graft*; Epidermal cellular grafting; Melanocyte* transfer; Melanocyte* keratinocyte* transplantation; Cell suspension transplantation; Epidermal cell suspension transplantation; Keratinocyte-melanocyte graft*; Epidermal graft*; Autologous epidermal graft*; Epithelial graft*; Punchgraft*; Punch graft*; Minigraft*; Mini graft*; Minipunchgraft*; Mini-punchgraft*; Mini-punch graft*; Mini punch graft; Minipunch graft*; Suction blister graft*; Suction blister transplantation; Suction blister epidermal graft*; Suction epidermal graft*; Split-thickness graft*; Miniature punch graft*; Miniature skin punch graft*; Split-thickness skin graft*; Split-skin graft*
MESH: Melanocytes/transplantation (MESH); Keratinocytes/transplantation (MESH); Skin transplantation/methods (MESH); Transplantation, autologous (MESH)

Terms for phototherapy
Non-MESH: Phototherapy; PUVA; PUVASOL; PUVA-SOL; Excimer; UV; UVA; UVB; NB-UVB; NBUVB; Narrowband; Ultraviolet; Ultraviolet-A; Ultraviolet-B; Light; Sunlight; Sun; Helium-Neon

MESH: PUVA Therapy (MESH); Lasers, Excimer/therapeutic use (MESH); Melanocytes/radiation effects (MESH); Ultraviolet Rays (MESH); Phototherapy (MESH); Ultraviolet; Therapy/instrumentation (MESH) ; Ultraviolet Therapy/methods (MESH)

Supplements S2 – Adapted Quality Appraisal tool for Case series

Title: **Author, year:**	
	Yes / no
Study objective	
1. Is the hypothesis/aim/objective of the study stated clearly in the abstract, introduction, or methods section?	
Study population	
2. Are the characteristics (age, gender, type of vitiligo) of the participants included in the study described?	
3. Were the cases collected in more than one centre?	
4. Are the eligibility criteria (inclusion and exclusion criteria) for entry into the study explicit and appropriate? (stability, type of vitiligo, treatment before study start)	
5. Were participants recruited consecutively?	
6. Did participants enter the study at a similar point in the disease? (stability)	
Intervention and co-intervention	
7. Was the intervention clearly described in the study? (type of UV treatment (duration, frequency) and transplantation technique)	
8. Were additional interventions (co-interventions) clearly reported in the study? (Prolongation UV, Wound dressing, local immunosuppressants)	
Outcome measure	
9. Are the outcome measures clearly defined in the introduction or methods section? (which outcome measure, blinded/nonblinded, method of measuring)	
10. Were relevant outcomes appropriately measured with objective and/or subjective methods?	
11. Were outcomes measured before and after intervention?	

Supplements S2 – Adapted Quality Appraisal tool for Case series (continued)
Statistical analysis
12. Were the statistical tests used to assess the relevant outcomes appropriate?
Results and conclusions
13. Was the length of follow-up reported?
14. Was the loss to follow-up reported?
15. Does the study provide estimates of the random variability in the data analysis of relevant outcomes?
16. Are adverse events reported?
17. Are the conclusions of the study supported by results?
Competing interests and sources of support
18. Are both competing interests and sources of support for the study reported?
Total yes:

Supplements S3 – Quality Appraisal Case series

Question Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total yes
Awad	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	Yes	No	No	Yes	Yes	Yes	**11**
Boersma	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	No	**11**
Chen	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**9**
El-Zawahry	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	**11**
Ghorpade	Yes	Yes	No	No	No	Yes	No	Yes	No	No	No	No	No	No	No	Yes	Yes	No	**6**
Gupta	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**8**
Hallaji	Yes	Yes	No	Yes	No	Yes	Yes	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**9**
Holla	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	Yes	**10**
Holla	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No	No	Yes	Yes	Yes	**11**
Krishnan	No	Yes	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	**6**
Lahiri	No	Yes	No	No	No	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	No	Yes	Yes	No	**9**
Lim	No	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	No	No	**6**
Majid	Yes	No	No	Yes	No	Yes	Yes	Yes	No	No	No	No	Yes	No	No	Yes	No	No	**7**
Malakar	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes	No	No	Yes	No	No	**6**
Matsuzaki	Yes	Yes	No	No	No	No	Yes	No	No	No	No	Yes	Yes	No	No	Yes	Yes	Yes	**8**
Olsson	No	Yes	No	Yes	No	No	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	Yes	**7**
Pai	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No	**4**
Piangiani	Yes	Yes	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	No	**11**
Sachdev	Yes	Yes	No	Yes	No	No	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**7**
Sahni	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**8**
Sarkar	Yes	Yes	No	Yes	No	Yes	No	No	No	No	No	No	No	No	No	Yes	Yes	No	**6**
Singh (1995)	No	Yes	No	Yes	No	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**7**
Singh (1997)	No	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	Yes	No	No	**4**
Suga	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	**2**
Tsuchiyama	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	No	Yes	Yes	No	**9**

Supplements S4 – Phototherapy regimen per study.

Author, year	Phototherapy a. Pre/post-surgery b. Frequency/duration c. Initial dose d. Dose adjustment e. Cumulative dose
Awad, 2008	K-UVA a. 1w post; b. 2-3 times/w; c. 2 J/m^2; d. 25%/treatment; e. unknown
Babu, 2008	PUVASOL a. 1w post; b. unknown; c. unknown; d. unknown; e. unknown
Barman, 2004	sPUVA a. 4w post; b. 2 times/w; c. unknown; d. unknown; e. unknown
Boersma, 1995	UVA a. 2w post; b. 2 times/w; c. 10 J/cm2; d. unknown; e. unknown
Budania, 2012	Sunlight a. 8d post; b. Daily (5-30 minutes); c. unknown; d. unknown; e. unknown
Chen, 2000	Sunlight a. 7-10 d post; b. unknown; c. unknown; d. unknown; e. unknown
Czajkowski, 2007	sPUVA a. post; b. 3 times/w, 6 mo; c. 0.5J/cm^2; d. unknown; e. unknown
De Leeuw, 2011	K-NBUVB a. 1w post; b. 3 times/w; c. 1 minute; d. 50%/2 sessions; e. unknown
Ebadi, 2015	MELa a. 2w post; b. 2-3 times/w; c. 200 mJ/cm^2; d. 0-100 mJ/cm^2; e. unknown
El-Zawahry, 2011	PUVA a. 3w post; b. 3 times/w; c. unknown; d. unknown; e. unknown
Ghorpade, 2004	PUVASOL a. 2w post; b. unknown; c. unknown; d. unknown; e. unknown
Gupta, 2002	sPUVA a. 8d post; b. 3-6 mo; c. unknown; d. unknown; e. unknown
Hallaji, 2003	tPUVA a. 3 mo post; b. 3 times/week; 4 mo; c. 15s; d. 10s/session; e. unknown
Holla, 2014	Sunlight a. 6-8 d post; b. Daily; up to 4 mo; c. 20 min; d. unknown; e. unknown
Holla, 2013	Sunlight a. 7-10 d post; b. Min. 4 mo; c. unknown; d. unknown; e. unknown
Khandpur, 2005	PUVASOL a. 2w post; b. 3-4times/week; c. unknown; d. unknown; e. unknown
Krishnan, 2012	PUVASOL a. 2w post; b. unknown; c. unknown; d. unknown; e. unknown

Supplements S4 – Phototherapy regimen per study. (continued)

Author, year	**Phototherapy** a. Pre/post-surgery b. Frequency/duration c. Initial dose d. Dose adjustment e. Cumulative dose
Lahiri, 2006	NB-UVB a. post; b. 2 times/w; c. 500mJ/cm^2; d. 10-20%/treatment; e. max: 2270mJ/cm^2
Lim, 1999	Pre-PUVA a. unknown; b. unknown; c. unknown; d. unknown; e. unknown Post PUVA a. unknown; b. 1 mo; c. unknown; d. unknown; e. unknown
Linthorst Homan, 2012	MELa a. 1w post; b. 2 times/w, 12w ; c. 0.05 J/cm^2; d. 0.025-0.10J/cm^2; e. Mean ± SD: 7.3 ± 3.5 J/cm^2 NBUVB a. 1w post; b. 2 times/w, 12w; c. 0.25 J/cm^2; d. 50 mJ/cm^2; e. Mean ± SD: 25.6 ± 11.6 J/cm^2
Majid, 2012	NB-UVB a. 10-14 d post; b. 3 times/w, 1-3 mo; c. 200mJ/cm^2; d. 0-20%/treatment; e. unknown
Malakar, 2004	PUVASOL a. post; b. unknown; c. unknown; d. unknown; e. unknown
Mapar, 2014	NB-UVB a. 1w post; b. 2 times/w; 6 mo; c. 0.2J/cm^2; d. 20%/treatment; e. unknown
Matsuzaki, 2013	Sunlight a. 3w post; b. Daily; 6 mo; c. 5-10 min; d. unknown; e. 30min-1h
Olsson, 1997	Sunlight a. post; b. unknown; c. unknown; d. unknown; e. unknown
Pai, 2002	Pre-PUVA a. pre; b. unknown; c. unknown; d. unknown; e. unknown Post PUVA a. 15d post; b. unknown; c. unknown; d. unknown; e. unknown
Pianigiani, 2005	NB-UVB a. 3w post; b. 2 times/w; 4 mo; c. 200mJ/cm^2; d. unknown; e. max: 1800mJ/cm^2
Sachdev, 2000	PUVA a. 6-8 w pre and 2 w post; b. 3 times/w; c. unknown; d. unknown; e. unknown
Sahni, 2011	Sunlight a. 8d post; b. Daily; c. unknown; d. unknown; e. max: 30 min

Supplements S4 – Phototherapy regimen per study. (continued)

Author, year	**Phototherapy** a. Pre/post-surgery b. Frequency/duration c. Initial dose d. Dose adjustment e. Cumulative dose
Sahni, 2011	Sunlight a. post; b. Daily; c. 30 min; d. unknown; e. unknown
Sarkar, 2001	PUVASOL a. post; b. unknown; c. unknown; d. unknown; e. unknown
Sheth, 2012	MELi a. 1w post; b. 3 times/w, 12w; c. 200mJ/cm^2; d. 50 mJ/treatment; e. unknown NBUVB a. 1w post; b. 3 times/w, 12w; c. 200mJ/cm^2; d. 15%/treatment; e. unknown
Singh, 1995	PUVASOL a. post; b. unknown; c. unknown; d. unknown; e. unknown
Singh, 1997	PUVASOL a. post; b. unknown; . unknown; d. unknown; e. unknown
Suga, 1996	tPUVA a. pre; b. unknown; c. 10-50 J/cm^2; d. unknown; e. unknown
Tegta, 2006	Sunlight a. post; b. Daily; c. 15-20 min. ; d. unknown; e. unknown
Tsuchiyama, 2016	MELa a. 1w post; b. 3 mo; c. unknown; d. unknown; e. unknown NBUVB a. 1w post; b. 3 mo; c. unknown; d. unknown; e. unknown
Verma, 2014	PUVASOL a. post; b. unknown; c. unknown; d. unknown; e. unknown
Zhang, 2014	preNBUVB a. 10w pre; b. 2 times/week; c. 0.40 J/cm^2; d. 0.10J/cm^2 per 2; e. 20 sessions postNBUVB a. 2w postv b. 2 times/week ; c. 0.40 J/cm^2; d. 0.10J/cm^2; e. 30 sessions

Supplements S5 – Results per non-comparative study.

Author, year	Study design	N	Follow-up	Outcomes	Intervention	Results
PUVA						
Awad, 2008	**CS**	20	2-3 mo	100% repigmentation	SBG + K-UVA	30%
Babu, 2007	RCT	18	1-3 mo	51-100% repigmentation	PG + PUVASOL	75%
					SBG + PUVASOL	60%
Czajkowski, 2007	RCT-IPC	40	6 mo	100% repigmentation	CAMT + sPUVA	50%
					SBG + sPUVA	98.7%
El-Zawahry, 2011	CS	25	6-17mo	I. 90-100% repigmentation II. 50-89% repigmentation III. 20-49% repigmentation IV. <20% repigmentation	NCAMT + PUVA	I. 23% II. 32% III. 27% IV. 18%
Ghorpade, 2004	CS	10	2 yr	I. Median pigment spread II. 100% repigmentation	PG + PUVASOL	I. 4.0 mm II.100%
Gupta, 2002	CS	10	3-6mo	>75% repigmentation	SBG + sPUVA	80%
Hallaji, 2003	CS	20	4 mo	I. 90-100% repigmentation II. 25-50% repigmentation III. 0% repigmentation	PG + tPUVA	I. 65% II. 15% III. 20%
Khandpur, 2005	RCT	64	3 mo	>75% repigmentation	PG + PUVASOL	44.1%
					STG + PUVASOL	83.3%
Krishnan, 2012	CS	26	5 mo	>90% repigmentation	NCAMT + PUVASOL	100%
Malakar, 2004	CS	108	3-5 mo	I. 90-100% repigmentation II. 70-90% repigmentation III. 50-70% repigmentation IV. 30-50% repigmentation V. 0-30% repigmentation VI. 0% repigmentation	PG + PUVASOL	I. 72.2% II. 1.9% III. 7.4% IV. 5.6% V. 1.9% VI. 9.3%
Pai, 2002	CS-IPC	29	n.a.	I. 75-100% repigmentation II. 50-75% repigmentation III. 25-50% repigmentation	PG + PUVA	I. 50% (lesions) II. 50% (lesions) III. 0% (lesions)
					SBG + PUVA	I. 0% (lesions) II. 60% (lesions) III. 40% (lesions)
					laserPG + PUVA	I. 68.8%(lesions) II. 31.3%(lesions) III. 0%(lesions)
					laserSBG + PUVA	I. 0%(lesions) II. 100%(lesions) III. 0%(lesions)

Supplements S5 – Results per non-comparative study. (continued)

Author, year	Study design	N	Follow-up	Outcomes	Intervention	Results
Sachdev, 2000	CS	13	32w	0% repigmentation	PUVA + PG + PUVA	14.4% (lesions)
Sarkar, 2001	CS	15	n.a.	I. 90-100% repigmentation II. 70% repigmentation III. 60% repigmentation IV. Mean pigment spread	PG + PUVASOL	I. 80% II. 13.3% III. 6.7% IV. 5.4 mm
Singh, 1995	CS	32	3-6 mo	I. Pigmentspread 1-4mm II. Pigmentspread 5-8mm III. Pigmentspread 9-12mm IV. Pigmentspread > 13mm	PG + PUVASOL	I. 22.2% (lesions) II. 42.2% (lesions) III. 28.9% (lesions) IV. 6.7% (lesions)
Singh, 1997	CS	55	6mo – 3yr	100% repigmentation	PG + PUVASOL	65.5%
Suga, 1996	CS	28	3 mo	I. 100% repigmentation II. 95-100% repigmentation III. 90-100% repigmentation IV. 85-100% repigmentation V. 80-100% repigmentation VI. 60-80% repigmentation VII. 20-100% repigmentation VIII. >10% repigmentation IX. <5% repigmentation	tPUVA + SBG	I. 25% II. 35.7% III. 39.3% IV. 50% V. 60.7% VI. 71.4% VII. 78.6% VIII. 89.3% IX. 10.7%
Verma, 2013	RCT	25	6 mo	I. > 70% repigmentation II. 31-70% repigmentation III. <31% repigmentation	CAMT + PUVASOL	I. 52% (lesions) II. 1% (lesions) III. 38% (lesions)
					NCAMT + PUVASOL	I. 62% (lesions II. 20% (lesions) III. 18% (lesions)
NBUVB						
De Leeuw, 2011	RCT-IPC	19	3-6mo	Mean repigmentation	SBG + K-NBUVB	65.3%
Lahiri, 2006	CS	66	18 mo	Mean pigment spread	PG + NBUVB	6.5mm
Majid, 2012	CS	40	3-12 mo	> 90% repigmentation	STG + NBUVB	83%
Mapar, 2014	RCT-IPC	25	6 mo	Mean pigment spread	PG + NBUVB	5.3 mm
					HFT + NBUVB	5.0 mm
Pianigiani, 2005	CS	93	18mo	I. 100% repigmentation II. 50-99% repigmentation	CAMT + NBUVB	I. 60% II. 30%

Supplements S5 – Results per non-comparative study. (continued)

Author, year	Study design	N	Follow-up	Outcomes	Intervention	Results
UVA						
Boersma, 1995	**CS**	19	3-12 mo	I. 80-100% repigmentation II. 50-79% repigmentation III. 0-50% repigmentation	PG + UVA	I. 38.9% (lesions) II. 27.8% (lesions) III. 33.3% (lesions)
Sunlight						
Budania, 2012	**RCT**	41	16w	I. 90-100% repigmentation II. 75-89% repigmentation III. > 75% repigmentation I.	NCAMT + Sunlight SBG + Sunlight	I. 71% II. 18% III. 89% I. 27% II. 58% III. 85%
Chen, 2000	**CS**	25	3-20mo	I. 95-100% repigmentation II. 65-94% repigmentation	CAMT + Sunlight	I. 84% II. 16%
Holla, 2013	**CS**	36	6-18mo	I. >75% repigmentation II. 50-75% repigmentation III. <50% repigmentation	NCAMT + Sunlight	I. 63.8% (lesions) II. 28.8% (lesions) III. 7.5% (lesions)
Holla, 2014	**CS**	31	6mo – 1yr	I. >75% repigmentation* II. > 90% repigmentation* III. 74-90% repigmentation* IV. 25-75% repigmentation* V. 0-25% repigmentation* VI. 0% repigmentation* *of leucotrichia	NCAMT + Sunlight	I. 45.2% (lesions) II. 28.6% (lesions) III. 16.7% (lesions) IV. 23.8% (lesions) V. 19% (lesions) VI. 11.9% (lesions)
Matsuzaki, 2013	**CS**	27	1yr	I. >90% repigmentation II. 50-90% repigmentation III. 0-50% repigmentation	CAMT + Sunlight	I. 51.9% II. 33.3% III. 14.8%

Supplements S5 – Results per non-comparative study. (continued)

Author, year	Study design	N	Follow-up	Outcomes	Intervention	Results
Olsson, 1997	**CS**	19	4-8mo	I. Mean repigmentation II. 100% repigmentation III. 95% repigmentation IV. 70% repigmentation V. 50% repigmentation VI. 20% repigmentation VII. 10% repigmentation VIII. 0% repigmentation	STG + Sunlight	I.75.1%(lesions) II. 49.0% (lesions) III. 16.3% (lesions) IV. 8.2% (lesions) V. 8.2% (lesions) VI. 2.0% (lesions) VII. 4.1% (lesions) VIII. 12.2% (lesions)
Sahni, 2011	**CS**	13	1 yr	I. > 90% repigmentation II. 75-90% repigmentation	NCAMT + Sunlight	I. 79% (lesions) II. 21% (lesions)
Sahni, 2011	**RCT**	25	16w	I. >75% repigmentation II. >90% repigmentation III. 76-90% repigmentation IV. 51-75% repigmentation V. 25-50% repigmentation VI. <25% repigmentation	NCAMT (saline) + sunlight	I. 66.7% (lesions); 64.3% (patients) II. 44.4% (patients) III. 22% (patients) IV. 11% (patients) V. 11% (patients) VI. 11% (patients)
					NCAMT (serum) + sunlight	I. 94.4% (lesions); 92.8% (patients) II. 88.8% (patients) III. 6% (patients) IV. 6% (patients) V. 0% (patients) VI. 0% (patients)
Tegta, 2006	**RCT**	20	12w	I. >75% repigmentation II. 51-75% repigmentation III. 26-50% repigmentation IV. 1-25% repigmentation V. 0% repigmentation	NCAMT (1:3) + Sunlight	I. 50% II. 20% III. 10% IV. 0% V. 20%
					NCAMT (1:5) + Sunlight	I. 0% II. 10% III. 20% IV. 40% V. 30%

8

Patients' perspective on current treatments and demand for novel treatments in vitiligo

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Abstract

Background
The treatment of non-segmental vitiligo (NSV) remains a challenge. Current treatments often achieve suboptimal clinical results. To improve these treatment results, several new therapies are being developed and investigated. There is, however, little evidence on the actual need for novel therapies.

Objective
To assess patients' perspective on current and novel therapies for vitiligo.

Methods
A prospective questionnaire study was conducted in a large cohort of vitiligo patients that consecutively visited the outpatient clinic of the Amsterdam University Medical Centre between April 2017 and January 2019. Patients were requested to fill in a digital questionnaire on patient characteristics, disease burden, quality of life, efficacy and satisfaction of current treatments and aspects regarding new treatments.

Results
A total of 325 vitiligo patients completed the questionnaire (60% response rate). Of the respondents, 94% believed that new and improved treatments are needed and 86% would be willing to participate in clinical trials investigating a new therapy. Sixty-nine percent would agree on taking weekly injections if it led to effective treatment results. Of the patients that had received therapy before, 49% reported that the current treatments were not effective and 50% was not satisfied with the current treatments. Sixty-seven percent of the patients experienced facial lesions as an extreme burden, whereas this was, 25%, 12% and 10% for lesions on the hands, trunk and feet respectively. The emotional burden score was significantly higher in dark skin types compared to light skin types (respectively 8 vs 5, U $p < 0.05$).

Conclusion
There is a substantial need for new vitiligo therapies. A considerable number of patients in our study is dissatisfied with current treatments and is emotionally burdened by the disease. Moreover, the vast majority demands novel treatments and is willing to participate in clinical trials.

Introduction

Vitiligo is the most common depigmenting skin disease affecting approximately 1% of the world population, regardless of gender, ethnicity or skin type.[1] Although problems like itch and a history of sunburns are common, vitiligo generally does not lead to physical complaints.[2] However, the disease is strongly associated with a significant negative effect on the quality of life, as many of the patients feel stressed and stigmatized by their condition.[3,4]

To date, there is no curative treatment available for vitiligo. For many years now vitiligo therapy consists of topical agents, phototherapy and surgical techniques, which all aim to minimize disease progression and/or stimulate repigmentation.[5] So far, current treatment results vary between patients and a considerable number of patients does not sufficiently benefit from treatment.

To improve these suboptimal treatment results, several new therapies are being developed and investigated.[5–8] However, for the clinical implementation of these new treatments it is crucial to know the vitiligo patients' need for novel treatment modalities. What are these novel therapies worth to a patient? And how satisfied are patients in fact with the current treatments? More knowledge of these factors could be of great value to various stakeholders, such as physicians, policy-makers and pharmaceutical companies.

Furthermore, many studies have shown that vitiligo is associated with a substantial disease burden, using various measurement tools.[9–11] In addition, a few studies have related psychological complaints and quality of life to the location of the vitiligo lesions, showing a higher impact on exposed areas, such as the face.[12–14] However, various other studies indicate that other body regions have larger impact on the quality of life and/or disease burden. For example, Linthorst Homan et al. showed that vitiligo particularly on the chest had an impact on the health-related quality of life, whereas lesions on the hands and face did not.[2] Another study found that lesions on the genital area were associated with a high burden.[11] Florez-Pollack et al. illustrated that lesions on the hands were even more bothersome compared to the face and neck.[15] We hypothesize that the disease burden and its relation to the location of the various vitiligo lesions could be correlated to the demand for new therapies.

To address these issues, we performed a cross-sectional prospective survey. The aim of this study was to assess the patients' perspective on current and novel therapies for vitiligo. Furthermore, we aimed to compare demographic features,

such as gender, skin type and location of lesions (e.g. facial vs. non-facial) in relation to the primary outcomes on satisfaction of current treatments, disease burden and demand for new therapies.

Patients and methods

Patients

This prospective questionnaire study was conducted in a cohort of vitiligo patients at the Netherlands Institute for Pigment Disorders (NIPD) of the Amsterdam University Medical Centre (UMC). This institute is a centre of expertise for vitiligo and has a nation-wide patient population. All patients with non-segmental vitiligo (NSV), aged 16 years or older that had consecutively visited our outpatient clinic between April 2017 and January 2019 were approached. Patients with segmental, focal and universal vitiligo were not included, since these subtypes differ in management, treatment and prognosis and cannot be compared to NSV for this study purpose.

Patients of whom the email address was registered in the electronic patient system received an email with a secured online questionnaire carried out by LimeSurvey version 2.6.7. If a patient did not respond, a reminder was sent after 1 month. Only fully completed responses were included in the final analysis. This study was not subject to the WMO (Medical Research Involving Human Subjects Act) as confirmed by the Medical Ethical Committee of the Amsterdam UMC (EC number W17_349).

Questionnaire

We developed an online questionnaire consisting of questions regarding; (i) demographics, (ii) disease burden, (iii) current treatment results, (iv) novel therapies and (v) quality of life (Supplementary Appendix I).

Demographic questions included age, gender, location of the vitiligo lesions and the Vitiligo Disease Activity (VIDA) score. Patients were requested to indicate the disease burden of their vitiligo lesions in relation to the location of these lesions. Furthermore, they were asked to score their emotional disease burden on a visual analogue scale from 0-10 (where 0 means no burden at all and 10 means an extreme burden). Patients were asked two questions in total about the effectiveness and satisfaction of current therapies. Three questions were included regarding novel therapies. First, patients were asked if they thought new and improved therapies for vitiligo are needed. Secondly, patients were requested to indicate whether they

would accept vitiligo therapy if it consisted of weekly injections. A third question was included about the willingness to participate in a clinical trial investigating a new vitiligo therapy, if participation would be for free. Finally, 10 questions of the Dermatology Life Quality Index (DLQI) were included to assess the quality of life.

Statistical analysis

Only fully completed responses were included in the final analysis. Descriptive statistics were used to analyse the demographics of the study population. To compare means and medians, respectively t-tests and Mann Whitney U tests (U) were performed. Spearman rank correlation coefficient (r_s) was used for correlation analysis and Pearson Chi-square tests ($\chi 2$) were used to analyse categorical data. A p-value of <0.05 was considered statically significant. All statistical analyses were performed in SPSS version 25.0 (IBM, Armonk, NY).

Results

Of the 920 patients with NSV, aged 16 or older that visited our outpatient-clinic, 542 patients had a functioning email address that was available to us. Questionnaires were sent to a total of 542 patients of whom 377 (70%) responded. Of these, 325 (60%) patients fully completed the questionnaire, 7 patients declined (1%) and 45 (9%) patients started the questionnaire, but did not complete it. Patient and vitiligo characteristics are presented in Table 1.

Table 1. Patient and vitiligo characteristics

Male : female ratio	133 : 192
Mean age ± SD (min-max) in years	41 ± 14 (16-77)
Mean age of onset ± SD (min-max) in years	31 ± 16 (0-76)
Disease duration median in years [IQR]	7 [3-16]
Location vitiligo N (%)	
face	278 (86)
hands	223 (69)
arms, legs	228 (70)
trunk	211 (65)
feet	153 (47)
Vitiligo disease activity (VIDA score) N (%)	
Activity in past 6 weeks	31 (10)
Activity in past 3 months	37 (11)
Activity in past 6 months	33 (10)
Activity in past year	104 (32)
Stable at least for 1 year	69 (21)
Stable for 1 year and spontaneous repigmentation	51 (16)

Table 1. Patient and vitiligo characteristics (continued)

Skin type N (%)	
I	11 (3)
II	202 (62)
III	58 (18)
IV	20 (6)
V	25 (8)
VI	9 (3)
Previous treatments N (%)	
Has had treatment before for vitiligo	323 (99)
Use of creams	322 (99)
Use of NB-UVB light therapy	221 (68)
Skin transplantations	25 (8)

Primary survey outcomes

The primary survey outcomes are shown in Table 2. Of all patients, 94% indicated that new and improved treatment modalities are needed for vitiligo. Half of the patients were not or little satisfied with the current therapies and 49% indicated that the current treatments were not or little effective. The demand for new treatments did not significantly differ between males and females ($\chi 2$ p=0.305). No association was found between the location of vitiligo lesions and the demand for new therapies (Fisher's exact test p=0.558). Dark skin type patients (Fitzpatrick 4-6) did not have a higher need for new therapies than light skin type patients (Fitzpatrick 1-3) (Fisher's exact test p=0.217). However, darker patients were significantly more willing to receive a new effective treatment if it consisted of weekly injections ($\chi 2$ p=0.029). No relations were found for gender, location of lesions and skin type with regards to the willingness to participate in clinical trials.

Table 2. Primary survey outcomes

Parameter	**N (%)**
Effectiveness of current treatments	
Not effective	56 (17)
Little effective	104 (32)
Effective	93 (29)
Very effective	55 (17)
Not applicable	17 (5)
Satisfaction regarding current treatments	
Not satisfied	63 (19)
Little satisfied	99 (31)
Satisfied	96 (30)
Very satisfied	45 (14)
Not applicable	22 (7)
Need for novel treatments: yes	305 (94)
Study participation: yes	281 (87)
New effective treatment if consisted of weekly injections: yes	224 (69)

Satisfaction with current treatments

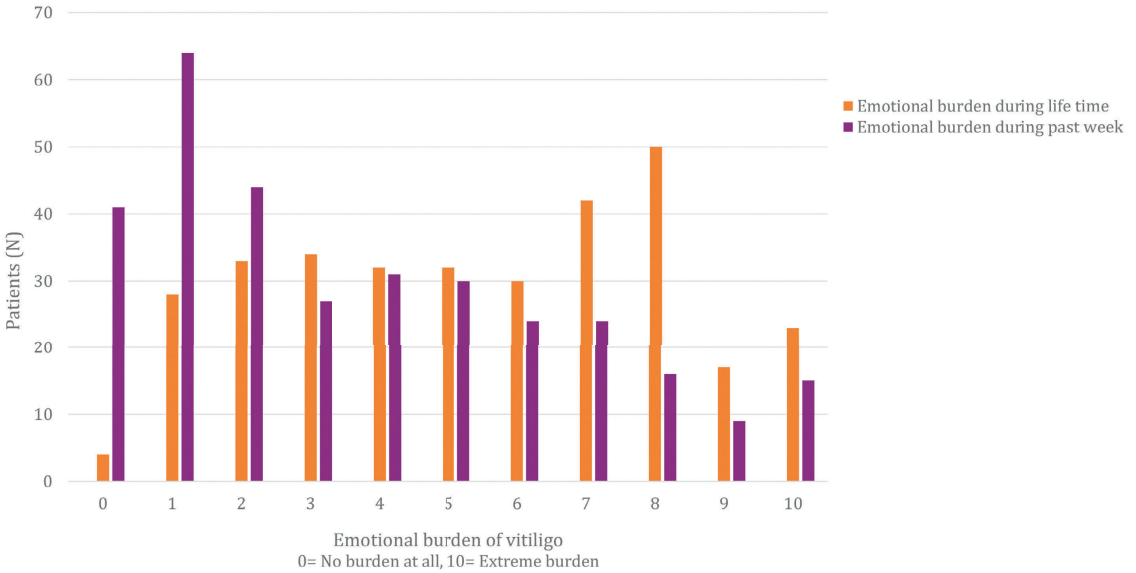
The lesser satisfied patients were with current treatments, the more they indicated a need for new and improved therapies (r_s -0.18, p=0.002). The satisfaction is significantly correlated to the effectiveness from patients view of current treatments (r_s = 0.778, p=0,00). Patients who had received UVB therapy were significantly more satisfied with treatment than patients who had not received UVB therapy ($\chi 2$ p=0.001). No relation was found between skin type and satisfaction with current therapies ($\chi 2$ p=0.19). Patients with facial vitiligo were significantly more satisfied with treatments than patients that did not have facial lesions ($\chi 2$ p=0.003). No difference in satisfaction was found between males and females ($\chi 2$ p=0.321).

Disease burden and Dermatology Life Quality Index

The median of the emotional disease burden score during lifetime was 5 [IQR 3-8] and over the past week was 3 [IQR 1-6] (Fig. 1). The burden score over the past week was significantly lower than the score during lifetime (Wilcoxon signed rank test, p=0.000). Of all our patients, 28% had a DQLI total score higher than 5, which indicates a moderate to severe impaired quality of life (Fig. 2). The emotional burden score of the past week was significantly correlated to the total scores of the DLQI (r_s = 0.607 p=0.000). The life time emotional burden score was significantly higher in darker skin types compared to lighter skin types (median 8 vs 5, U p=0.000). In addition, female patients had a significantly higher emotional burden score than male patients (t-test, p=0.002).

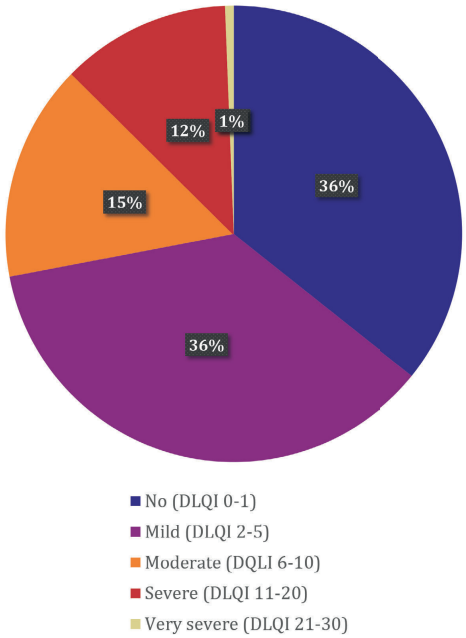
In figure 3 the burden of the disease is shown for each body region. Sixty-seven percent of the patients experienced their facial lesions as an extreme burden, whereas this was 25%, 12% and 10% for lesions on the hands, trunk and feet respectively. Patients that experienced facial lesions as an extreme burden were significantly more convinced that new and improved vitiligo treatments are needed ($\chi 2$ p=0.033). Furthermore, the emotional burden score of the past week had a significant relation with (i) the demand for novel treatment (r_s = 0.147 p=0.002), (ii) the willingness to participate in clinical trials (r_s = 0.131 p=0.018) and (iii) the acceptance of taking invasive therapy (r_s = 0.263 p=0.000). The same applies to the DLQI total scores with the demand for new treatment (r_s = 0.226 p=0.000), willingness to participate in clinical trials (r_s = 0.175 p=0.002) and acceptance of taking invasive therapy (r_s = 0.327 p=0.000).

Figure 1. Emotional burden of vitiligo



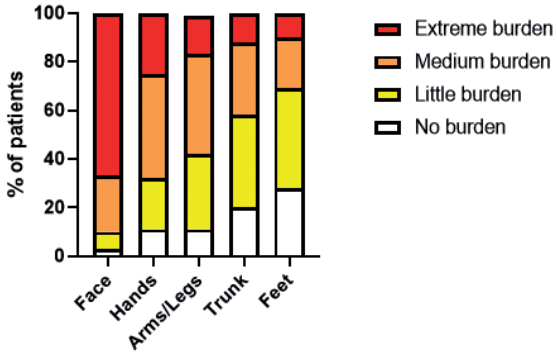
The bar graph represents the emotional burden that vitiligo patients have experienced during their lifetime (orange bars) and during the past week (purple bars). On the x-axis the numbers are shown for the degree of emotional burden, in which 0 means no burden at all and 10 means an extreme burden (presented to patients in a visual analogue scale). The y-axis represents the number of patients.

Figure 2. Dermatology Quality Life Index (DLQI) total scores



Dermatology Life Quality Index (DLQI) total scores distribution between the patients. Percentage of total number of patients (n = 325) shown. DLQI total scores of 0-1 represent no effect at all on patient's life, 2-5 represent a mild effect, 6-10 represent a moderate effect, 11-20 a severe effect and 21-30 a very severe effect on a patient's life.

Figure 3. Patient burden of disease per location



The stacked bar chart shows the patient burden of disease per body region. Red illustrates an extreme burden, orange a medium burden, yellow is little burden and white is no burden at all. On the x-axis the specific body regions are shown, and the y-axis represents the percentage of total number of patients (n = 325).

Discussion

Our study clearly demonstrates the substantial demand of vitiligo patients for new therapies. Ninety-four percent of the vitiligo patients included in this study indicate that new and better treatments are needed for vitiligo. Consequently, a majority of these patients is willing to participate in clinical trials for improvement of vitiligo therapy. Moreover, approximately half of the patients felt that their current treatment was not effective and was not satisfied with it.

We analysed factors with potential influence on patient satisfaction. We found that patients who had received UVB therapy were significantly more satisfied than patients who had only received topical treatment, which is in line with previous studies.[16,17] Patients with facial vitiligo also seemed to be more satisfied with current treatments. This could be clarified by the fact that facial lesions often respond better to therapy than lesions on other body regions.[18]

Our results also show that patients that are little or not satisfied with current treatments, are more demanding for new treatments. It is however remarkable that even patients who were (very) satisfied with current treatments, still expressed the opinion that new treatments are needed for vitiligo.

Moreover, we found that the emotional burden was significantly higher in dark skin type patients (Fitzpatrick skin type 4-6). This is in accordance with our previous study which showed that vitiligo particularly has more impact on the quality of life of patients with dark skin than patients with a light skin (Fitzpatrick skin type 1-3).[2] In addition, our results show a clear difference in experiencing burden of vitiligo lesions related to their location. The majority of the patients reported that they experienced their facial lesions as an extreme burden, followed by lesions on the hands in a quarter of the patients. This is not unexpected, since these body sites are usually exposed areas. On the contrary, lesions on the feet and on the trunk are experienced as little or no burden at all by the majority of the patients, probably since these usually involve covered areas. Thus, the degree of burden seems to be correlated to the visibility of the specific body region, which is in line with previous studies.[12–14,19] Furthermore, this study shows that a higher burden and impaired quality of life were significantly associated with a higher demand for new therapies, but also with a higher willingness to participate in clinical trials and a higher acceptance of invasive treatment for their vitiligo.

To our knowledge, our study is the first to analyse the demand for new therapies in a large cohort of vitiligo patients. As our collected data were recent up to October

2019, it represents an up-to-date view of patients' perspective. The strength of our study is that all patients were approached consecutively from our outpatient department, thereby including patients from daily practice. Moreover, this survey is completed by a relatively large cohort of patients all over the Netherlands. However, there are some limitations to this study. Firstly, this questionnaire was constructed specifically for this study and was not validated. Secondly, the response rate was 70% but only 60% completed the full questionnaire, which may raise a bias, due to the lack of data available on the non-responding patients. Moreover, since patients were included at a national referral centre for pigment disorders, the population of vitiligo patients in this study may have a relative high disease burden and may not fully reflect the larger community of people with vitiligo who do not seek care or are not referred to our expert centre.

The results of this exploratory study underline the demand for improved and novel vitiligo therapies. A considerable fraction of patients in our study is dissatisfied with current treatments and emotionally burdened by the disease. In addition, the disease burden seems to be dependent on the visibility of the location of the vitiligo lesion. Moreover, the vast majority of all patients included demands novel treatments and is willing to participate in clinical trials.

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Supplementary Appendix 1. Short survey vitiligo treatment

This survey consists of 24 (multiple)choice questions. We would like to know your opinion about current treatments and about the development of new therapies for vitiligo. In case you have any questions, you may address these to your attending physician.

Dear patient,

We would like to invite you to fill in this questionnaire regarding new treatments for vitiligo. This would take about 5 minutes.

Currently, medicines that are used for other auto-immune diseases, might have a favourable effect on vitiligo. For this reason, the Netherlands Institute for Pigment Disorders has started a questionnaire study to see if there is an actual need for new vitiligo therapies. With this research we also hope to gain more insight on the current situation of patients.

Your personal data will be analyzed confidentially and anonymously. This means that your treating physicians will not know your answers. The results of this study may be presented at medical congresses and published a scientific paper.

Thank you for participating.

Do you agree on completing this questionnaire?

I understand that filling in this questionnaire is voluntary

My answers will not have an influence on my regular treatment

My privacy will be respected at all times

I give my permission to participate in this research.

□ Yes I agree □ No I do not agree

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1. What is your age? ……years

2. Are you male or female?: □Male □Female

---

3. How old were you when your vitiligo started?

I was …… years old.

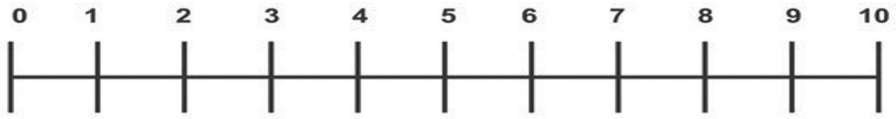
4. Where is your vitiligo located? (multiple answers possible)

□ Face
□ Hands
□ Arms, legs
□ Trunk
□ Feet

5. How much emotional burden did you experience from your vitiligo?

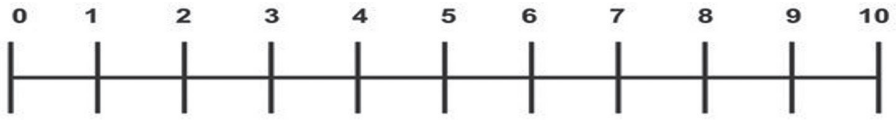
Please indicate your emotional burden during <u>your life time</u> and <u>over the past week</u> on a scale from 0 to 10.

The emotional burden during my life time was (encircle your answer):



No burden Extreme burden

The emotional burden over the past week was (encircle your answer):



No burden Extreme burden

6. How much burden do you experience of the location of the vitiligo lesions?:
If you do not have any vitiligo lesions on the body regions mentioned below, please indicate the burden you might experience if vitiligo lesions would occur on that region.

Face burden	◻ No burden	◻ Little burden	◻ Burden	◻ Extreme
Hands burden	◻ No burden	◻ Little burden	◻ Burden	◻ Extreme
Arms, legs burden	◻ No burden	◻ Little burden	◻ Burden	◻ Extreme
Trunk burden	◻ No burden	◻ Little burden	◻ Burden	◻ Extreme
Feet burden	◻ No burden	◻ Little burden	◻ Burden	◻ Extreme

7.Was your vitiligo active during the past period? So this means: have the white spots got bigger or have new white spots appeared?
◻ Active in the past 6 weeks
◻ Active in the past 3 months
◻ Active in the past 6 months
◻ Active in the past 1 year
◻ Stable for at least 1 year
◻ Stable for at least 1 year and spontaneous repigmentation

8. Which treatment(s) did you receive until now for your vitiligo? (multiple answers possible)
◻ Creams ◻ Phototherapy ◻ Skin transplantation ◻ Not applicable

9. How effective was the treatment for your vitiligo until now?
◻ Not effective ◻ Little effective ◻ Effective ◻ Very effective ◻ Not applicable

10. How satisfied are you with the treatment results until now?
◻ Not satisfied ◻ Little satisfied ◻ Satisfied ◻ Very satisfied ◻ Not applicable

11. Do you think new and improved therapies are needed for vitiligo?
□ Yes □ No

12. Would you choose for a new and effective therapy for vitiligo if this consisted of weekly injections?
□ Yes □ No

13. If a new treatment would be tested for vitiligo in a clinical trial, would you consider participating if this new treatment would be for free?
□ Yes □ No

14. Do you want us to contact you if a new treatment is being investigated for vitiligo?
□ Yes □ No

DERMATOLOGY LIFE QUALITY INDEX

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick þ one box for each question.

1.	Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?	Very much A lot A little Not at all	□ □ □ □
2.	Over the last week, how **embarrassed** or **self-conscious** have you been because of your skin?	Very much A lot A little Not at all	□ □ □ □
3.	Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?	Very much A lot A little Not at all Not relevant	□ □ □ □ □
4.	Over the last week, how much has your skin influenced the **clothes** you wear?	Very much A lot A little Not at all Not relevant	□ □ □ □ □
5.	Over the last week, how much has your skin affected any **social** or **leisure** activities?	Very much A lot A little Not at all Not relevant	□ □ □ □ □

6.	Over the last week, how much has your skin made it difficult for you to do any **sport**?	Very much A lot A little Not at all Not relevant	□ □ □ □ □
7.	Over the last week, has your skin prevented you from **working** or **studying**?	Yes No Not relevant	□ □ □
	If "No", over the last week how much has your skin been a problem at **work** or **studying**?	A lot A little Not at all	□ □ □
8.	Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?	Very much A lot A little Not at all Not relevant	□ □ □ □ □
9.	Over the last week, how much has your skin caused any **sexual difficulties**?	Very much A lot A little Not at all □ Not relevant	□ □ □ □
10.	Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?	Very much A lot A little Not at all Not relevant	□ □ □ □ □

Please check you have answered EVERY question. Thank you.

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Discussion

Discussion

Vitiligo is an acquired cutaneous condition associated with white patches caused by destruction of melanocytes and it has a significant negative effect on the quality of life. In this thesis, we explore **light and color** in the field of vitiligo; the overall aim of this thesis is to improve the care of vitiligo by broadening the knowledge about vitiligo, with specific focus on imaging (using UV colored light), outcome measures and treatment including narrow band ultraviolet (NB-UVB) light treatment and surgical techniques.

Imaging

Ultraviolet (UV) light is an essential tool to assess the extent, spreading and staging of vitiligo. The Wood's lamp, which emits UV-A rays, is often used for diagnosis of vitiligo.[1] It is also used to enhance contrast between vitiligo lesions and normal skin, to assess the progression during follow-up and for research purposes. Different UV emitting set-ups can be used. In **chapter 2a** we found that quality scores of images varied largely between 4 different UV set-ups. The high output flash lamp scored highest for both small and large areas on all criteria. Furthermore, this device scored best for ease of use. For small areas the Wood's lamp and camera flash had average scores, while soft boxes scored low on quality for both small and larger areas. For small areas the Wood's lamp and camera flash were acceptable. All set-ups scored badly for shadows. To create optimal illumination without shadows possibly two high output flash lights could be used, but our estimate is that this would increase the costs of the Wood's lamps by more than a 10-fold. When not considering the costs, we recommend the use of a (or multiple) high output flash lamp for photographing vitiligo, since it performs superior to other investigated UV light set-ups. A disadvantage of using multiple high output flash lamps is the increased UV exposure.

UV photography uses a special UV sensitive camera capable of passing UV light and filtering out visible light. Hence, it captures a large part of the UV light reflected by the skin which is supposed to improve the imaging of vitiligo. UV photography and its potential within dermatology have been described in multiple studies.[2–4] Mustakallio and Korhonen already described the use of UV photography for pigment disorders including vitiligo in 1966.[4] In **chapter 2b** we report that both vitiligo experts and unexperienced medical interns unanimously agree that the image quality of the UV photography images were of superior quality than the conventional digital camera images. Furthermore, we found very high intraclass correlation coefficients when comparing the UV photography method with other measurement instruments, as well as for multiple measurements by the same

or different physicians, supporting validity and reliability of this measurement instrument. Further research is needed to confirm the validity and reliability of this measurement instrument. This would ideally take place in a larger and international setting, measuring target sizes with other techniques. Next to improved image quality, another benefit of UV photography is the ease of use. A disadvantage is that UV photography cannot be used for assessment of target lesions localized on curved skin. Nevertheless, it can be resolved by using multiple cameras or other 3D image analysis systems.[5] A UV photography image captures more information than the physician can see by eye, since the UV light is outside the visible spectrum. Some images showed pinpoint or confetti lesions that were not or barely visible with UV light. This might imply that UV photography could ameliorate the assessment of vitiligo activity. Future studies could explore this hypothesis, by comparing activity assessment of conventional camera images with UV camera images. Still, the digital UV enhanced camera is a very promising tool for assessing vitiligo both in clinical practice as well as in research.

Outcome measures

Measurements are essential in medicine to diagnose diseases, assess results of treatment and for prognosis. Assessing outcomes is also one of the most important components in research: researchers have to decide what to measure and how to measure the outcomes. If different studies about the same subject use different measurement instruments is can be very difficult to compare them. International consensus on what should be measured in research on vitiligo has been reached in 2015.[6] For the evaluation of overall repigmentation of vitiligo two new outcome instruments were developed to measure the extent of vitiligo: the Vitiligo Extent Score (VES) and the Self-Assessment Vitiligo Extent Score (SA-VES).[7,8] The VES and SA-VES have been validated and are feasible and user friendly outcome measurement instruments for assessing the overall extent of vitiligo.[7,8] To improve the interpretability of these measurement we determined the minimal important change (MIC) in **chapter 3**. We found an anchor-based MIC of 0.53% decrease body surface area (BSA) for the VES, compared to a previously found smallest detectable change (SDC) between 3.81% and 4.68%.[7] For the SA-VES we found a MIC of -0.2% BSA, compared to a previously found SDC of 4.49%.[8] In both situations the SDC's are larger than the (absolute) MIC's. This means that a decrease in body surface area measured by the SA-VES as great as the MIC might be considered as important for patients (or physicians for the VES), but this change cannot be distinguished from measurement error.[9] In an ideal situation the MIC is larger than the SDC, so that any change in outcome as large as the MIC can be interpreted as being clinically relevant. In future clinical studies the intent should be to reduce the SDC to become smaller than the MIC by increasing sample size, as for groups of

patients the SDC is reduced by a factor √n, when a number of n patients is being studied.[10] Future studies could assess whether the MIC varies among different countries. Our findings can be used for larger studies and power analyses. Our results improve the interpretability of these measurement instruments and can be used to facilitate sample size calculations in future studies using the VES and SA-VES as outcome instruments.

Information on how to translate the obtained VES scores into disease severity strata from the physicians' point of view has been described recently while this is still lacking for the patient reported version (SA-VES).[8] In **chapter 4** we validated a Patient Reported Global Assessment (PR-GA) for severity, extent and impact using a simple scoring system based on a global assessment question. The PR-GA is an intuitive and simple measure that is often used in clinical trials. The PR-GAs were used to stratify the numeric score obtained by the SA-VES. Stratification of outcome measures is crucial to interpret the obtained scores and to perform research on homogenous study populations. In this study, we confirmed the intrarater reliability and construct validity of 3 PR-GAs based on hypotheses testing. Using the validated PR-GA-anchor questions the possible strata per category could be assessed for the SA-VES score. Based on the cut off values, an affected body surface area (SA-VES) of more than 1.05% and 6.45% was already considered as moderate extensive and extensive, respectively. The median SA-VES score for moderate extensive and extensive were 2.745 and, 10.25 respectively. Cut-off values for PR-GA severity were different compared to PR-GA extent, emphasizing the importance of separating these 2 aspects in future trials. Severity assessment requires an additional dimension from patient's point of view including additional influencing factors such as location of the lesions or patients skin photo type especially for a pigment disorder like vitiligo. Insight into patients' perception is crucial to properly define severity in a way that is both useful to the physician and reflective of the patient's status. A thorough investigation within a large vitiligo patient population may offer additional criteria to better define vitiligo severity. Impact assessment involves a different dimension as it measures the individual experience of the influence of the disease on daily life. This may be a crucial factor in the therapeutic choice during a vitiligo consultation. Interestingly >1/3 patients rated impact and severity differently indicating that patients recognize that the more general concept of severity may result in a different subjective impact from one patient to another. Stratification based on PR-GA for impact indicated that even a low affected BSA (SA-VES) of 3.35% can have a high to very high impact on the quality of life. Conversely, a very high SA-VES can have a moderate impact. Our findings can be used for the interpretation and stratification of scores obtained with other Patient Reported Outcome Measures (PROMs) and can guide treatment

decisions in vitiligo management. To date, our knowledge on the stratification and the PR-GA is limited to the Belgium population with vitiligo from a single expert center. Future studies in other countries, skin types and ethnicities are mandatory as the PR-GA and the strata concerning extent, severity and impact are probably highly dependent on the aforementioned factors.

Previous studies have confirmed the validity and reliability of the VES and SA-VES, we improved the interpretability.[7,8] Furthermore the responsiveness of both tools has been confirmed as well in a small population.[11] Concluding, with our study on the interpretability research has been performed on all measurement properties of the VES and SA-VES, confirming high quality of these outcome measurements for the assessment of disease extent in non-segmental vitiligo. Furthermore, we validated a stratification based on patient reported global questions for severity, extent and impact for the SA-VES, which is an intuitive and simple measure that can used in clinical trials.

Treatment

To date, there is no curative treatment available for vitiligo. For many years now vitiligo therapy consists of topical agents, phototherapy and surgical techniques, which all aim to minimize disease progression and/or stimulate repigmentation.[12] Topical therapy with corticosteroids or calcineurin inhibitors are suggested as first-line treatment.[13] Furthermore phototherapy using narrow band ultraviolet B (NB-UVB) is considered to be an important modality for the treatment of generalized NSV.[12] NB-UVB is preferred to other UV therapy regimens like psoralen combined with UVA (PUVA) due to its equal or better efficacy while adverse effects are decreased.[14,15] In **chapter 5** we examined patient reported outcomes regarding repigmentation and satisfaction in non-segmental vitiligo patients after completing NB-UVB therapy. This study shows that the twice-weekly and thrice-weekly regimen in combination with topical agents seem to be comparable regarding patient reported repigmentation, satisfaction and occurrence of short term adverse effects. Although patients seem to notice an earlier onset of repigmentation in the intensified group, this difference is not significant. Finally, the start of repigmentation in the intensified group significantly correlates with the satisfaction after completing the NB-UVB therapy. The intensified treatment might be more effective to speed up the onset of repigmentation, but costs and burden should be taken into account. Larger prospective studies using validated outcome measures are needed to corroborate these findings. A direct comparison between twice weekly and thrice weekly NB-UVB in vitiligo patients is still needed to determine what the optimal frequency is. It is possible that the additional topical treatment is responsible for the earlier repigmentation in the intensified group.

Thus, prospective studies are required, since it is difficult to critically investigate these regimens in retrospective study designs due to the possible variations in dosimetry, irradiation time, recall bias and other outcome measures. A twice-weekly regimen might be more convenient regarding time and effort for patients. However, this could be accompanied by psychological distress experienced by patients due to slow responses, leading to reduced therapy compliance.[16]

Next to topical and UV therapy, adding epidermal cells including melanocytes locally could boost the standard treatment to achieve optimal repigmentation in non-segmental vitiligo. In **chapter 6a** we found no significant benefit for additional autologous cell suspension transplantation (CST) in patients with non-segmental vitiligo with lesions unresponsive to standard of care (NB-UVB, topical corticosteroids). Only two patients out of 17 patients showed successful repigmentation (>75% repigmentation). Our results are contradictory to a number of publications in this field, which show successful repigmentation varying from 52% to 89%.[17–19] In these studies only patients were included with stable vitiligo. In contrast, we included patients who were already receiving standard of care, mostly having an active vitiligo before the start of NB-UVB combined with topical therapy. CST was performed 9 months after the start of NB-UVB therapy and we expected to have induced stability due to immunosuppressive treatment, allowing the grafts to successfully induce repigmentation. This did not occur, with only two patients with successful repigmentation of the treatment area. So, stability induced by therapy may be misleading; our findings suggest that stability of vitiligo without therapy is essential for successful CST and that standard of care treatment does not contribute enough to enable successful repigmentation upon CST. In line with our findings one study on punch grafting observed that a group of patients only showed repigmentation during NB-UVB therapy; after the NB-UVB therapy the punch grafts depigmented.[20] We agree with their recommendation to only select those patients for CST who have a stable vitiligo for 1 to 2 years without therapy, because NB-UVB therapy given before transplantation may lead to an incorrect selection.[20]

Another reason for the low repigmentation rates may be due to the design of the study. We included patients with lesions that were still depigmented after 9 months of standard of care and thereby applied a strong selection for the most difficult and unresponsive lesions. We did this because there is a strong medical need for repigmentation in this group of patients. These kind of lesions might have an intrinsically low potential of repigmentation to any kind of treatment, including melanocyte transplantation. Yet, the guidelines for vitiligo mention that melanocyte transplantation should be considered in patients unresponsive to

conventional treatment.[21] With the results of our study, we propose that guidelines adjust their recommendations; melanocyte transplantation for patients with non-segmental vitiligo should preferably be performed in patients with stable non-segmental vitiligo without treatment or in lesions that are responsive to topical and or UV therapy. The locations of the treated lesions could also have influenced our results. Many of our patients were treated on the wrists (n=6) or hands (n=2). The hands are known to respond inferior to transplantation compared to other locations.[22,23] Separate results for wrists are not often mentioned in studies, but other joints like elbows and knees are also known to be difficult to repigment upon transplantation.[22,23]

We used punch grafting to assess stability and predict outcomes of CST. In other studies punch grafting has been proposed as an indicator to determine disease stability and predict successful repigmentation after surgical therapies.[24,25] In contrast to their findings our results suggest that punch grafting is not a good indicator to assess possible efficacy of CST. Interestingly even though all our randomized patients had a successful punch grafting test, most of them failed CST. Possible explanations could be that with preparation of the acceptor site with laser treatment the vitiligo was activated due to koebnerization. This partially contradicts previous studies using laser therapy in combination with NB-UVB therapy to induce repigmentation.[26,27] But these studies used fractional laser ablation while we used full surface ablation. Another possible explanation could be the medium or lack of tissue architecture of skin cell suspensions during transplantation. Melanocytes might benefit from intercellular contact with other epidermal cells for cell survival and growth.[28] This interaction is lost in a cell suspension, whereas transplantation of a punch graft has an intact tissue environment. In conclusion, our findings in this chapter indicate that non-segmental vitiligo lesions that are unresponsive to prolonged standard of care will generally not benefit by adding CST. We therefore recommend to use the punch graft test only to predict results for tissue grafts but not for cellular grafting techniques. Other methods determining disease activity, like markers in the blood, skin or other tissues are needed to improve patient selection for CST.

In **chapter 6b** we found, to our surprise, that repigmentation upon CST in segmental vitiligo and piebaldism is not directly determined by graft cell/melanocyte content or viability, as successful repigmentation was seen both in patients transplanted with high or low numbers of melanocytes per mm^2 and vice versa. Only the proportion of total viable cells correlated to repigmentation. In the analysis of the suspension for chapter 6a we also did not find a significant relationship between graft cell/melanocyte density or cell viability and repigmentation. These

findings are in contrast with two previous studies, where the minimum number of melanocytes needed for visible repigmentation was estimated at 190 and 210/mm^2.[8,9] However, the study by Olsson and Juhlin used an estimation on a previous calculation by Rosdahl and Rorsman[31] to determine the 190 melanocytes per mm^2 and did not determine the number of melanocytes in the grafts in their own study.[29] The study by Tegta et al. randomized 22 patients in 2 groups with 2 different donor-recipient site ratios (3-fold versus 5-fold).[30] They found a significantly higher repigmentation in the 3-fold group (transplanted melanocytes 181-275 per mm$^{2)}$ compared to the 5-fold group (transplanted melanocytes 108-180 per mm^2), with >50% repigmentation in 70% and 10% respectively. In this study patients with stable non-segmental vitiligo were also included. The differences between Tegta's and our findings show that repigmentation might also be determined by other factors than the number of transplanted cells per mm^2. Moreover, patients' characteristics like vitiligo disease activity or skin type could play a role. Our results emphasize that further research is needed to find out which factors other than cell content, are essential for inducing successful repigmentation after cell suspension transplantation. Maybe other markers for disease activity could correlate with transplantation success. Our findings suggest that the proportion of viable cells in the cell suspension is a more important factor than the absolute number of cells or melanocytes. Factors other than graft composition seem to be responsible for the clinical outcomes.

In chapter 6a we did not find a relationship between the percentage of viable cells in the suspension and repigmentation, while we did find this in chapter 6b. Possibly, this difference is because the studies included different patient populations, non-segmental vitiligo in chapter 6a and segmental vitiligo and piebaldism in chapter 6b. This difference is not very surprising, since non-segmental vitiligo is defined by autoimmune activity more so than in segmental vitiligo and in piebaldism autoimmunity does not seem to play a role. Maybe in non-segmental vitiligo more is needed than only living cells in a transplantation to achieve successful repigmentation. Moreover, in the study with non-segmental vitiligo, clinical outcomes were generally so poor, that a relation between clinical outcome and composition of cell suspension would be difficult to find.

To date, there is no consensus on the role of phototherapy in the surgical treatment of vitiligo.[13] The objective of **chapter 7** was to identify whether phototherapy improves the outcome of melanocyte transplantation in vitiligo. We reviewed 39 studies investigating 1624 patients with vitiligo. We found 2 RCTs, 1 clinical trial and 1 case series comparing phototherapy (monochromatic excimer light or laser, PUVA, NB-UVB) with no phototherapy after melanocyte transplantation.[32–35]

Our review confirm the benefit of phototherapy for melanocyte transplantation. Recent guidelines and a recent systematic review also have recommended combination therapy (NB-UVB + topical treatment) in the treatment of vitiligo and state that combination therapies are associated with more repigmentation than monotherapies.[13,15,36,37] Four studies directly compared phototherapy modalities. These studies found no significant differences in repigmentation between phototherapy modalities. The eligible studies showed high heterogeneity in terms of outcome measures, transplantation techniques, stability of vitiligo, phototherapy regimen, quality and follow-up duration. We also found a high variation in measurement instruments, which further impedes the comparison between studies. Therefore, we recommend a fast introduction of a core set of measurement instruments, especially on the assessment of repigmentation, possibly including the VES and SA-VES. A recent systematic review and meta-analysis shows that monotherapy with NB-UVB is even as effective and in some studies more effective than PUVA in vitiligo.[38] Therefore, we would recommend NB-UVB as the standard phototherapy after melanocyte transplantation. The immunosuppressive effects and safety profile of NB-UVB make this modality the most preferred phototherapy option, especially in non-segmental vitiligo. In conclusion, the results of our review suggest that phototherapy improves the outcome of melanocyte transplantation in vitiligo. We recommend NB-UVB as standard phototherapy after melanocyte transplantation for segmental and non-segmental vitiligo. More prospective randomized controlled studies are needed to investigate the additional benefit of the different phototherapy modalities and the preferred moment of phototherapy for non-segmental and segmental vitiligo separately.

Future

Recent insights into the pathophysiology of vitiligo, like the important role of the cytokine interferon gamma and the intracellular JAK-STAT pathway have led to investigations for new treatment modalities in the field of vitiligo. Several new therapies are being developed and investigated.[12,39,40] However, for the clinical implementation of these new treatments it is crucial to know the vitiligo patients' need for novel treatment modalities. **Chapter 8** clearly demonstrates the substantial demand of vitiligo patients for new therapies. Ninety-four percent of the vitiligo patients included in this study indicate that new and better treatments are needed for vitiligo. Consequently, a majority of these patients is willing to participate in clinical trials for improvement of vitiligo therapy. Moreover, approximately half of the patients felt that their current treatment was not effective and was not satisfied with it. In this study we also found that patients with facial vitiligo also seemed to be more satisfied with current treatments. This

could be clarified by the fact that facial lesions often respond better to therapy than lesions on other body regions.[41] Furthermore, we found that patients that are little or not satisfied with current treatments, are more demanding for new treatments. It is however remarkable that even patients who were (very) satisfied with current treatments, still expressed the opinion that new treatments are needed for vitiligo. The results of this exploratory study underline the demand for improved and novel vitiligo therapies. In addition, the disease burden seems to be dependent on the visibility of the location of the vitiligo lesion. Summarizing, the vast majority of all patients included, demands novel treatments and is willing to participate in clinical trials.

In conclusion, this thesis sheds light on how to interpret outcome measures and investigated how to optimally regain color in depigmented lesions with transplantation and light therapy. We recommend the use of a high output UV-flash light and find the results of UV photography in vitiligo imaging very promising. We determined the MIC of the VES and SA-VES and severity strata for the SA-VES which enabled the translation into a global vitiligo grading for extent, severity and impact. Furthermore, we could not find additional benefit for an intensified regimen of NB-UVB light therapy or cell suspension transplantation in non-segmental vitiligo. We suggest phototherapy improves the outcome of melanocyte transplantation in vitiligo. Lastly, we demonstrate a substantial demand of vitiligo patients for new therapies. Future studies will hopefully color the blanks and shed light upon remaining questions further optimizing the treatment of vitiligo.

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10a

Summary

Light and Color

Imaging, Outcome Measures and Treatment in Vitiligo

Vitiligo is an acquired cutaneous condition associated with white patches caused by destruction of melanocytes and it has a significant negative effect on the quality of life. The overall aim of this thesis is to improve the care of vitiligo by broadening the knowledge about vitiligo, with specific focus on imaging (using UV colored light), outcome measures and treatment including NB-UVB light treatment and surgical techniques.

Imaging

Ultraviolet (UV) light is an essential tool to assess the extent, spreading and staging of vitiligo. Different UV light set-ups are used for vitiligo photography, including: (i) hand-held Wood's lamps; (ii) softboxes with UV lamps; (iii) UV flash attached to the camera; (iv) high output UV flash. The objective of **chapter 2a** was to compare UV light set-ups for vitiligo photography regarding image quality and ease of use. Patients with vitiligo lesions with unclear borders were included. Two images were made with all four UV set-ups per patient, for a large and a small area. Three blinded vitiligo experts separately scored image quality on five criteria: overall quality, clearness of borders, contrast, sharpness and for larger areas the shadows. The two professional medical photographers scored the ease of use for each set-up. A total of 88 photos were assessed from 11 patients. For larger areas the frequency of a 'good' or 'very good' overall quality rating was 12.1% (Wood's), 6.1% (softboxes), 15.2% (camera flash) and 78.8% (high output flash). For smaller areas the score 'good' or 'very good' was given to 54.5%, 3%, 66.6% and 84.8% in the same order. For the shadow criteria, each set-up scored below 40% on a 'good' or 'very good' score. The photographers scored the high output flash as most easy to use. We concluded that the UV set-ups strongly influenced the quality scores of the obtained images. The high output flash scored best for both small and large areas and for ease of use. For small areas, the Wood's lamp and camera flash were acceptable. All set-ups scored badly for shadows, more research is needed to find the optimal exposure to avoid shadows.

Conventional cameras are insensitive for UV light. UV photography uses a camera capable of preferentially detecting UV rays and filtering out visible light. Hence, it captures all rays emitted by the UV lamps, which possibly improves the imaging of vitiligo. UV photography might improve image quality and ameliorate the assessment of target lesions in vitiligo. The objective of **chapter 2b** was to determine image quality and the validity and reliability of UV photography for the assessment of vitiligo target lesions. Images of patients with vitiligo were made

with UV photography, a conventional camera and lesions were drawn on graph paper and transparent sheets. Vitiligo experts and medical interns scored image quality. The intraclass correlation coefficients (ICCs) of the lesion size determined with UV photography combined with digital surface measurement and the other techniques were hypothesized to be above 0.6. The ICCs between UV images taken by the same physician and between two different physicians were calculated for determining inter- and intra-reliability. In total 31 lesions of 17 patients were included. Image quality was assessed as good or very good for 100% and 26% for UV photography and the conventional camera respectively. ICCs of UV photography and the conventional camera, drawing the lesions on transparent sheets and graph paper were respectively 0.984, 0.988 and 0.983, confirming our hypotheses. The ICCs of the intra-rater and inter-rater were 0.999 and 0.998 respectively. The results of this study indicate that the use of UV photography for the assessment of vitiligo lesions improves image quality and is valid and reliable.

Outcome measures

For the evaluation of overall repigmentation of vitiligo, two new outcome instruments were developed to measure the extent of vitiligo: the Vitiligo Extent Score (VES) and the Self-Assessment Vitiligo Extent Score (SA-VES). The VES and SA-VES have been validated and are feasible and user-friendly outcome measurement instruments for assessing the overall extent of vitiligo. To improve the interpretability of these measurement instruments we determined the minimal important change (MIC) in **chapter 3.** The MIC is the smallest change in score that patients (or clinicians) perceive as important. Patients with non-segmental vitiligo, starting therapy were included in hospitals in Egypt, Korea, Singapore and the Netherlands. The MIC of the VES and SA-VES was determined using the anchor-based mean change method. In total 135 patients completed the study, with a mean age of 42.3 years, 79 (58.5%) were female. We found a MIC of -0.53% and -0.20% for the VES and SA-VES respectively. The MIC's we found are smaller than previously reported smallest detectable changes of these outcome measurements. The size of subgroups were too small to make reliable estimates of the MIC for the hands. Our results improve the interpretability of these measurement instruments showing the smallest change in SA-VES and VES scores that patients and clinicians perceive as important. Our results can be used to determine number of responders in future studies.

For the SA-VES, a patient reported outcome measure, information on how to translate the obtained score into extent, severity and impact strata (mild-moderate-severe) was still lacking. Stratification is helpful to define inclusion criteria for trials, enables comparison and pooling of trial results and can be used

for epidemiological research. The aim of **chapter 4** was to develop extent, severity and impact strata for the SA-VES based on validated anchor based questions. In total, 316 patients with non-segmental vitiligo (age ≥16) recruited at the Ghent University Hospital (Belgium) completed a questionnaire that was conducted in cooperation with the Dutch Society for Vitiligo patients to ensure content validity. First three anchor questions included in the questionnaire [Patient Reported Global Assessment (PR-GA) for vitiligo extent, severity and impact] were assessed for content validity, construct validity and intrarater reliability. Subsequently, the PR-GAs were used to stratify the SA-VES based on ROC analysis. For all PR-GAs (PR-GA severity, PR-GA extent, PR-GA impact), at least 75% of hypotheses evaluated for construct validity were confirmed. Intrarater reliability of all PR-GAs were good to excellent (ICCs PR-GA severity: 0.828; PR-GA extent: 0.623; PR-GA impact: 0.851). This study provides the first guide for the interpretation of the numerical output obtained by the SA-VES (vitiligo extent) and enables the translation into a global vitiligo grading for extent, severity and impact.

Treatment

Treatment of non-segmental vitiligo remains a challenge. For many years now, vitiligo therapy consists of topical agents, phototherapy and surgical techniques, which all aim to minimize disease progression and/or stimulate repigmentation. Efficacy of NB-UVB light treatment may increase with more frequent use or in combination with topical agents. Currently, data on the most effective treatment regimen are lacking. The objective of **chapter 5** was to retrospectively compare NB-UVB treatment regimens for non-segmental vitiligo. Patients with non-segmental vitiligo treated with NB-UVB treatment were included in two time periods. Group I received NB-UVB treatment twice a week (conventional treatment) and group II received NB-UVB thrice a week, combined with topical agents (intensified treatment). Patients completed a questionnaire regarding the degree and onset of repigmentation, satisfaction and side effects. Repigmentation scores did not differ significantly between the two groups. Onset of repigmentation in the first three months seemed higher in group II, but this difference was not significant (23.4% vs 51.1%; p=0.11). In both groups, the majority of the patients were moderately to very satisfied (group I: 70.2% group II: 73.3%). The occurrence of adverse effects was comparable. This study indicates that conventional and intensified treatment for non-segmental vitiligo seem to be comparable. The intensified treatment might be more effective to speed up the onset of repigmentation, but larger prospective studies are needed to objectify these findings.

Autologous cell suspension transplantation (CST) is an effective surgical treatment method in segmental vitiligo and piebaldism. However, the efficacy in non-segmental vitiligo is not yet sufficiently validated in randomized controlled trials. Stability of vitiligo for 12 to 24 months is generally regarded essential for this therapy, which does not occur in all patients. The objective of **chapter 6a** was to assess the efficacy and safety of CST in patients with non-segmental vitiligo receiving concurrent standard of care immunosuppressive therapy (NB-UVB treatment + topical anti-inflammatory). A prospective, randomized, observer blinded, within-patient controlled trial was performed in patients with non-segmental vitiligo receiving standard of care (311 nm UVB treatment and topical anti-inflammatory therapy) for at least 6 months. To indicate disease stability, a punch grafting test was performed and evaluated after 3 months; patients were randomized only if the punch grafting test was successful. Subsequently, two comparable depigmented regions per patient were randomized to receive CST or no grafting, while standard of care was continued. Cellular composition of the graft was analyzed by flow cytometry. Outcomes were assessed after 3 and 6 months. The median repigmentation in 17 patients of treated and control areas were 17.3% and 9% after 3 months and 5.3% and 8.3% after six months (p=0.11), respectively. Repigmentation failure could not be attributed to graft composition. Twelve patients had progressive disease before starting NB-UVB treatment and topical therapy. Two patients showed repigmentation of more than 75%. The repigmentation of the treated lesion was scored as good to very good by three of the patients versus one patient for the control lesion. The study was discontinued due to insufficient effect in the evaluated patient population. Our findings indicate that non-segmental vitiligo lesions that are unresponsive to prolonged standard of care will generally not benefit from adding CST to standard of care and stability of vitiligo without therapy might be required for successful CST.

The objective of **Chapter 6b** was to determine whether the number of transplanted (viable) melanocytes and overall cell numbers correlate with repigmentation after CST for patients with segmental vitiligo and piebaldism. Twenty patients with segmental vitiligo or piebaldism were treated with suspension grafts from split-skin grafts using a cell-harvesting device. Correlations between repigmentation six months after transplantation and melanocytes, viable melanocytes, or total transplanted cell numbers were determined. Twenty patients had a median repigmentation of 78%. No significant correlations were found between the absolute number of (viable) melanocytes or (viable) total cells and repigmentation. Successful repigmentation was induced by both low and high numbers of melanocytes transplanted per mm^2. The proportion of viable cells during CST correlated significantly with repigmentation rate (p=0.015). Our findings suggest

that repigmentation is determined predominantly by the proportion of viable cells in the cell suspension, rather than melanocyte content or absolute cell number.

Frequently CST is combined with phototherapy to improve results. However, it is not known whether there is an additional benefit of phototherapy and which phototherapy modality combined with melanocyte transplantation leads to optimal repigmentation. In **chapter 7,** we performed a systematic review to determine whether phototherapy improves the outcome of melanocyte transplantation in vitiligo patients. We searched and screened for eligible studies in the databases of MEDLINE, EMBASE and CENTRAL. We included all clinical studies investigating melanocyte transplantation techniques combined with phototherapy. We included 39 eligible clinical studies, based on the selection criteria, comprising 1624 patients in total. The eligible studies investigated several phototherapy modalities, such as NB-UVB (n=9), PUVA (n=19), UVA (n=1), MEL (n=4) and active sunlight exposure (n=9). The studies directly comparing phototherapy versus no phototherapy, confirmed the added value of phototherapy for melanocyte transplantation. No significant differences in repigmentation between studies directly comparing phototherapy modalities were found. The overall quality of the studies was moderate to poor and showed high heterogeneity between studies. We conclude that phototherapy improves the outcome of melanocyte transplantation in vitiligo. More studies should be performed investigating the additional benefit of different types of phototherapy, the preferred timing of phototherapy and also the safety of phototherapy upon melanocyte transplantation for vitiligo.

Next to the above mentioned conventional treatment modalities, several new therapies are being developed and investigated. There is, however, little evidence on the actual need for novel therapies. The objective of **chapter 8** was to assess patients' perspective on current and novel therapies for vitiligo. A prospective questionnaire study was conducted in a cohort of vitiligo patients that consecutively visited the Amsterdam UMC between 2017 and 2018. Patients were requested to fill in a questionnaire on disease burden, quality of life, satisfaction of current treatments and aspects regarding new treatments. A total of 325 vitiligo patients completed the questionnaire (60% response rate). Of the respondents, 94% believed that new and improved treatments are needed and 86% would be willing to participate in clinical trials investigating a new therapy. Sixty-nine percent would agree on taking weekly injections if it led to effective treatment results. Of the patients that had received therapy before, 49% reported that the current treatments were not effective and 50% was not satisfied with the current treatments. Sixty-seven percent of the patients experienced facial lesions as an extreme burden, whereas this was, 25%, 12% and 10% for lesions on the hands,

trunk and feet respectively. The emotional burden score was significantly higher in dark skin types compared to light skin types (respectively 8 vs 5, $p < 0.05$). We conclude that there is a substantial need for new vitiligo therapies. A considerable number of patients in our study is dissatisfied with current treatments and is emotionally burdened by the disease. Moreover, the vast majority demands novel treatments and is willing to participate in clinical trials.

In conclusion, this thesis sheds light on how to interpret outcome measures and investigated how to optimally regain color in depigmented lesions with transplantation and NB-UVB light treatment. Future studies will hopefully color the blanks and shed light upon remaining questions further optimizing the treatment of vitiligo.

10b

Samenvatting (Nederlands)

Licht en Kleur

Beeldvorming, Uitkomstmaten en Behandeling van Vitiligo

Vitiligo is een verworven huidaandoening geassocieerd met witte vlekken veroorzaakt door vernietiging van melanocyten en heeft een significant negatief effect op de kwaliteit van leven. Het doel van dit proefschrift is om de vitiligo zorg te verbeteren door de kennis over vitiligo te verbreden, met specifieke focus op beeldvorming (met UV-gekleurde straling), uitkomstmaten en behandeling, waaronder NB-UVB behandeling en chirurgische technieken.

Beeldvorming

Ultraviolet (UV) lampen zijn een essentieel hulpmiddel om de uitgebreidheid en verspreiding van vitiligo te beoordelen. Er worden verschillende UV-opstellingen gebruikt voor vitiligo-fotografie, waaronder: (i) Wood's lampen; (ii) softboxen met UV-lampen; (iii) UV-flitser bevestigd aan de camera; (iv) UV-flitser met hoog vermogen. Het doel van **hoofdstuk 2a** was het vergelijken van UV-opstellingen voor vitiligo fotografie met betrekking tot beeldkwaliteit en gebruiksgemak. Patiënten met onscherp begrensde vitiligo laesies werden geïncludeerd. Er werden per patiënt twee afbeeldingen gemaakt met alle vier de UV-instellingen, van een groot en een klein gebied. De beeldkwaliteit werd afzonderlijk beoordeeld door drie geblindeerde vitiligo experts op vijf criteria: algehele kwaliteit, duidelijke randen van de laesies, contrast, scherpte en voor grotere gebieden schaduwvorming. Twee professionele medische fotografen scoorden voor elke opstelling het gebruiksgemak. In totaal werden 88 foto's beoordeeld van 11 patiënten. De algemene kwaliteit van afbeeldingen van grotere gebieden werd als 'goed' of 'zeer goed' beoordeeld in 12,1% (Wood's), 6,1% (softboxen), 15,2% (cameraflitser) en 78,8% (high-output flitser) van de gevallen. Voor kleinere gebieden werd de score 'goed' of 'zeer goed' gegeven aan 54,5%, 3%, 66,6% en 84,8% in dezelfde volgorde. Elke opstelling scoorde in minder dan 40% van de gevallen een 'goede' of 'zeer goede' score voor de schaduwvorming. De flitser met hoge output vonden de fotografen het gebruiksvriendelijkst. De UV-opstellingen kunnen de kwaliteitsscores van de verkregen afbeeldingen dus sterk beïnvloeden. De flitser met hoge output scoorde het beste voor zowel kleine als grote gebieden en voor gebruiksgemak. Voor kleine gebieden waren de Wood's lamp en cameraflitser acceptabel. Alle opstellingen scoorden slecht qua schaduwvorming; er is meer onderzoek nodig om de optimale belichting te vinden om schaduwvorming te voorkomen.

Conventionele camera's zijn ongevoelig voor UV-straling. UV-fotografie maakt gebruik van een camera die UV stralen kan doorlaten en zichtbaar licht eruit kan

filteren. Deze vorm van fotografie vangt alle stralen op die door de UV-lampen worden uitgezonden, wat mogelijk de beeldvorming van vitiligo verbetert. UV-fotografie kan de beeldkwaliteit verbeteren en de beoordeling van specifieke laesies bij vitiligo verbeteren. Het doel van **hoofdstuk 2b** was om de beeldkwaliteit, validiteit en betrouwbaarheid van UV-fotografie te bepalen voor de beoordeling van specifieke vitiligo laesies. Afbeeldingen van patiënten met vitiligo werden gemaakt met UV-fotografie, een conventionele camera en laesies werden getekend op ruitjespapier en transparante vellen. De beeldkwaliteit werd gescoord door vitiligo-experts en coassistenten. De intraclass-correlatiecoëfficiënten (ICC's) van de gemeten laesie oppervlakten bepaald met UV-fotografie gecombineerd met digitale oppervlaktemeting en de andere technieken werden verondersteld hoger te zijn dan 0,6. De ICC's tussen twee foto's gemaakt door dezelfde arts en tussen twee verschillende artsen werden berekend voor het bepalen van inter-beoordelaar en intra-beoordelaar betrouwbaarheid. In totaal zijn 31 laesies van 17 patiënten onderzocht. De beeldkwaliteit werd beoordeeld als goed of zeer goed voor respectievelijk 100% en 26% voor UV-fotografie en de conventionele camera. ICC's van UV-fotografie en de conventionele camera, de transparante vellen en ruitjespapier, waren respectievelijk 0,984, 0,988 en 0,983, wat onze hypothesen bevestigt. De ICC's van de intra-beoordelaar en inter-beoordelaar betrouwbaarheid waren respectievelijk 0,999 en 0,988. De resultaten van dit onderzoek geven aan dat het gebruik van UV-fotografie voor de beoordeling van vitiligo-laesies, de beeldkwaliteit verbetert en valide en betrouwbaar is.

Uitkomst maten

Voor de evaluatie van de algehele repigmentatie van vitiligo zijn twee nieuwe meetinstrumenten ontwikkeld om de mate van vitiligo uitgebreidheid te meten met behulp van vragenlijsten: de Vitiligo Extent Score (VES) en de Self-Assessment Vitiligo Extent Score (SA-VES). De VES en SA-VES zijn gevalideerd en gebruiksvriendelijke meetinstrumenten voor het meten van de algehele uitgebreidheid van vitiligo. Om de interpreteerbaarheid van deze metingen te verbeteren, hebben we de minimale belangrijke verandering (*Minimal Important Change* - MIC) bepaald in **hoofdstuk 3**. De MIC is de kleinste verandering in score die patiënten (of clinici) als belangrijk beschouwen. Dit is onderzocht bij patiënten met non-segmentale vitiligo, bij aanvang van behandeling in ziekenhuizen in Egypte, Korea, Singapore en Nederland. De MIC van de VES en SA-VES werd bepaald met behulp van de anker methode voor gemiddelde verandering. In totaal voltooiden 135 patiënten het onderzoek, met een gemiddelde leeftijd van 42,3 jaar, 79 (58,5%) waren vrouwen. We vonden een MIC van -0,53% en -0,20% voor respectievelijk de VES en SA-VES. De MIC's die we hebben gevonden zijn kleiner dan eerder gerapporteerde minimaal detecteerbare veranderingen door

deze uitkomstmaten. De grootte van subgroepen was te klein om betrouwbare schattingen te maken van de MIC voor de handen. Onze resultaten verbeteren de interpreteerbaarheid van deze meetinstrumenten en laten zien wat de kleinste verandering in SA-VES en VES-scores is die patiënten en clinici belangrijk vinden. Onze resultaten kunnen worden gebruikt om het aantal respondenten in toekomstige studies te bepalen.

Voor de SA-VES, een door de patiënt gerapporteerde uitkomstmaat, ontbrak nog informatie over hoe de verkregen score in uitgebreidheid, ernst en impact strata (licht-matig-ernstig) moest worden vertaald. Stratificatie is nuttig om inclusiecriteria voor onderzoeken te definiëren, het maakt vergelijking en poolen van resultaten mogelijk en kan worden gebruikt voor epidemiologisch onderzoek. Het doel van **hoofdstuk 4** was het ontwikkelen van uitgebreidheid-, ernst- en impact-strata voor de SA-VES op basis van gevalideerde ankervragen. In totaal zijn er 316 patiënten met non-segmentale vitiligo (leeftijd ≥16) gerekruteerd in het Universitair Ziekenhuis Gent (België), zij hebben een vragenlijst ingevuld die werd ontwikkeld in samenwerking met de Nederlandse Vereniging voor Vitiligo-patiënten om de validiteit van de inhoud te waarborgen. De eerste 3 ankervragen in de vragenlijst [*Patient Reported Global Assessment* (PR-GA) voor uitgebreidheid, ernst en impact van vitiligo] werden beoordeeld op inhoudsvaliditeit, constructvaliditeit en intra-beoordelaar betrouwbaarheid. Vervolgens werden de PR-GA's gebruikt om de SA-VES te stratificeren op basis van een ROC-analyse. Voor alle 3 PR-GA's werd ten minste 75% van de hypothesen, die waren beoordeeld op constructvaliditeit, bevestigd. De intra-beoordelaar betrouwbaarheid van alle PR-GA's was goed tot uitstekend (ICC's PR-GA-ernst: 0,828; PR-GA-uitgebreidheid: 0,623; PR-GA-impact: 0,851). Deze studie geeft een eerste aanzet tot de interpretatie van de numerieke output verkregen door de SA-VES (vitiligo-uitgebreidheid) en maakt de vertaling mogelijk naar een globale vitiligo-beoordeling voor uitgebreidheid, ernst en impact.

Behandeling

Behandeling van non-segmentale vitiligo blijft een uitdaging. Vitiligo behandeling bestaat al vele jaren uit lokale middelen, NB-UVB behandeling en chirurgische technieken, die er allemaal op gericht zijn de ziekteprogressie te minimaliseren en/ of repigmentatie te stimuleren. De werking van NB-UVB behandeling kan verbeteren door herhaaldelijk gebruik of in combinatie met lokale middelen. Momenteel ontbreken gegevens over het meest effectieve behandelregime. Het doel van **hoofdstuk 5** was om NB-UVB behandelingsregimes voor non-segmentale vitiligo retrospectief te vergelijken. Patiënten met vitiligo behandeld met NB-UVB behandeling werden opgenomen in twee tijdsperioden. Groep I kreeg tweemaal

per week een NB-UVB behandeling (conventionele behandeling) en groep II kreeg driemaal per week een NB-UVB behandeling, gecombineerd met lokale middelen (intensieve behandeling). Patiënten vulden een vragenlijst in over de mate en aanvang van repigmentatie, tevredenheid en bijwerkingen. Repigmentatie scores verschilden niet significant tussen de twee groepen. Het vroeg beginnen van repigmentatie in de eerste drie maanden leek hoger in groep II, maar dit verschil was niet significant (23,4% versus 51,1%; p = 0,11). In beide groepen was de meerderheid van de patiënten matig tot zeer tevreden met de behandeling (groep I: 70,2% groep II: 73,3%). Het optreden van bijwerkingen was vergelijkbaar. Deze studie geeft aan dat conventionele en geïntensiveerde behandeling voor non-segmentale vitiligo vergelijkbaar lijken. De geïntensiveerde behandeling kan effectiever zijn om het begin van repigmentatie te versnellen, maar grotere prospectieve studies zijn nodig om deze bevindingen te bevestigen.

Autologe celsuspensietransplantatie (CST) is een effectieve chirurgische behandelmethode bij segmentale vitiligo en piebaldisme. De werkzaamheid bij non-segmentale vitiligo is echter nog onvoldoende gevalideerd in gerandomiseerde gecontroleerde studies. Stabiliteit van vitiligo gedurende 12 tot 24 maanden wordt over het algemeen als essentieel beschouwd voor het slagen van deze therapie, maar dit komt niet bij alle patiënten voor. Het doel van **hoofdstuk 6a** was om de werkzaamheid en veiligheid van CST te beoordelen bij patiënten met non-segmentale vitiligo tijdens standaardbehandeling met immunosuppressieve therapie (NB-UVB behandeling gecombineerd met lokale behandeling). Een prospectieve, gerandomiseerde, voor de observator geblindeerde, binnen de patiënt gecontroleerde studie werd uitgevoerd bij patiënten met non-segmentale vitiligo die standaardzorg kregen (311 NB-UVB behandeling en lokale ontstekingsremmende therapie) gedurende ten minste 6 maanden. Om de stabiliteit van de ziekte te evalueren, werd een minigraft transplantatie test uitgevoerd en geëvalueerd na 3 maanden, patiënten werden alleen gerandomiseerd voor deelname als de minigraft transplantatie succesvol was. Vervolgens werden twee vergelijkbare gedepigmenteerde regio's per patiënt gerandomiseerd om CST of geen transplantatie te ontvangen, terwijl de zorgstandaard werd voortgezet. De cellulaire samenstelling van het transplantaat werd geanalyseerd door middel van flowcytometrie. Resultaten werden beoordeeld na 3 en 6 maanden. De mediane repigmentatie bij 17 patiënten in behandelde en controlegebieden was respectievelijk 17,3% en 9% na 3 maanden en 5,3% en 8,3% na zes maanden (p = 0,11). Slechts twee patiënten vertoonden een repigmentatie van meer dan 75% in het behandelde gebied. Het falen van de behandeling kan niet worden toegeschreven aan de samenstelling van het transplantaat. Twaalf patiënten hadden progressieve ziekte voordat ze met NB-UVB behandeling en lokale

therapie begonnen. De repigmentatie van de behandelde laesie werd als goed tot zeer goed beoordeeld door drie van de patiënten versus één patiënt voor de controle laesie. De studie werd stopgezet vanwege onvoldoende effect. Onze bevindingen geven aan dat non-segmentale vitiligo laesies die niet reageren op langdurige standaardbehandeling over het algemeen geen baat zullen hebben bij het toevoegen van CST aan deze behandeling en dat stabiliteit van vitiligo zonder therapie vereist is voor succesvolle CST.

Het doel van **Hoofdstuk 6b** was om te bepalen of het aantal getransplanteerde (levende) melanocyten en het totale cel aantal te correleren was met repigmentatie na CST bij patiënten met segmentale vitiligo en piebaldisme. Twintig patiënten met segmentale vitiligo of piebaldisme werden behandeld met CST. Correlaties tussen repigmentatie zes maanden na transplantatie en melanocyten, levende melanocyten of totaal aantal getransplanteerde cellen werden bepaald. Twintig patiënten hadden een mediane repigmentatie van 78%. Er werden geen significante correlaties gevonden tussen het absolute aantal (levende) melanocyten of (levende) totale cellen en repigmentatie. Succesvolle repigmentatie was aanwezig bij zowel een laag als een hoog aantal getransplanteerde melanocyten per mm^2. Het aandeel levende cellen tijdens CST correleerde significant met het percentage repigmentatie ($p = 0,015$). Onze bevindingen suggereren dat repigmentatie voornamelijk wordt bepaald door het aandeel levende cellen in de celsuspensie, in plaats van het melanocytgehalte of het absolute cel aantal.

Vaak wordt melanocyttransplantatie gecombineerd met lichtbehandeling om de resultaten te verbeteren. Het is echter niet bekend of lichtbehandeling een bijkomend voordeel heeft en welke lichtbehandelingsmodaliteit in combinatie met melanocyttransplantatie leidt tot optimale repigmentatie. In **hoofdstuk 7** hebben we een systematische review uitgevoerd om te bepalen of lichttherapie de uitkomst van melanocyttransplantatie bij vitiligo patiënten verbetert. Met behulp van de databases MEDLINE, EMBASE en CENTRAL, hebben we alle publicaties van klinische onderzoeken opgenomen waarin transplantatietechnieken van melanocyten of huidcelsuspensies werden gecombineerd met lichttherapie. We hebben 39 publicaties geselecteerd, op basis van de selectiecriteria, met in totaal 1624 patiënten. Deze studies onderzochten verschillende modaliteiten voor lichttherapie, zoals NB-UVB ($n = 9$), PUVA ($n = 19$), UVA ($n = 1$), MEL ($n = 4$) en blootstelling aan actief zonlicht ($n = 9$). De studies die geen lichttherapie direct vergeleken met lichttherapie bevestigden de toegevoegde waarde van lichttherapie voor melanocyttransplantatie. Er werden geen significante verschillen in repigmentatie gevonden in studies die lichttherapie modaliteiten rechtstreeks vergeleken. De algehele kwaliteit van de onderzoeken was matig tot slecht en

vertoonde een hoge heterogeniteit tussen de onderzoeken. We concluderen dat lichttherapie de uitkomst van melanocyttransplantatie bij vitiligo verbetert. Er moeten meer onderzoeken worden uitgevoerd naar het aanvullende voordeel van verschillende soorten lichttherapie, de voorkeurstiming van lichttherapie en ook naar de veiligheid van lichttherapie bij melanocyttransplantatie voor vitiligo.

Naast de bovengenoemde conventionele behandelmodaliteiten, worden er verschillende nieuwe therapieën voor vitiligo ontwikkeld en onderzocht. Er is echter weinig bewijs voor de daadwerkelijke behoefte van patiënten aan nieuwe therapieën. Het doel van **hoofdstuk 8** was om het perspectief van patiënten op huidige en nieuwe therapieën voor vitiligo te beoordelen. Een prospectieve vragenlijststudie werd uitgevoerd in een cohort van vitiligopatiënten die tussen 2017 en 2018 achtereenvolgens het Amsterdam UMC bezochten. Patiënten werd verzocht een vragenlijst in te vullen over ziektelast, kwaliteit van leven, tevredenheid over huidige behandelingen en aspecten van nieuwe behandelingen. In totaal vulden 325 vitiligo-patiënten de vragenlijst in (60% respons). Van de respondenten was 94% van mening dat er nieuwe en verbeterde behandelingen nodig zijn en 86% van de respondenten zou bereid zijn deel te nemen aan klinische onderzoeken naar een nieuwe therapie. Negenenzestig procent zou instemmen met het nemen van wekelijkse injecties als dit tot effectieve behandelresultaten zou leiden. Van de patiënten die eerder therapie hadden gekregen, meldde 49% dat de huidige behandelingen niet effectief waren en was 50% niet tevreden met de huidige behandelingen. Zevenenzestig procent van de patiënten ervoer laesies in het gezicht als een extreme last, terwijl dit respectievelijk 25%, 12% en 10% was voor laesies aan de handen, romp en voeten. De score voor emotionele belasting was significant hoger bij donkere huidtypes in vergelijking met lichte huidtypes (respectievelijk 8 versus 5, $p < 0,05$). We concluderen dat er grote behoefte is aan nieuwe vitiligo-therapieën. Een aanzienlijk aantal patiënten in ons onderzoek is ontevreden over de huidige behandelingen en wordt emotioneel belast door de ziekte. Bovendien wenst de overgrote meerderheid nieuwe behandelingen en is bereid deel te nemen aan klinische studies.

Concluderend werpt dit proefschrift nieuw licht op de interpretatie van uitkomstmaten en hoe de kleur van gedepigmenteerde laesies optimaal kan worden hersteld met transplantatie en NB-UVB behandeling. Toekomstige studies zullen hopelijk de witte plekken kleuren en licht werpen op de resterende vragen om de behandeling van vitiligo verder te optimaliseren.

Appendices

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Ultraviolet photography in vitiligo: image quality, validity and reliability

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Assessing the minimal important change in the vitiligo extent score and the self-assessment vitiligo extent score

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Validation of a Patient Global Assessment for extent, severity, and impact to define the severity strata for the Self Assessment Vitiligo Extent Score (SA-VES)

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Patient Reported Outcomes for Intensified versus Conventional NB-UVB Treatment in Non-Segmental Vitiligo

S.E. Uitentuis: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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Addition of cell suspension transplantation to standard of care in non-segmental vitiligo: a randomized controlled study
S.E. Uitentuis: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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Impact of graft cell density and viability on repigmentation upon non-cultured autologous cell suspension transplantation in vitiligo and piebaldism

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The role of phototherapy in the surgical treatment of vitiligo: a systematic review.

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Patients' perspective on current treatments and demand for novel treatments in vitiligo

V.S. Narayan: Contributions to conception and design, acquisition of data, analysis and interpretations of data, drafting the article and revising it critically for important intellectual content and final approval of the version to be published. Corresponding author.

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List of publications

Publications in this thesis:

Lommerts JE, Uitentuis SE, Bekkenk MW, de Rie MA, Wolkerstorfer A. The role of phototherapy in the surgical treatment of vitiligo: a systematic review. *Journal of the European Academy of Dermatology and Venereology* 2018; **32**: 1427–1435.

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Other publications:

Wanders LK, East JE, Uitentuis SE, Leeflang MMG, Dekker E. Diagnostic performance of narrowed spectrum endoscopy, autofluorescence imaging, and confocal laser endomicroscopy for optical diagnosis of colonic polyps: A meta-analysis. *The Lancet Oncology* 2013; **14**: 1337–1347.

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PhD Portfolio

Name PhD-student	Sanne Elisabeth Uitentuis
PhD Period	December 2016 – November 2020
Promotores	prof. dr. R.M. Luiten
	prof. dr. M.A. de Rie
Co-promotores	dr. A. Wolkerstorfer
	dr. M.W. Bekkenk

	Year	Workload ECTS
1. PhD Training		
General courses and seminars		
Practical Biostatistics	2015	1.1
Scientific Writing in English for Publication	2016	1.5
Electronic Basic Course Legislation and Organization for Clinical Researchers (eBROK)	2017	1.0
Citation analysis and impact factors	2017	0.1
The AMC World of Science	2017	0.7
Clinical data management	2017	0.9
Clinical Epidemiology: Randomized Clinical Trials	2017	0.6
Observational Clinical Epidemiology: Effects & Effectiveness, exam: 9	2017	0.6
SELF (Students Experienced in Lifestyle and Food)	2018	1.0
Weekly scientific meeting of department	2016-2020	3
	Total	**10.5**
Presentations		
International Pigment Cell Conference, Denver, U.S.A. *Oral presentation:* Impact of graft cell density and viability on repigmentation upon non-cultured autologous cell suspension transplantation in vitiligo	2017	0.5
International Pigment Cell Conference, Denver, U.S.A. *Oral and poster presentation:* Development of a mobile application technique for the follow up of target lesions in vitiligo	2017	1.0
Annual meeting European Academy of Dermatology and Venereology, Paris, France *Poster presentation:* Patient reported outcomes for intensified versus conventional treatment in non-segmental vitiligo	2018	0.5
Annual meeting Dutch Society for Experimental Dermatology, Lunteren, The Netherlands *Oral presentation*: The ultraviolet light camera, a promising measurement instrument for lesion assessment in Vitiligo	2019	0.5

	Year	Workload ECTS
Annual meeting American Academy of Dermatology, Washington, U.S.A. *Oral presentation:* The ultraviolet light camera, a promising measurement instrument for lesion assessment in vitiligo	2016	0.5
International Pigment Cell Conference, online booklet (due to COVID) *Selected as oral presentation:* Assessing the minimal important change in the vitiligo extent score and the self-assessment vitiligo extent score	2020	0.5
International Pigment Cell Conference, online booklet (due to COVID) *Selected as poster presentation:* Autologous cell suspension transplantation concurrent with UVB in non-segmental vitiligo patients: A randomized controlled study	2020	0.5
	Total	**4.0**
(Inter)national conferences		
Vitiligo International Symposium, Rome	2016	0.75
Annual meeting Dutch Society for Experimental Dermatology, Lunteren	2017	1.0
International Pigment Cell Conference, Denver	2017	1.25
CSG-COUSIN and Annual Cochrane Skin Group meeting	2018	0.5
Annual meeting Dutch Society for Experimental Dermatology, Lunteren	2018	0.5
Annual meeting European Academy of Dermatology and Venereology, Paris	2018	1.0
Annual meeting Dutch Society for Experimental Dermatology, Lunteren	2019	0.5
Annual meeting American Academy of Dermatology, Washington	2019	1.25
2. Teaching		
Refereeravond Dermatologie LUMC: Chirurgische therapie bij vitiligo	2017	0.2
Supervising master student for master thesis, V.S. Nayaran	2017	1.0
Supervising master student for master thesis, M.N. Heilmann	2016	1.0
Supervising bachelor student for bachelor thesis, S.G. Ruige	2016	1.0
Histopathology teaching for course Clinical Cell Biology master students	2017 - 2018	0.5
	Total	**3.7**
3. Parameters of esteem – Awards		
Travel grant, International Pigment Cell Conference, 2016, Denver, U.S.A.		

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Curriculum Vitae

Sanne Elisabeth Uitentuis werd geboren op 7 november 1986 te Groningen. In haar eerste levensjaar verhuisde zij naar Grouw. Sanne volgde haar middelbare school opleiding aan het Piter Jelles Stedelijk Gymnasium in Leeuwarden en rondde daar in 2005 het profiel Natuur en Techniek met economie af – daarmee kon ze alle richtingen op behalve Geneeskunde. Na het afronden van haar bachelor Economie aan de Universiteit Utrecht, besloot zij toch Geneeskunde te willen studeren. Na het aanvullen van de ontbrekende vakken werd zij in 2010 decentraal geselecteerd aan de UvA. Zij rondde in 2016 de Geneeskunde studie af. Tijdens bachelor- en masterfase volgde Sanne het Honours programma waarbij zij al vroeg in aanraking kwam met onderzoek doen. Haar interesse voor dermatologie werd gewekt tijdens de coschappen; onder begeleiding van dr. M.W. Bekkenk deed zij twee onderzoeken naar Merkelcelcarcinoom. In december 2016 is zij onder leiding van prof. dr. R.M. Luiten, prof. dr. M.A. de Rie, dr. A. Wolkerstorfer en dr. M.W. Bekkenk gestart met een promotieonderzoek naar vitiligo. In februari 2019 is zij met de opleiding tot dermatoloog gestart. Naast haar studie en werk is Sanne actief wedstrijdzeiler.