Scleroderma: diagnosis and experimental therapy
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Citation for published version (APA):
Chapter 3

Treatment of patients with systemic sclerosis with extra-corporeal photochemotherapy (photopheresis)

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Published in:

*J Am Acad Dermatol* 1999;41:915-922
SUMMARY

Background: Effective treatment modalities for systemic sclerosis, a life-threatening and disabling disease, are still lacking. Possible efficacy of photopheresis has been reported in several studies. Because of the complexity of the treatment, placebo-controlled trials are difficult to perform.

Objective: We investigated the effect of photopheresis on clinical parameters (skin score and internal organ functions), immunological parameters and quality of life.

Methods: Nineteen patients with progressive systemic sclerosis of less than five years’ duration were randomized into 2 groups. One group (group A) received photopheresis for one year, the other group (group B) received no treatment at all. After 1 year the groups switched (crossover design). Photopheresis was performed on 2 consecutive days every 4 weeks; the psoralens were administered parenterally. The main outcome parameter was the skin score after 1 year of treatment compared with that of the control group.

Results: The average skin score improved with 5.4% (standard error [SE], 20.8%) in group A and deteriorated with 4.5% (SE, 13.8%) in group B (not significant; p = 0.71). Before crossover, the average increase in skin score was 5.3% (means of entire group). No change was observed in other clinical parameters. Approximately 1 year after cross-over, the skin score reversed to what would have been expected with an average increase of 5.3% per year. There was also no effect on immunological parameters. Quality of life did not change during treatment.

Conclusion: We were not able to show that photopheresis, performed as described above, is an effective treatment in systemic sclerosis. The difference in average skin score was statistically and clinically insignificant. Despite the small sample size, we concluded that the magnitude of the observed changes is too small to justify photopheresis as a regular treatment.
INTRODUCTION

Systemic sclerosis (SSc) is a rare chronic disease of unknown etiology, associated with a high morbidity and mortality.\textsuperscript{1-4} It is characterized by excessive deposition of collagen in all involved organs (skin, gastrointestinal tract, kidneys, heart, lungs). This leads to sclerosis of the skin and fibrosis in internal organs (lungs, kidney, heart), which often determines the prognosis of the disease.\textsuperscript{5} Initial symptoms are Raynaud phenomenon, skin thickening, and hardening of the skin. In most cases the first alterations start at the acral areas (hands, feet, face). To date there is no proven adequate treatment of systemic sclerosis.\textsuperscript{6-9}

Photopheresis is the repeated extracorporeal exposure of peripheral blood lymphocytes to UVA (320-400 nm) in the presence of psoralens (8-methoxypsoralen [8-MOP]). The lymphocytes are then reinfused into the patient. It has been recommended as an experimental treatment in patients with SSc.\textsuperscript{10,11} Photopheresis can be regarded as a modified extracorporeal form of PUVA treatment. PUVA treatment is an established modality for dermatological disorders such as psoriasis, atopic dermatitis, and cutaneous T-cell lymphoma (CTCL). In PUVA treatment only the T-cells that are present in the inflammatory infiltrate in the skin are exposed to UVA and psoralens. In photopheresis, the entire T-cell fraction is exposed to UVA and psoralens. It has been suggested that the treatment generates CD8 suppressor T-cells capable of efficiently suppressing auto-reactive T-cell clones, and that exposure of activated human T cells to photoactivated 8-MOP increases display of antigens recognizable by these CD8+ T cells.\textsuperscript{12} Photopheresis was initially developed for treating CTCL patients.\textsuperscript{13} It has now been approved by the U.S. Food and Drug Administration for this indication.

The working mechanism of photopheresis in systemic sclerosis and its possible effects on T cells and the human immune system have not been clarified. There have been some previous studies, but until now the number of patients treated with photopheresis is too low to draw definitive conclusions.\textsuperscript{7,11,14-16} Building up the evidence is difficult because systemic sclerosis is a rare disease, and photopheresis is an expensive, and time-consuming treatment. To study the effectiveness of photopheresis in systemic sclerosis, in terms of improvement of skin involvement, internal parameters, immunologic parameters and quality of life, we performed a randomized controlled study.
PATIENTS AND METHODS

Patients
Patients with progressive systemic sclerosis of less than 5 years duration (first symptom attributable to scleroderma) were eligible for this trial. The diagnosis of systemic sclerosis was made on the basis of the American Rheumatism Association criteria and confirmed by histopathological examination of lesional skin. The disease was considered to be progressive in case of an increase in skin score between two consecutive measurements with an interval of at least 3 months. Patients with advanced pulmonary involvement (CO-diffusion capacity less than 40%), advanced cardiac involvement (ejection fraction less than 40%) or advanced renal involvement (serum creatinine level twice the normal limit) were excluded. Patients receiving immunosuppressive drugs or drugs that might interfere with the synthesis or turnover of collagen (D-penicillamine, steroids, colchicine) were asked to discontinue these drugs for at least 3 months in the case of D-penicillamine, or 1 month in the case of steroids. The study was approved by the local ethics committee. All patients received written information about the trial and gave their informed consent.

Study design
The patients were randomized either to receive photopheresis treatment during the first year and no treatment during the second year, or no treatment during the first year and photopheresis treatment during the second year. The main outcome parameter was the average change in skin score after 1 year of treatment, compared with that of the control group.

Photopheresis procedure
Photopheresis was performed with UVAR Photopheresis Equipment (Therakos Ltd, West Chester, Pa, USA), as described before in detail. Photopheresis treatment was given on 2 consecutive days every 4 weeks for 1 year. Instead of using oral psoralens, 200 μg of stabilised aqueous 8-MOP solution (Gerot, Vienna, Austria) was injected directly into the treatment bag, according to Knobler et al. The psoralen levels in the treatment bag were monitored to make sure that they were above the recommended concentration of 60 ng/mL.
Assessment of clinical efficacy
In all patients every 3 months the skin score, oral aperture, and a hand mobility measurement (finger to palm distance and maximal width between digits I and V) was recorded by an independent dermatologist/investigator, who was not informed about the treatment schedule. A validated 4-scale skin scoring method (0 = normal skin, 1 = mild induration, 2 = moderate induration, 3 = severe induration) was used.\textsuperscript{20-22} The sum of all skin scores obtained from 74 areas of the body (see Fig. 1) was used as outcome parameter.\textsuperscript{23}

Figure 1. Improvement of skin score during photopheresis treatment. Skin score was assessed every 3 months, in 74 body areas. Black: severe induration (score 3), dark grey: moderate induration (score 2), light grey: mild induration (score 1), white: normal skin (score 0).
To determine and follow up visceral involvement, 4 months before treatment (screening period), within the week of the first treatment and after the last treatment, esophagus manometry, a lung function test (including carbon-monoxide (CO) diffusion capacity) and a cardiac output test (gate synchronized angiography using $^{99m}$Tc labelled erythrocytes) were performed.

**Laboratory studies and effects on the immune system**

Every 3 months routine blood parameters including autoantibodies and complement factors were analyzed. Biopsy specimens were obtained from lesional and non-lesional skin before and after therapy. To determine whether there could be an immunomodulatory effect of photopheresis in patients with systemic sclerosis, the following panel of immunological tests was performed: leukocyte phenotyping, including surface markers (CD3, CD4, CD8, CD19, CD57), activation molecules (HLA-DR, CD25), and adhesion molecules (CD11a, CD11b, CD11c, CD18, VLA-1, VLA-2, VLA-3, VLA-4, CD2), lymphocyte proliferation tests, and cytokine secretion patterns (sCD27, sCD25, sFcgammaRIII, Interleukin [IL]-6, interferon-gamma, endothelin, tumor necrosis factor-α, IL-2, IL-4, IL-5, IL-10, IL-12). Cellular and humoral immune responses in vivo were investigated using a standard delayed-type hypersensitivity test (Multitest IMC/CMI, Pasteur Merieux, Paris, France). The humoral immune response was tested by using the antigens Keyhole Limpet Haemocyanin and tetanus toxoid. To rule out the possibility that photopheresis induces complement activation, blood samples of 5 patients were screened for early components of the complement cascade. Possible induction of apoptosis in leukocytes was analyzed by using two techniques, in situ-nick translation and gel electrophoresis. All aforementioned investigations were performed before, during and after treatment.

**Quality of life assessment**

General health, psychological and physical distress, functional status and well-being were assessed by means of regular interviews (every 3 months) by an epidemiologist. Specific quality of live and health questionnaires were used, such as the Rotterdam Symptom Checklist, the MOS short-form general health survey, the Frenchay activity index, and the Barthel-index. 24-28

**Estimation of the costs of photopheresis treatment**

All costs directly related to the treatment (eg. equipment, disposables, medication, laboratory and other tests required for treatment, personnel, use of daycare unit) were calculated.
Statistical analysis
The primary outcome parameter was the decrease in skin involvement compared to baseline after 12 treatments (44 weeks), as evaluated by the skin score. The average relative decrease in each group was calculated and compared between groups by means of a standard t test. All analyses were performed based on the intention-to-treat principle. In case of missing data, the last observation was used (last observation carried forward).

RESULTS

Patients
Nineteen patients were included. Their baseline characteristics are summarized in Table 1; the individual parameters are shown in Table 2. Only patients with cutaneous involvement proximal of the metacarpophalangeal joints or on the trunk were included. Most patients, 15 of 19, equally distributed between the photopheresis group (8 patients) and the control group (7 patients), had scleroderma proximal to the elbows or knees, which is a recently suggested criterion for trials in diffuse systemic sclerosis. Most patients (16 of 19) had a disease duration of less than 3 years. The skin score at baseline varied between 10 to 120 (average 47.7) in the photopheresis group and between 13 to 96 in the control group (average 52.0).

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group A (photopheresis first, control in 2nd year)</th>
<th>Group B (control first, photopheresis in 2nd year)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 9)</td>
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<tr>
<td>male / female</td>
<td>3 / 7</td>
<td>1 / 8</td>
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<tr>
<td>mean age (years, ± s.e.*)</td>
<td>45.7 (6.5)</td>
<td>44.6 (4.0)</td>
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<td>minimum - maximum age</td>
<td>19-85</td>
<td>28-69</td>
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<tr>
<td>mean disease duration (month, ± s.e.)</td>
<td>23.3 (7.0)</td>
<td>10.4 (2.5)</td>
</tr>
<tr>
<td>mean baseline skin score (± s.e.)</td>
<td>47.7 (12.5)</td>
<td>52.0 (9.9)</td>
</tr>
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</table>

* s.e.: standard error
### Table 2. Patient characteristics (individual parameters)

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex (year)</th>
<th>Disease Duration (months)</th>
<th>Antibodies</th>
<th>Lung function (CO-diffusion) (%)</th>
<th>Esophagus Involvement</th>
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<tr>
<td>1</td>
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<td>85</td>
<td>2</td>
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<td>2</td>
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<td>54</td>
<td>48</td>
<td>ANA, Scl-70</td>
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<td>81</td>
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<td>ANA</td>
<td>86</td>
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<td>45</td>
<td>19</td>
<td>ANA, RF</td>
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</table>

**Clinical Efficacy**

The main outcome parameter was the total skin score, defined as the sum of the scores of 74 body areas (Fig. 1). Fig. 1 shows an example of a patient in whom the total skin score improved from 139 to 54 during photopheresis. This patient (male, age 85 years) had progressive skin involvement since 6 months just before inclusion. The skin of hands, forearms, feet, legs and buttocks became sclerotic and he had Raynaud phenomenon and a mild respiratory insufficiency.
Photopheresis in systemic sclerosis

(CO-diffusion capacity: 64%). Antinuclear antibodies, rheumatoid factor and anti-SS-A were present. In the majority of patients, however, such an impressive improvement was not observed. In the group that received photopheresis immediately (group A), the average skin score improved after 12 treatments by 5.4% (standard error [SE], 20.8%). In the same period, the skin score in the control group (group B) deteriorated by 4.5% (SE, 13.8%). The difference was not significant (p = 0.71). The individual curves, summarizing 175 skin score evaluations in 19 patients (Fig. 2), show that there is no distinct pattern of improvement in the course of disease during the period that photopheresis was administered.

By means of a mixed-model analysis of variance for repeated measurements, the average increase in absolute skin score was calculated to be 0.052 point per week in untreated patients, which is approximately 5.3% per year. During treatment, a small reduction was observed ( -0.29 point per week in group A, -0.12 point per week in group B). Approximately 1 year after crossover, the skin score reversed to what would have been expected with an average increase of 5.3% per year.

No changes, neither improvement nor deterioration, were observed in the oral aperture measurements and hand mobility measurements, esophagus manometry, cardiac function, renal function or routine laboratory tests during or after therapy. Lung function tests showed stabilization or a slight deterioration after 44 weeks in both groups. Photopheresis did not have any beneficial effect on this parameter. The lesional skin biopsy specimens showed no changes in the extend and depth of collagen deposition after treatment.

**Immunological effects of Photopheresis**

We were not able to detect statistically significant changes during or after therapy in any of the immunological parameters mentioned above. Lymphocyte proliferation tests and cytokine secretion patterns did not change during or after therapy. The cellular and humoral immune responses before and after treatment did not alter. There was no complement activation during therapy. The only significant observation we made was the induction of apoptosis in leukocytes by photopheresis\(^{30}\); the meaning of this observation is not yet clear. The same observation has been made by Yoo et al\(^{31}\) in patients with CTCL.

**Quality of life**

As expected patients scored low in the quality of life questionnaires. No change was observed during or after treatment.

**Costs**

The average total cost of photopheresis treatment, given at 2 consecutive days per month, was 67.693 NLG ($ 33,850) per patient per year.
Fig 2. Individual curves reflecting the disease activity, measured with the skin score. In
group A (upper graph), a slight average reduction (-5.4%) during the photopheresis
period was observed. In group B (lower graph) where no treatment at all was given in
this period, a slight average increase was observed (+4.5%).
DISCUSSION

Diffuse systemic sclerosis, especially the rapidly progressive subtype, is a devastating disease. To date, no drug or combination of drugs has been proven to be of value in adequately controlled prospective trials or is generally accepted as being useful. Therefore new treatment modalities that might offer even the slightest improvement or could stabilize the disease should be considered carefully. This study reported a slight improvement in skin score (+5.4%) in the photopheresis group compared with the control group (-4.5). The difference was not statistically significant. For an overall interpretation of the results one should take into account the magnitude and the clinical relevance of the observed effect, the statistical significance, the internal and external consistency of the results, the possibility of placebo-effects, the occurrence of side-effects, and the costs related to treatment. Furthermore it would be helpful if there were a theoretical background for the treatment.

The magnitude of the effect, in this study defined as the average percentage change in skin score, was low (9.9% difference between groups). The clinical relevance of a difference of 10% in skin score is limited: for the average scleroderma patient scored with the 74-body area method this would mean a reduction from score 2 to 1 in only 4-5 hand palm-size body areas (see Fig. 1). Internal consistency of data would be present if several different evaluation methods yield the same results and conclusions. We were not able to show any improvement in the more objective disease parameters like lung function, cardiac output, esophagus motility, or any of the immunological markers that could support the skin score measurements. The skin score is an accepted evaluation method for scleroderma research, but the inter-observer reliability is relatively low.22,23

In addition, the quality of life measurement and health care questionnaires did not show any consistent outcomes.

In previous studies, an overall improvement in skin score of 15% of the patients (n = 31) compared with treatment with D-penicillamin was reported.10,11 In general, these studies only mentioned improvement of the skin score, not of the more objective parameters.

An influence on the results of this and previous investigations by a placebo effect cannot be ruled out completely. Usually, the photopheresis treatment is quite impressive for the patients. Through psychologic mechanisms, the technical equipment, the high costs and the time and efforts invested by patients and health care workers make patients believe in the treatment. The patients receive a lot of attention during the treatment and have the possibility to discuss all their problems in detail with the operator nurse and the attending physician.
It is difficult to design a placebo controlled study. Currently, a randomized multicenter study is performed in which patients receive either photopheresis or 'sham-treatment'. The UVAR equipment is hidden behind a curtain, blood is taken from the patients and returned, but they can not see whether it is actually going through the machine. Trials like this and the combination of trial results in meta-analyses will soon provide sufficient evidence to evaluate the value of photopheresis in scleroderma properly.

Side-effects were not seen at all, apart from some mild symptoms of hypovolemia during treatments. The cost of treatment is considerable, mainly because of the price of the disposables and the personnel costs. Currently, new prototypes of the photopheresis machines are being developed. The new generation machines are more automatically operated, which can be time saving.

The theoretical background for the efficacy of photopheresis in scleroderma is not yet clear. The first problem is that the pathogenesis of scleroderma is not fully understood. Several mechanisms have been proposed, such as vascular alterations with microvascular endothelial cell damage, an autoimmune response, and disturbances in the control of connective tissue synthesis. The vascular alterations include increased vasopermeability, increased diapedesis of mononuclear cells into the tissue leading to formation of perivascular infiltrates, and endothelial cell damage, which may cause expression of adhesion molecules and release of cytokines. Clinically, the vascular component is demonstrated by the presence of Raynaud's syndrome, organ damage, damaged nail capillaries, and digital ulcers and necrosis.

Autoimmune mechanisms are suspected because of the presence of circulating autoantibodies against nuclear and cellular antigens. Antinuclear antibodies are present in over 90% of patients, and more than 30% have antibodies against scleroderma associated Scl-70 (anti-topoisomerase I). It has been suggested that there could be an overactive clone of pathogenic T cells in systemic sclerosis, probably CD4+ helper T cells and that the ratio T-helper/T-suppressor cells is elevated because of a decrease in T-suppressor lymphocytes.

Abnormal production or turnover of collagen is the third pathogenetic component. Research is focused at intrinsic or temporarily induced disturbances of fibroblast function, collagen turnover, and metalloproteinases and their inhibitors. It has been shown that collagen production by fibroblasts can be enhanced by cytokines such as transforming growth factor β, released from inflammatory cells.
So, under the assumption that T cells are important in the pathogenesis of systemic sclerosis, it was a logical step to evaluate the effect of photopheresis, which is reported to be an effective treatment in diseases caused by expanded populations of pathogenic T cells. Some investigators hypothesized that T cells of the malignant or pathogenetic clone are altered, lethally damaged by photopheresis. It has been shown that photopheresis indeed causes apoptosis in T-cells. The altered cells may initiate through their surface antigens an immune response and cause the host to recognize the pathogenetic clone, resulting in a favorable immune response. In vitro and animal studies support this theory, but it is not certain whether it will be the final theory behind photopheresis.

**Conclusions**

We were not able to conclude that photopheresis twice a month for 1 year, with parenteral administration of the psoralens, is an effective treatment in systemic sclerosis. There is an imbalance between the observed minor average improvement in skin score and the duration, intensity and costs of the treatment. Because no changes in objective internal parameters, quality of life, or any effect on the immune system were observed, we concluded that, for the present, photo-pheresis given in the frequency as described above should not be considered as suitable therapy for systemic sclerosis.

On the basis of this report and the current literature, the Dutch minister of public health decided in November 1997 not to approve nor to reimburse costs of photopheresis for systemic sclerosis.
REFERENCES

Photopheresis in systemic sclerosis


