Scleroderma: Diagnosis and Experimental Therapy

Dory Enomoto

Stellingen

behorend bij het proefschrift:

Scleroderma: diagnosis and experimental therapy

1. Diffuse huidsclerose heeft een slecht voorspellende waarde voor de prognose van sclerodermie.

2. Er bestaat geen genezende therapie voor systemische sclerodermie.

3. De aanwezigheid van het Raynaud fenomeen is een belangrijk hulpmiddel bij het stellen van de diagnose systemische sclerodermie.

4. Indien er géén sprake is van sclerose aan de handen, moet een andere diagnose dan systemische sclerodermie overwogen worden.

5. Studying therapies for scleroderma is a frustrating experience for many clinical investigators *(Rook AH et al. J Rheum 1993;20:1081).*

6. Het placebo effect van technisch gecompliceerde en kostbare behandelingen kan nooit uitgesloten worden.

7. Onkunde gewint vaker zelfvertrouwen dan kennis *(Charles Darwin).*

8. Elk nadeel heb zijn voordeel *(Johan Cruyff).*

9. Het is een algemeen maatschappelijk belang dat voor vrouwelijke specialisten (in opleiding) de mogelijkheid bestaat werk en het krijgen van kinderen te combineren. Het feit dat zij slechts gedurende een zeer beperkte tijd niet inzetbaar zijn in het arbeidsproces, weegt immers niet op tegen de positieve inbreng van vrouwelijke artsen binnen de patiëntenzorg *(Vrouwelijke arts in zwang. Rapport van de Commissie Positie Vrouwelijke Artsen).*

10. Het is algemeen bekend dat Nederland een plat land is. Het is derhalve onjuist om te spreken over het "platteland" zodra iemand 100 km naar het noorden verhuist.

Dory Enomoto, 27 november 2000

SCLERODERMA: DIAGNOSIS AND EXPERIMENTAL THERAPY

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Aan mijn ouders

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Chapter 1

General introduction and aims of the study

Enomoto DNH.

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Behandeling van chronische progressieve sclerodermie
met behulp van fotoferese
Uitgave van de ZiekenfondsRaad
Amstelveen, april 1998 (Editie 92/005)

INTRODUCTION

Systemic sclerosis, especially the rapidly progressive diffuse systemic sclerosis variant, can be a devastating disease, with a high morbidity and mortality. The etiology is unknown, and until now no adequate treatment seems to be available. Within a few years, previously healthy patients have to cope with progressive hardening (sclerosis) of the skin, joint immobility, pain, sclerosis of facial skin, changes in facial expression, pigmentation disorders, Raynaud's phenomenon, painful ulcers at the fingertips, shortness of breath caused by reduced lung function, renal disorders, hypertensive crises, cardiac failure, esophageal dysmotility, and intestinal obstruction. Many patients have to quit their work and need special medical and paramedical care, and special facilities in or around their homes. In addition to this, the disease causes great psychological distress, because of its insidious and progressive nature, and because elementary questions like 'what is causing this disease and what will be my prognosis' cannot be answered. Even more difficult to accept, both for patients and doctors, is the lack of therapeutical possibilities.

In this perspective, it is understandable that the medical specialists that deal with this disease, dermatologists, rheumatologists, internists and pulmonologists are interested in investigating any possible new treatment modality. At the same time, a responsible approach is required to avoid that patients, who are desperate and willing to try anything, are given false hope or are exposed to experimental treatments that are potentially dangerous or ineffective. Another important requirement is a good cooperation between all involved specialists.

In the period 1989 -1991, some preliminary case reports and abstracts were published suggesting that photopheresis (extracorporeal photochemotherapy), a treatment modality developed for the treatment of cutaneous T-cell lymphoma, might be effective in systemic sclerosis.[401,402] Photochemotherapy, or PUVA therapy, is a familiar concept within the dermatological specialty, routinely used in psoriasis, atopic dermatitis, and cutaneous T-cell lymphoma. For these indications, the patients are prescribed oral psoralens and the skin is being exposed to ultraviolet A radiation. In extracorporeal photochemotherapy, blood is drawn from the patient, centrifuged, the lymphocyte fraction is exposed to UVA in the presence of psoralens, and then returned to the patient. We were highly interested in investigating this newly developed treatment modality in patients with systemic sclerosis. But soon it became clear that the costs involved with starting up a photopheresis unit (costs of the equipment, the disposables and sufficient personnel to operate the unit) were far too high to be financed from our regular hospital budget.

In the same period, the Dutch health care insurance council (ZiekenfondsRaad) in cooperation with the Ministry of Health launched a research program, known as 'developmental medicine' (FOGM, Ontwikkelingsgeneeskunde) which made it possible to apply for a research grant. The evaluation of a new, complex and expensive treatment like photopheresis fitted well within the research target of this program, and the grant application was approved.

Goal of the study
The primary goal of the study was to investigate the effect of photopheresis, given once every 4 weeks at 2 consecutive days, on clinical parameters like sclerosis of the skin and internal organ function.

Study design
A randomized controlled cross-over design was used. The main outcome parameters were skin score (skin induration), lung function, heart function, and kidney function. Only patients with progressive diffuse (type II or III, see table 2) systemic sclerosis of less than 5 years duration were included. The patients were observed for 4 months to measure the slope of their disease progression, and then randomized into two groups. One group received photopheresis for 1 year, the control group received no treatment at all. After 1 year the control group and treatment group switched. Every 3 months skin sclerosis was scored by an independent observer. Clinimetry, quality of life assessment, and routine and immunological laboratory tests were performed regularly.

Secondary goals
Secondary goals were to investigate the effect of photopheresis on the immune system, to investigate the impact of the treatment on the quality of life, and to calculate the costs that would be involved when this treatment modality would be implemented in the Dutch health care system.

In this introductory chapter, the relevant literature on issues like classification, epidemiology, diagnostic criteria, etiology, and treatment of scleroderma, and the principles behind photopheresis will be reviewed.

In *chapter 2*, some closely related clinical conditions are discussed to illustrate the pitfalls in establishing the diagnosis of systemic sclerosis.

In *chapter 3*, the main results of the photopheresis study are presented, with the focus on clinical efficacy.

RELATED RESEARCH SUBJECTS

During the study, a number of investigations were performed in an effort to elucidate the working mechanism of photopheresis in systemic sclerosis. In the photopheresis equipment, only the white cell fraction is exposed to PUVA, and within this white cell fraction the lymphocytes are being considered as the target cell type. One of the hypotheses was that the lymphocytes are modified during PUVA-exposure (a change in cell membrane rigidity, modifications in cell surface receptors, or induction of the release of cytokines) and elicit an immune-response after reinfusion to the patient. One important issue in this respect was the viability of the PUVA exposed cells. This was investigated by determining whether photopheresis induces apoptosis (programmed cell death) in lymphocytes. The results of this study are described in *chapter 4*.

In systemic sclerosis, there is a correlation between the extent of sclerosis of the skin and the final prognosis of the disease. For this reason, skin sclerosis is usually one of the primary outcome parameters in trials. In most studies a clinical scoring system known as the skin score is being used. The skin score is a subjective parameter, based on how a human observer rates several features of scleroderma skin, like edema, induration, fixation to the underlying tissue, inability to pinch the skin into a skinfold, and resistance to lateral movement. Because this subjectivity introduces observer bias and inter-observer variability, there is a need for alternative measurement methods. Alongside with the clinical studies, we investigated 3 newly developed methods to quantify cutaneous sclerosis: skin elasticity measurement, laser scatter profilometry, and fast Fourier transform. The experiences with these methods are described in *chapter 5 and 6*.

The new methods to quantify sclerosis were used simultaneously in some patients with systemic sclerosis, and subsequently in a study in patients with morphea, a form of localized scleroderma, who were treated with medium-dose ultraviolet A1. The results of this study are discussed in *chapter 7*.

CLASSIFICATION OF SCLERODERMA

Scleroderma can be regarded as a spectrum disease. It comprises *localized scleroderma*, restricted to the skin only, without internal organ involvement (morphea, linear scleroderma, and generalized morphea) and *generalized scleroderma* or systemic sclerosis, with organ involvement, which can be divided in a benign limited form (lSSc) and an aggressive diffuse form (dSSc).

In 1980, generally accepted preliminary diagnostic criteria for systemic sclerosis were formulated by the American Rheumatism Association (The ARA criteria).[13] As major criterium, the presence of *proximal scleroderma* (sclerosis of the skin, proximal of the metacarpo- or metatarsophalangeal joints) was defined.[7] Minor criteria were sclerodactyly, digital pitting scars, loss of substance of the distal finger pad, and bibasilar pulmonary fibrosis. The diagnosis systemic sclerosis required the major criterium or two minor criteria.

Table 1. ARA criteria for systemic sclerosis

Major criterium:	proximal scleroderma
Minor criteria:	sclerodactyly
	digital pitting scars
	pulp loss
	bibasilar pulmonary fibrosis

The ARA criteria are 97% sensitive and 98% specific for systemic sclerosis, but they do not discriminate between benign or progressive subgroups. Barnett et al.[30] introduced a subdivision in type I, II, and III, based on the early extent of skin sclerosis. Type I (limited extent) indicates sclerodactyly only. The sclerosis remains limited to acral regions for years. Type II (moderate extent) indicates sclerosis proximal to the metacarpophalangeal joints but excluding the trunk. Type III (extensive) indicates diffuse skin sclerosis including the trunk. This type is usually expanding rapidly, and is associated with internal organ involvement.

Table 2. Clinical subsets of systemic sclerosis (Barnett et al.[30])

Type I	Acrosclerosis (sclerodactyly only)
Type II	Scleroderma skin changes proximal to the MP joints with sparing of the trunk
Type III	Diffuse scleroderma skin changes including the trunk

This subdivision was soon accepted with a few small modifications by a number of European groups,[9,15,26,30,53,61,63] and was also used by us to define the inclusion criteria for the photopheresis study. Only type II or III systemic sclerosis patients were included.

Many other classification systems have been proposed, all designed to discriminate between the limited form of systemic sclerosis, which is usually limited to the acral regions (hands, forearms, face), and the diffuse form. A very suitable criterium appeared to be the presence of truncal sclerosis. Patients with limited SSc usually have multiple telangiectases and may have all other symptoms of the CREST[78] syndrome (calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia), but the course of the disease tends to be more benign, a slow development of symptoms over a number of years, and a low incidence of internal complications. Patients with diffuse SSc have sclerotic regions on the proximal extremities and / or the trunk, usually rapidly progressive, and they have a higher incidence of internal organ involvement, associated with a higher morbidity and mortality.

This last group, the more severe cases with the worst prognosis, is the target population for clinical trials. White et al.[81] suggested in their paper on guidelines for clinical trials in SSc that patients should have diffuse SSc only (clinically apparent involvement of the skin on the extremities proximal to the elbows or knees or on the trunk), of recent onset (disease duration < 2 years from the onset of the first symptom). The main features of limited versus diffuse sclerosis are summarized in table 3, adapted from Perez and Kohn.[21,61,81]

Table 3. Clinical subsets of systemic sclerosis

Subtype:	autoantibodies:	prognosis:
lSSC Limited systemic sclerosis (Calcinosis, Raynaud, Esophageal dysmotility, Sclerodactyly, Telangiectasia)	Anti-centromere	benign course; low incidence of renal and pulmonary involvement, 10 years survival approximately 70%
dSSc Diffuse systemic sclerosis (Rapid cutaneous sclerotic progression from distal to truncal sclerosis, few telangiectases)	Anti-topoisomerase-I (anti-Scl-70)	poor course; renal, pulmonary, gastrointestinal, neurological, and cardiac involvement; 10 years survival 22-26%

Regarding the afore mentioned classification systems, the extent of the skin sclerosis is of major importance concerning the incorporation in the different subgroups and is likewise of importance in determining the prognosis of the disease. The skin involvement in systemic sclerosis seems to be a reliable identification criterium.

EPIDEMIOLOGY

Several studies on the incidence, prevalence and mortality have been published.[66,84,85,87,92,93] The data show some variation due to differences between countries, different patient populations, different year cohorts, and different recording methods, which makes them difficult to extrapolate to the current situation in The Netherlands. In most studies, the ARA-criteria were used to define systemic sclerosis.[13] The ARA-criteria have been selected in 1980 to combine a high sensitivity (97%) with a high specificity (98%), but they do not discriminate between subclasses I (acroscleroderma), type II (acroscleroderma with progression of the sclerosis to proximal areas such as the arms and legs), and type III (diffuse scleroderma, usually starting on the trunk, with rapid progression to other areas including the extremities).[9,11,26,30,53,67]
The incidence (number of new cases per 1.000.000 individuals per year) was reported to be between 2-10 per 1.000.000 (0.6-2.3 of hospital diagnosis, 6.3-12 of all diagnosis, up to 18.7 per 1.000.000 during active recruitment for a clinical trial.[84,85] The prevalence (number of patients with active disease per 1.000.000 individuals per year) was estimated to be between 31 and 126.[66,84,85,87] The source data for the incidence and prevalence figures are shown in table 4.

Table 4. Summary of reported incidence and prevalence of systemic sclerosis

Prevalence	Period	Incidence	Period	Country	Region
31	1986	3.7	1980-85	UK	West Midlands
100	1980-81	-		GER	Leipzig
105	1968	10	1950-79	USA	Rochester
126	1977-79	-		DEN	multihospital
		6.3	1970-79	NWZLAND	Auckland
		10	1963-72	USA	Pittsburg
		7	1961-69	CZE	Piestany

To estimate the number of available patients in the Netherlands, the following assumptions were made: it was assumed that the prevalence would be comparable with the prevalence in Germany (100), USA (105) or Denmark (126). To be on the safe side, an estimated prevalence of 80 (31-126) was used for further calculations. It was assumed that the incidence would be somewhere between 6.3 and 10 (median: 8.15), comparable with the incidence in USA (10), New Zealand (6.3) and Czechoslovakia (7).

The average mortality was assumed to be approximately 2.5 per million per year, based on studies in the USA and the UK (table 5).[84,85]

Table 5. Mortality of systemic sclerosis

male	female	average	country
1.3	2.2	1.75 per million	USA 1949-63
1.0	2.1	1.55 per million	USA 1959-61
1.0	4.0	2.50 per million	USA 1963-68
1.5	3.5	2.50 per million	USA 1969-77
1.0	4.0	2.50 per million	UK 1974-85

The majority of these deaths are expected in the patient groups type II and type III, the more severe cases. About 70-80% of patients are classified as type I, 15-20% as type II, and 5-10% as type III. Based on the total population in the Netherlands at the start of the study, 15.2 million, the number of patients with type II or III scleroderma could be estimated (table 6).

Table 6. Estimation of number of patients with type II or III scleroderma

	Prevalence	Incidence	Survival		Death/Year	
	80	8.15	5 jr	1 jr	3.1%	38
Total number of patients:	1216	124				
type I: (80%)	972	99	85%	98%	2%	19
type II: (15%)	183	19	75%	94%	6%	11
type III: (5%)	61	6	50%	87%	13%	8

According to the original inclusion criteria, only patients with type II (19 new patients per year) or type III (6 new patients per year) were eligible for the study. The maximum disease duration was 5 years. By combining incidence and death rates for type II and III, it was estimated that during the inclusion phase, approximately 85 patients with type II and 33 patients with type III scleroderma were available in the Netherlands.

CUTANEOUS SYMPTOMS OF SYSTEMIC SCLEROSIS

Sclerosis

Systemic sclerosis or generalized scleroderma is characterized by deposition of abnormal amounts of collagen in the skin and in internal organs (gastro-intestinal tract, kidneys, heart, lungs). The increase in collagen content seems to be preceded or accompanied by microvascular damage. Also an edematous component is present, especially in the early phase. Due to the excessive deposition of collagen, the skin hardens, becomes thicker, tighter, more fixated to the subcutaneous layers ('hidebound'), a condition clinically described as 'cutaneous sclerosis' or 'scleroderma'. Gintrac, in 1847, was probably the first to name this condition scleroderma. The clinical picture, especially of the diffuse form is so outstanding that it must have drawn the attention of physicians in the past ages. Hippocrates (460-370 BC) and Galen (130-299 AD) described conditions of skin "so indurated that it could not be pinched" and "obstruction of pores with thickening, pigmentation and absence of sweat glands". Curzio of Naples is considered to be the first to describe a convincing case of scleroderma.[3] Matsui was the first to describe the systemic nature of this disease (1924). He described fibrosis of the lungs, the gastrointestinal tract and the kidneys.[14]

The skin

The skin changes in systemic sclerosis [7,9,11,15,21,24] can roughly be divided in 3 phases. During the first phase there is edema of the involved skin, on the hands it is noticed as stiff and swollen fingers. During the second phase sclerosis of the skin develops, the so characteristic and diagnostic stage of the disease. The skin is tight and shiny, and can cause restriction of movement especially nearby joints. The skin can not be normally picked up or pinched because of the induration. The normal aspect of the skin is changed and in the involved area there is less or no hair growth. Pigment changes (hypopigmentation as well as hyperpigmentation) can already be seen during this phase. It is most easily noticed in dark skin and it typically occurs in the neck area. The changes in pigment can have a patchy or a diffuse appearance; however it occurs always perifollicular. Finally the third phase can occur, the skin can become atrophic and soft.

In the very beginning when there is only edema of the skin it is very difficult and sometimes impossible to differentiate between other connective tissue diseases. Other criteria are required such as the existence of Raynaud's phenomenon, laboratory abnormalities and periungual telangiectases (see further). Often the diagnosis of carpal tunnel syndrome is made.

The recognition of the disease is relatively easy when the skin becomes tight and indurated. The distribution of the affected skin is diagnostic for systemic sclerosis.[30,34,37,41,56,61] It is the most important feature for the classification of this disease and it is of important prognostic value. The skin changes in systemic sclerosis always start at the hands and face. If the skin changes do not involve the hands, another diagnosis should be considered. In the face, the skin around the eyes becomes tight, wrinkles disappear and the nose is small and pinched. The lips are thin, the opening of the mouth is restricted (microstomia) and there are radial furrows around the mouth. These facial changes are characteristic for patients with systemic sclerosis. Proximal extension of the skin changes is a sign of progression of the disease. Noticeable is the fact that the skin in the lower lumbar region and the buttocks is seldom involved.[34]

Telangiectases

Telangiectases in the face, on the hands, on the lips, and the upper chest are seen in the whole spectrum of the disease; they cannot differentiate between lSSc, dSSc and the CREST-syndrome. However, they are observed mostly in patients with the CREST-syndrome or lSSc. The telangiectases are often multiple and large ('macula-like'). The skin around the nails can show an abnormal capillary pattern which can be detected by nailfold capillary microscopy. These are the periungual telangiectases.[71,240,241]

Calcinosis cutis

Calcinosis is most often found in patients with the CREST syndrome, it can nevertheless be found in the whole spectrum of the disease.[138] Calcinosis commonly occurs on the volar aspect of the fingertips but also over joints of elbows and knees and in the soft tissues of the forearms, buttocks and legs. The deposits are irregular and firm, they can vary from one nodus to multiple large packets. The overlying skin may become red because of friction or infection and can sometimes ulcerate with discharge of white chalky material. The extent of the depositions can be disclosed by X-ray examination. The cause of calcinosis is unknown, serum calcium, phosphorus and alkaline phosphatase levels are normal.

Raynaud's phenomenon

Raynaud's phenomenon is defined as episodic attacks of well-demarcated blanching or cyanosis of the fingers or toes, usually provoked by triggers such as changes in temperature (cold) and emotions.[274,276,277,280] The clinical manifestations of this phenomenon are pallor of the affected skin due to vasospasm, followed by cyanosis because of venous stasis and finally rubor due

to reactive hyperemia because of the return of the blood flow. During the attack the digits are cold and sometimes there is a numbness. Pain of the involved digits is almost always present. Usually only the fingers and toes of the patients are involved, sometimes only one or two digits are included. Noticeably often the thumbs are spared. Raynaud's phenomenon is common in the general population (overall prevalence 3-4%, 20-30% amongst young women), but is highly associated with connective tissue diseases. It occurs in 98% of patients with systemic sclerosis, and in 70% it was the first symptom. This phenomenon is of diagnostic importance in differentiating between the limited and diffuse form of scleroderma. In the limited form (lSSC), Raynaud's phenomena is present for years without sclerosis of skin or viscera. In contrary, in the diffuse form (dSSc) Raynaud's phenomenon is of recent onset or starts together with the skin problems.

In all patients Raynaud phenomenon causes serious morbidity, it can range from discomfort and pain to ulcerations and digital loss. The vasospasms due to Raynaud's phenomenon are superposed on the smaller lumina of the blood vessels. These lumina are considerably narrower due to intima proliferation and fibrosis. This can cause chronic and therapy resistent ulcera on the fingertips, which heal with pitting scars. This problem can cause gangrene and amputation of one or more fingertips. These pitting scars are seen in the whole spectrum of systemic sclerosis often combined with the loss of substance of the finger pads. The fingers become pointed and tapered, probably due to diminished blood flow in the tissue because of the combination of Raynaud's phenomenon and the existent narrowing of the blood vessels. Sometimes the terminal phalanges are bulbous, in other cases they become atrophic and deformity is clear.

INTERNAL ORGANS INVOLVEMENT

Tractus digestivus

Gastrointestinal disease is commonly recognized as visceral manifestation in systemic sclerosis.[21,65,249,268,270] The symptoms are dysphagia and heartburn due to the loss of esophageal motility and esophageal reflux. This can lead to esophagitis and strictures of the esophagus. These abnormalities can be detected by X-ray with contrast, manometry of the esophagus or endoscopic procedures. These peristaltic abnormalities can lead to delayed gastric emptying and changed motility of the small and large intestines, which can cause pseudo-obstruction or malabsorption secondary to bacterial overgrowth. Other gastrointestinal manifestations include megacolon, transverse and sigmoid colon volvulus, stenosis and diverticular ulcerations. The macula-like telangiectases in the gastrointestinal tract can sometimes cause severe bleeding.

Kidneys

Renal problems are often associated with the diffuse form of systemic sclerosis.[237,249,251,267] Clinical manifestations are hypertension and proteinuria. Since the use of angiotensin converting enzyme inhibitors and the development of haemodialysis, mortality due to renal failure in systemic sclerosis has been drastically declined. The histopathology shows subendothelial intima proliferation. Risk factors for the onset of renal crises are rapid progression of skin fibrosis, congestive heart failure and therapy resistant hypertension. It is also known that high doses of corticosteroids may initiate renal crises.[263]

Lungs

Pulmonary disease[254,256,257,258,260,261] is currently considered to be the major cause of death in patients with systemic sclerosis. Fibrosing alveolitis and pulmonary vascular disease are the two major manifestations of lung involvement. Fibrosing alveolitis is probably due to increased collagen deposition and the preceding inflammation reactions. Pulmonary hypertension is the result of the vascular abnormalities in systemic sclerosis. Alveolitis is especially found in the diffuse form of systemic sclerosis, pulmonary hypertension especially in the limited form. Patients complain of exhaustion and dyspnea on exertion which is often accompanied by a non-productive cough. X-ray and CT-scan are of importance in diagnosing fibrosis in an early stage of the disease. Lung function tests are also important diagnostic tools, carbon monoxide diffusion capacity is often low.

Bronchoalveolar lavages can show an active alveolitis because of an increased percentage of neutrophils, eosinophils, lymphocytes and macrophages. Pulmonary hypertension can be diagnosed by Doppler echocardiography.

Heart

Although rare, symptomatic complaints caused by cardial disorders may occur. These symptoms are often non-specific and therefore not recognized. Tiredness, dyspnea and palpitations can be the symptoms. Fibrosis of the conduction system can cause arrythmias. Pericarditis and myocardial fibrosis are regularly seen. Right-sided heart failure due to fibrosis or pulmonary hypertension may be a sign of disease progression. Follow-up of cardiac disease can be managed by 24-hour continuous electrocardiogram, exercise testing and cardiac output measurement. The ejection fraction of the left ventricle can be measured by GSA (gated synchronized angiography) and is an index of the function of the heart.

Joints

The joints and muscles may be affected.[249] Flexion contractures, arthralgias and disabling arthritis are sometimes associated with systemic sclerosis. Occasionally, it can be the first sign of the disease, in combination with Raynaud's phenomenon. Sometimes flexion contractures are seen, caused by fibrosis of the skin and tendons. Inflammation of the joints, involvement of the joint capsule, collateral ligaments and tendon sheaths can also cause contractures. This phenomenon gives rise to the so-called "palpable tendon friction rubs".[75] The latter can be palpated over the flexor and extensor tendons of fingers and lower arms.

Myositis, which is difficult to distinguish from polymyositis can sometimes be found. This is probably due to microvascular damage. Proximal muscle weakness, an abnormal electromyography (EMG) and an increased creatinine phosphokinase are found in systemic sclerosis with myositis.

Neurological disorders

Neurologic involvement is uncommon in systemic sclerosis.[468,473] Trigeminal neuralgia is reported in 4% of all patients. Carpal tunnel syndrome is sometimes diagnosed before the onset of systemic sclerosis and can be due to recent onset of Raynaud's phenomenon (pain and numbness).

Laboratory investigations in patients with systemic sclerosis

There is no single diagnostic test for systemic sclerosis. In patients suffering of systemic sclerosis a number of serological abnormalities can be found. At presentation antinuclear antibody, anticentromere and anti-DNA topoisomerase I (Scl-70) antibody tests should be performed.

A complete blood count, renal function tests, creatinine phosphokinase level and urinalysis should also be done.[2,9,11,21,65,90,104]

Quality of life measurements in systemic sclerosis

Several disease-specific and non-specific health surveys have been used in the past to evaluate quality of life in patients with systemic sclerosis. Progressive systemic sclerosis causes a number of limitations, physical limitations like joint immobility, tiredness, painful ulcerations at the fingertips, or shortness of breath. The disease also causes psychological distress.[9,34] To evaluate the impact of these events on the patients' quality of life, daily functioning, general health and general well being, we used 4 different questionnaires: the Barthel-Index, the Frenchay Activity Index (FAI), the Rotterdam Symptom Checklist, and an adapted version of the de Rand Health Insurance Study Questionnaire, known as the MOS-24 (MOS: Medical Outcome Study).

The Barthel-Index is a validated instrument to measure limitations in daily functioning. Using 10 questions, it inquires whether patients experience problems in simple basic daily activities (like walking stairs, eating, dressing, bathing, personal care).[324] The Frenchay Activity Index consists of 15 questions and evaluates limitations in more complex daily activities, at home, work/leisure, and social activities.[330] The MOS-24 is an extended version of the MOS-20.[331] The MOS-20 is a validated questionnaire to measure general health in adults and young adults. It contains 24 questions on subjects as mental health, physical health, role functioning, social functioning, physical pain, energy. The results are compared with a reference group (2595 patients visiting medical specialists, but without a chronic disease). The Rotterdam Symptom Check List consists of 30 questions on physical health and mental health.[325]

All patients were interviewed using these health questionnaires, on a regular basis, before, during, and after treatment. Not surprisingly systemic sclerosis patients scored low compared to control groups. Quality of life measurement has become an important tool to evaluate new treatment modalities; it is considered by many to be as important as clinical and economical evaluations.

QUANTIFICATION OF SCLEROSIS IN SCLERODERMA PATIENTS

Histologic examination

In the early phase of the disease there is a dense mononuclear infiltrate around the vessels. The infiltrate consists of lymphocytes and neutrophils. In the late phase there is no infiltrate and there is an increased amount of collagen in the dermis. The collagen bundles are parallel oriented, thickened and tightly packed. The adnexa are surrounded by homogeneous collagen and the number of adnexa is markedly decreased.[203]

Skin score

Of all the involved organs in systemic sclerosis, the skin changes are the most easy to access, the extent and progression of the skin induration is easy to monitor. Exact evaluation of the distribution and severity of the skin problems are important for the classification and prognosis of the disease.[336,340,341] Until now it is the only parameter to follow up disease activity and therefore it is an important tool in the evaluation of efficacy in trials. The skin score is an internationally used and validated semi-quantitative measure of cutaneous involvement in systemic sclerosis.[355] Cutaneous sclerosis is assessed on a 0 to 3 scale (0 = normal skin thickness, 1 = mild skin thickness, 2 = moderate skin thickness, 3 = severe skin thickness with inability to pinch the skin into a fold). This semi-quantitative measurement has a low inter observer and intra observer reliability but it is still regarded as a suitable outcome variable. A number of objective measurements can be added, such as maximal oral aperture measurement. Patients have difficulty in opening their mouth due to sclerosis of the face, the maximal width between the teeths can be measured. The possibility to bend the fingers (finger to palm distance) can also be measured. Sclerosis of the hands is an early sign of this disease; progression of the sclerosis may lead to restriction of finger movement. The distance between the palm of the hand and the fingertip of the middle finger is measured (normally this distance is 0). The maximal spreading of the fingers is another objective parameter; the distance between thumb and little finger can be measured.

Skin Elasticity Measurement

An objective measuring device to quantify skin sclerosis is the SEM 474 Cutometer. This device measures elasticity of the skin. The elasticity of the skin in systemic sclerosis is changed due to collagen accumulation in the dermis. As a result of the increase of collagen in the skin it becomes impossible to pinch the skin into a normal skin fold. This 'fixation' of the skin can be translated into 'less' elasticity. The system consists of a unit containing a vacuum pump, a measurement probe with a defined diameter and a personal computer. The skin is lifted in the probe because of the vacuum. The depth of penetration into the probe is determined by a noncontact optical measure system. This measurement can be expressed in millimeters.

ETIOLOGY OF SCLERODERMA

The pathogenesis of systemic sclerosis is still unclear. Research is hampered by the low prevalence of the disease, the variability in expression of systemic sclerosis and the fact that there is no consensus regarding the classification. There are several pathogenic processes which occur in systemic sclerosis and they are thought to be closely related. These processes are (micro)vascular changes, increased deposition of extracellular matrix components and activation of the immune system.[2,6,8,10,11,18,25]

Endothelial and microvascular disorders

Endothelial damage is an early feature of systemic sclerosis. Raynaud's phenomenon[274] and the abnormal capillary pattern of the nail folds, often observed in systemic sclerosis, can both be regarded as a consequence of the microvascular abnomalities. Edema in the skin, an early sign of this disease, can be an indication of dysfunction of the endothelial cells with abnormal vascular permeability. Histologically, proliferation of intima cells can be seen. This causes intravascular obliteration. Early in the disease infiltrates around the blood vessels are found. The role of the endothelial cells in the pathogenesis is unclear.[94] On the one hand they can be damaged due to external influences such as viruses or immunological activity, on the other hand they can induce inflammatory or immunological processes. Damage to endothelial cells can be caused by multiple factors such as (retro) viruses,[235] activated adhesion molecules,[246,247] free radicals, and granzymes. Signs of endothelial damage are raised plasma levels of factor VIII (von Willebrand) and increased platelet aggregation.[236,237] Finally, deficiencies in complement regulatory molecules with a protective function, such as membrane cofactor protein and decay-accelerating factor, may contribute to the vascular damage.[164]

Collagen metabolism

The production of normal collagen type I and III by fibroblasts is increased in systemic sclerosis. This means that there is an excessive deposition of collagen and other connective tissue matrix proteins.[182,183,201,215,220] This increase results in fibrosis of affected organs and causes dysfunction of the affected organs such as skin, joints, gut, heart, lungs and kidneys. Apart from the increased production of collagen type I and III, there is also over-expression of collagen type IV, V and VI, fibronectine and proteoglycans. A range of cytokines and growth factors seem to influence the overproduction of fibroblasts, for example transforming growth factor beta (TGFß), platelet-derived growth factor (PDGF),

interleukine 1, 4, 6 and 8, fibroblast growth factor (FGF) and interferon gamma (IFN).[186,190,191,195,204,207-209,211]

Immunological mechanisms

A few autoantibodies are associated with systemic sclerosis.[90,94,103,118,143] Anticentromere antibody (ACA) is found in 70-90% of patients with the limited form of systemic sclerosis (lSSc). Anti-topoisomerase I (Scl-70) is found in approximately 30 % of patients with the diffuse form of systemic sclerosis (dSSc). These antibodies are specific for systemic sclerosis. Antinuclear antibodies (ANA), although very nonspecific, are almost always present. The presence of ANA together with Raynaud's phenomenon is useful when the diagnosis of systemic sclerosis is suspected.

Activated T-cells (T-helper CD4+)[108] are found in tissue as well as in peripheral blood. In the blood there are also increased amounts of IL-2 and IL-2 receptors.[101] Complement factors such as C4a and C4b seem to be increased in systemic sclerosis.[92,154]

The vascular abnormalities are most obviously expressed in the limited form of systemic sclerosis. These patients have a long history of Raynaud's phenomenon, digital scars and ulcers, telangiectases and pulmonal hypertension. The fibrotic component with the accumulation of collagen is best seen in the diffuse form of systemic sclerosis. These patients have widespread sclerosis of the skin and visceral organs, this sclerosis is also accompanied by a vascular component.

The endothelial cell and the fibroblast seem to be the targets for the triggers (immunological, viral, environmental factors) that initiate the cascade of events eventual leading to sclerosis, such as endothelial damage, increased microvascular permeability, and enhanced adherence of lymphocytes to endothelial cells facilitated by adhesion molecules. These adhesion molecules help lymphocytes to adhere to the (damaged) endothelium and so facilitate their migration into the interstitium. Possibly, cytokines are then upregulated, which creates an environment suitable for fibroblasts to synthesize, uncontrollably, extracellular matrix components.[144] This gives rise to an increased amount of collagen and results in sclerosis of the different target organs.

The relationship between endothelial damage and fibroblast activation is not yet elucidated. It could be two separate processes, reacting on the same stimulus or it could be two consecutive processes in which damage to the endothelial cell is a stimulus for fibroblast activation.

THERAPEUTICAL OPTIONS IN SYSTEMIC SCLEROSIS

Evaluation of the efficacy of various treatments in systemic sclerosis is difficult because this disease is heterogeneous in severity and progression rate. There is also a tendency towards spontaneous improvement of skin sclerosis in some patients. Furthermore, the pathogenesis of systemic sclerosis is still uncertain. Finally, there are only a few objective parameters to monitor disease progression/regression and their relevance is disputable.

General measures and symptomatic treatment[302,311,312,314,321,323]

The skin. The skin in scleroderma can be dry and pruritic. The use of topical emollients can be effective. In early active systemic sclerosis pruritus is often intense and is usually confined to the skin of forearms and hands. Topical corticosteroids can provide partial relieve. This intense pruritus is usually self-limiting.

Patients with the limited form of systemic sclerosis often have large, so-called macula-like *telangiectases*. These are often localized on the face and hands and are seldom of clinical importance. However these telangiectases can cause significant cosmetic distress. Laser therapy (pulse dye) provides very good results.

Calcinosis cutis lesions do not necessarily cause morbidity, but sometimes the skin around a lesion becomes infected and painful. In some cases antibiotics are useful, together with a vasodilating agent, preferably calcium-channel blockers. Large masses of calcinosis cutis or calcifications located at a strategic site such as the thumb or the ulnar surface of the forearm can be treated by surgical excision or 'debulking' performed by experienced surgeons.

Raynaud's phenomenon is present in nearly all patients with systemic sclerosis. The morbidity is considerable, especially in patients with the limited form of systemic sclerosis. Preventive measurements are of extreme significance in preventing recurrent attacks. Avoidance of exposure to cold temperatures or changes in temperature is very important. These facts should be overemphasized to the patients. Calcium-channel blockers are the first-line therapy for Raynaud's phenomenon in systemic sclerosis. Nifedipine has been shown to improve digital blood flow and to induce healing of digital ulcers.[297] In practice, the sustained-release preparations are easy to use. If this is not effective, intermittent dosing with shorter-acting preparations can be tried, or switching to a different calcium-channel blocker, for instance amlodipine. Other vasodilating drugs, such as nitrates[279] or sympatholytic agents such as prazosin have been tried. They are not as effective as calcium-channel blockers and the side-effects are considerable. Sometimes they are used in combination with a

calcium-channel blocker. There are some reports in which the usefulness of intravenous prostaglandines has been described.[284,285] Sometimes severe attacks of Raynaud's phenomenon result in ischemia of the digits and ulcerations. Efforts to create maximum blood flow in the involved digit should be tried.

Treatment of associated complications

Tractus digestivus. The gastrointestinal tract is almost always involved in systemic sclerosis.[299,302] Reflux and dysmotility can cause ulceration, Barret's esophagus and strictures. Some patients have mild symptoms but already significant disease. Therefore, evaluation for esophageal complications should be performed without hesitation. Standard barium swallow examination can rule out strictures, endoscopy is necessary for all other complications.

Symptoms of the gastrointestinal tract can result from esophageal dysmotility and/or delayed gastric emptying.

Reflux symptoms can be improved by taking practical measures such as elevation of the head of the bed and avoidance of food that lowers the gastroesophageal sphincter tone (coffee, alcohol). Mild symptoms can be treated conservatively with frequent small meals.

Pharmacological treatment of esophagus symptoms is effectively done by the use of proton pump inhibitors such as omeprazole.[301] H_2-blockers and antacids are less effective. Delayed gastric motility can be treated with cisapride, low dose erythromycin acts as a motilin receptor agonist and can sometimes be a useful drug of choice.

Decreased motility of the small bowel can result in abdominal bloating, distention, alternating diarrhea and constipation, pseudo-obstruction and malabsorption.[316] Medications such as calcium-channel blockers can aggravate symptoms of small bowel hypomotility. Bacterial overgrowth can be the result of hypomotility, this problem can be adequately treated by taking antibiotics (ampicillin) 2 weeks each month. Patients with significant malabsorption problems should be supplied with fat-soluble vitamins and extra protein. Cisapride should always be tried in symptomatic patients. Acute pseudo-obstruction is best treated conservatively with nasogastric tube placement and bowel rest.

Severe involvement of the gastrointestinal tract can result in malnourishment in some patients. Total parental feeding is therefore sometimes required.

The kidneys. Renal involvement used to be the major cause of mortality in patients with systemic sclerosis due to scleroderma renal crisis. In the beginning of the 1980s angiotensin-converting enzyme (ACE) inhibitors were introduced. This resulted in dramatical improvement and reversal of scleroderma renal

crises.[319] These renal crises are often preceded by elevation of the blood pressure. It typically occurs in patients with rapidly progressive, diffuse systemic sclerosis. The onset of hypertension can be the first sign of renal crises, therefore it should be treated aggressively with ACE inhibitors. Frequent monitoring is required, because scleroderma renal crisis is considered to be a medical emergency. Captopril is the drug of choice. Other antihypertensive medications such as thiazides, ß-blockers and calcium-channel blockers can also be used together with the ACE inhibitors to control blood pressure.

The lungs. Pulmonary manifestations can be divided into separate forms: pulmonary hypertension and interstitial lung disease. Pulmonary hypertension occurs mostly in patients with the limited form of systemic sclerosis. It can also develop in patients with the diffuse form of systemic sclerosis, as a result or complication of advanced pulmonary fibrosis. Interstitial lung disease develops generally in diffuse systemic sclerosis, although it may sometimes occur in limited systemic sclerosis.
Pulmonary hypertension is a major cause of mortality in systemic sclerosis. Therapy for this organ problem is very difficult. Vasodilators, as with Raynaud's phenomenon, may be successful, especially calcium-channel blockers. However these medications should be used carefully in patients with severe pulmonary hypertension because a rapid decrease in systemic blood pressure to less than the pulmonary pressure can have devastating consequences. Prostaglandines have been also shown to be effective in severe pulmonary hypertension.[284,289]
The severity and extent of interstitial lung disease can be examined by high-resolution computed tomography (HRCT). A ground-glass appearance may correlate with active inflammation. Bronchoscopic alveolar lavage in patients with interstitial lung disease can show a neutrophilic alveolitis. This sign of pulmonary inflammation together with the ground-glass appearance on HRCT is believed to be indicative of progressive pulmonary involvement. Several studies have shown encouraging effects of cyclophosphamide in treating alveolitis, active lung inflammation, but not in established lung fibrosis.[286]

The heart. Arrythmias and conduction disturbances can be treated with standard antiarrythmic medication and pacemaker placement. Symptomatic pericarditis can be treated by non-steroidal anti-inflammatory agents.

Joints. Arthralgias can be disabling in patients with systemic sclerosis. Nonsteroidal anti-inflammatory medication (NSAID) is helpful in some patients. It should be used cautiously in patients with diffuse systemic sclerosis

because of the potential reduction in renal blood flow. Inflammatory myositis responds to high doses of corticosteroids.

Control of disease

Unfortunately, to date no standard drug or combination of drugs has been of value for systemic sclerosis in adequately controlled trials.[510] Many drugs have been tried, usually in open, uncontrolled trials in small numbers of patients.

The processes involved in the pathogenesis are inflammation, vascular dysfunction and as end result fibrosis.

In the past years treatment was especially focused on the fibrotic component. Recently, the inflammation and immunological process and the vascular component are targets of future therapies.

Since the early 1960's D-penicillamine was the drug of choice in treating systemic sclerosis.[317,318] This medication is believed to alter the process of fibrosis by inhibiting the cross-linking of collagen. A recent controlled trial demonstrated no beneficial effects of D-penicillamine in systemic sclerosis.[293]

There have been studies evaluating the efficacy of interferon-gamma.[298] Interferon-gamma inhibits active collagen synthesis but has no effect on established fibrosis. This medication is associated with considerable side-effects (renal failure, digital infarction) and no impressive beneficial effects.

Among the immunomodulatory agents, there is an increased use of methotrexate. This medication is well known and often used in other rheumatological and dermatological diseases. The side-effects are considered to be relatively mild. A randomized, double-blinded trial has been performed and showed favorable (statistical not-significant) outcomes in skin scores, general well-being and creatinine clearance. [303]

Several studies have been conducted to evaluate the beneficial effects of anti-thymocyte globulin, until now no positive effects have been shown.[320]

Corticosteroids are still used by some clinicians in the inflammatory phase of systemic sclerosis, especially inflammatory myositis, pericarditis and alveolitis.[315] The induction of scleroderma renal crises by the use of high-dose corticosteroids remains a topic of discussion.[263]

Cyclosporine has been tried in only a few patients,[292,307] but the potential of nephrotoxicity makes this agent not very popular in the treatment of systemic sclerosis. Recently, procedures using purified autologous stem cells to eliminate potentially autoreactive T-cells, showed promising results.[309]

PHOTOPHERESIS

General principle

In photopheresis, blood is drawn from the patient and centrifuged. The leukocyte fraction is exposed to a high dose of ultraviolet A, in the presence of psoralen, and then reinfused into the patient.

Description of the UVAR equipment

Photopheresis is being performed using the UVAR Photopheresis unit (Therakos, West Chester, PA). Basically, the machine consists of an ultraviolet A chamber, a set of rotation pumps, clamps, a centrifuge chamber, and a control panel. The system does not operate fully automatic, an operator is required during the process. Prior to treatment, the system is packed with a sterile disposable set consisting of the infusion and reinfusion system, bags for the collection of blood, a centrifuge bowl, and a 1 mm thin transparent cassette (photoceptor) which is placed between the UVA bulbs. After heparinizing these components, the patient is connected to the machine, usually by accessing an arm vein. In some patients it is difficult to obtain vascular access. Since a good venous flow rate (25-50 ml/min) is required for the treatment, it is necessary to use femoral vein cannulation in these patients.

The blood, mixed with heparin, is centrifuged in a continuous process. In the centrifuge bowl the blood is separated in erythrocytes, the buffy coat (leukocytes) and serum. The serum flows into a separate bag. The leukocytes are collected in the buffy coat bag or 'treatment bag'. Then the erythrocytes and the remaining serum are returned to the patient. This cycle is repeated six times. After six cycles, the treatment bag is filled with approximately 240 ml of leukocyte-enriched blood, mixed with 300 ml of the patients plasma, and 200 ml saline plus approximately 10.000 U of heparin. The final buffy coat preparation contains 25-50% of the total peripheral blood mononuclear cell compartment. The treatment bag also contains psoralen. The liquid form (stabilized aqueous 8-MOP solution, Gerot, Vienna, Austria) is used, 200 μg of liquid psoralen is injected directly in the treatment bag after the first collection cycle.

From the treatment bag, the leukocytes are continuously pumped through the thin photoceptor, which is positioned between the UVA light bulbs. After completion of the sixth cycle, the cells are irradiated for another 1.5 hour. In total, an average exposure per lymphocyte of 2 J/cm2 is accomplished. After the whole procedure, which takes about 3.5 hours, the content of the treatment bag is returned to the patient. The whole procedure is summarized in Fig. 1.

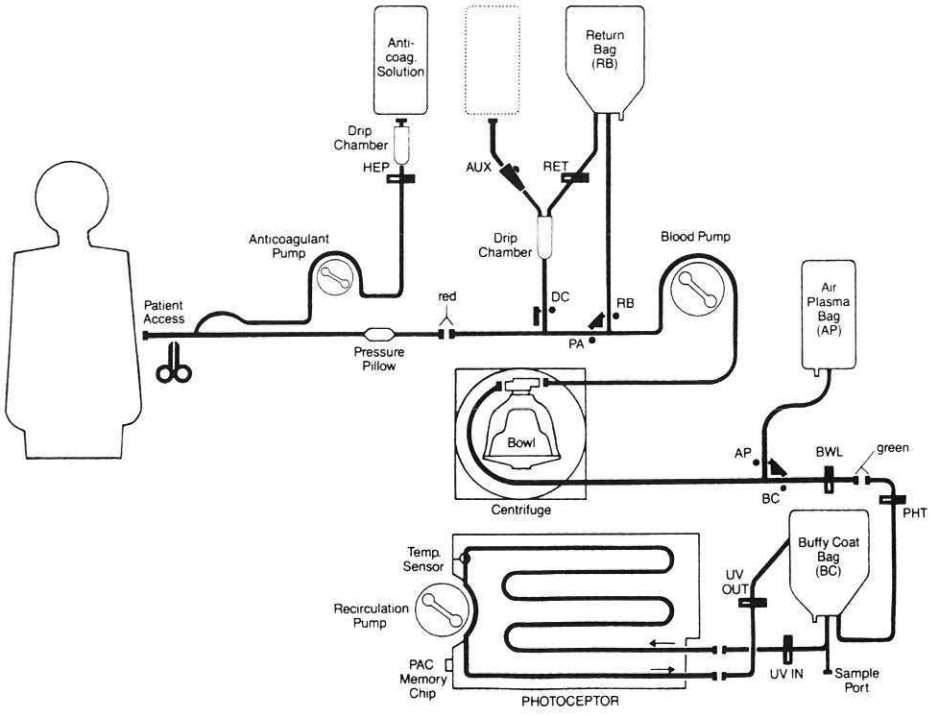

Fig. 1. Schematic representation of the photopheresis procedure

Organizational aspects

In order to treat all patients on a two days per month - basis, and taking into account that the machines are not continuously available because they need service occasionally, two UVAR machines were required. These two machines produce a lot of heat, and also noise (caused by the forced ventilation in the UVA chamber). So a separate, well ventilated and air conditioned two beds - room was specially equipped as photopheresis facility. To operate the machines one full time and two part time trained nurses were employed, and one medical doctor. Because of the difficulties to get venous access in systemic sclerosis patients, the procedures were often delayed and took longer dan 3.5 hours.

Photopheresis in Cutaneous T-cell Lymphoma

Photopheresis was first developed for cutaneous T-cell lymphoma (CTCL). In the initial stages of CTCL, the malignant (CD4+) helper T lymphocytes are predominantly present in the superficial layers of the skin, were they cause erythemato-squamous lesions, plaques, tumors or erythroderma. In these stages, the disease can be controlled by local treatment modalities such as ultraviolet A photochemotherapy (PUVA-treatment), electron beam radiation, or topical mechlorethamine. In later stages with extracutaneous involvement (lymph nodes, peripheral blood, bone marrow), treatment of the skin only is not any longer sufficient, and usually chemotherapy has to be started, which is associated with a high degree of morbidity. Because of the side-effects of chemotherapy, it seemed worthwhile to investigate whether UVA photochemotherapy could be used systemically, by exposing the blood to UVA in the presence of psoralens. In 1987, the first large multicenter trial on extracorporeal photochemotherapy or photopheresis was reported by Edelson et al.[412] In this study, the use of photopheresis on two successive days every month yielded highly beneficial results and little adverse effects. Twenty-four of 29 (83%) of the patients with erythrodermic CTCL showed improvement, and in the peripheral blood a drop in the numbers of CD4 positive malignant cells and atypical cells with a cerebriform nuclear morphology (Sezary cells) was observed. Since then, many studies on photopheresis in CTCL were published, and photopheresis was approved by the American Food and Drug Association for this indication.[411-415,418,419,421,422-425,427,428]

Photopheresis in scleroderma

The first case reports that photopheresis might be effective in systemic sclerosis appeared in 1990. Since then, several studies were published, [392-404,406,408,409,410] but most were of limited value because the number of patients was too small or the data were collected retrospectively. Until now there has been only one randomized controlled prospective trial, the study of Rook et al.[403] In this study photopheresis was compared with D-penicillamine. Photopheresis was given at 2 days per month, as in the CTCL studies. After 6 months of treatment, a significant softening of the skin was observed in 21 of 31 (68%) patients receiving photopheresis, compared to 8 of 25 (32%) patients receiving D-penicillamine. Significant worsening of the skin score was seen in 3 of 31 (10%) patients in the photopheresis group against 8 of 25 (32%) in the D-penicillamine group. After 6 months and after 10 months treatment with photopheresis, the mean skin score, oral aperture, and hand closure measurements improved from baseline. Compared to control, an improvement

of approximately 15% in mean skin score was observed. Internal organ involvement did not improve.

Photopheresis in other conditions

After the initial studies with photopheresis in CTCL and systemic sclerosis, the treatment was tried in several other conditions in which T-cells were supposed to be the pivotal cell type.[429-451] Photopheresis was tried in psoriasis vulgaris,[449] psoriatic arthritis,[437,438,447] rheumatoid arthritis, pemphigus vulgaris,[433,443,444] AIDS-related complex,[429] chronic lymphatic leukemia,[434] scleromyxoedema,[430] juvenile dermatomyositis,[450] atopic dermatitis,[441] Lyme arthritis,[442] graft versus host disease,[431,440] renal allograft rejection,[446] rejection of lung allografts,[445] heart transplantation,[435] and epidermolysis bullosa aquisita.[436] In most of these studies, although success stories have been reported, the number of treated patients is still to low to draw definite conclusions. An exception should be made for the most recent area of photopheresis research, the suppression of organ transplant rejections. The results presented until now are quite impressive.

Theoretical background of photochemotherapy

Psoralens like 8-MOP and 5-MOP are naturally occurring aromatic tricyclic molecules. Their planar structure enables them to intercalate with nucleic acid base pairs.[388] The aromatic structure enhances their ability to absorb UVA. After exposure to UVA, photoadducts are formed that are able to bind to DNA, especially with the pyrimidine bases thymine and uracil. As a result an interstrand cross-link is formed and DNA replication is inhibited. These direct inhibitory and toxic effects on DNA are thought to be the most important explanation for the therapeutic effects of PUVA treatment in psoriasis and cutaneous T-cell lymphoma. Psoralens do not only bind to nuclear DNA. After oral ingestion, free 8-MOP is distributed throughout the cell: in cell membranes, in the cytoplasm, as well as in the nucleus, and binds to cell membrane-DNA,[452] lipids, and proteins. After exposure to UVA, photoactivated 8-MOP binds and photomodifies several intracellular proteins, with unknown consequences. Several additional effects of PUVA treatment have been reported, such as the up regulation of TNF-alpha,[390] changes in cytokine profiles (induction of IL-2 and interferon),[388] and enhancement of the synthesis of the major histocompatibility complex (MHC) Class I.[456]

Another consequence of the interaction between photo-activated 8-MOP and DNA is an increased occurrence of cytogenetic aberrations, an enhancement of sister chromatid exchange,[386] and the induction of apoptosis (programmed cell death).[462]

In regular PUVA treatment, psoralens are taken systemically and the entire skin is exposed to UVA. In psoriasis, other mechanisms such as a direct effect of UVA on the skin immune system, mediated through Langerhans cells or cytokines produced by UVA-exposed keratinocytes may contribute to the mechanism of action. In cutaneous T-cell lymphoma, the cytotoxic effect on the T-cell infiltrate may be sufficient to explain the results; it is important to realize that in cutaneous T-cell lymphoma, because of the strong epidermotrophic properties of these cells, a large proportion of the T-cell fraction is continuously present in the upper layers of the skin, and therefore accessible to irradiation.
In systemic sclerosis, this is not the case. The entire system is not exposed to psoralens (a small volume is injected in the treatment bag), the skin is not exposed to UVA, and only a small proportion of the total white cell fraction, approximately 2-5%, is exposed to the treatment.[391] This rules out the concept of simple elimination of a disease specific T-cell clone. Edelson et al. suggested that the malignant or disease specific T-cell clone is susceptible to lysis by autologous cytotoxic T-cells. In animal studies (experimental allergic encephalitis in rats) the concept of modification of disease specific T-cells has been substantiated.[122] The pathogenetic T-cells causing allergic encephalitis can be isolated. After injection of the pathogenetic clone in a healthy animal, encephalitis is induced. If these T-cells are treated with PUVA, encephalitis does not occur, but more importantly, the animals are protected against the development of encephalitis. This suggests the induction of clone-specific suppressor T-cells by PUVA treatment. However, this hypothesis has not been confirmed in human studies. Another explanation may be the observation that photopheresis induces the release of inflammatory mediators from mononuclear cells, such as TNF.[390]

At this moment, many questions remain to be answered. The first goal seems to be to identify the proper indications for photopheresis. To date, cutaneous T-cell lymphoma and allograft rejection are regarded as the major indications. The next step would be to elucidate the working mechanism of photopheresis.

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Chapter 2

Diagnostic features of skin induration: systemic sclerosis, morphea, eosinophilic fasciitis and scleredema.

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SUMMARY

There are several diseases in which indurated or sclerotic skin is the most important feature. We describe four patients with specific clinical features of systemic sclerosis, morphea, eosinophilic fasciitis and scleredema respectively. These disorders are relatively rare and as such can easily be misidentified. The clinical picture, histology and laboratory manifestations of each disease are described.

INTRODUCTION

Systemic sclerosis, morphea (localized scleroderma), eosinophilic fasciitis and scleredema share several clinical features which can make it difficult to differentiate between them. Because of the wide range of associated symptoms, patients fall within the scope of many different specialities. A good dermatological examination is helpful, because the proper diagnosis of the abovementioned diseases can be made on the basis of dermatological signs and symptoms. The most important common feature in all conditions is the induration of the skin. Subtle differences in clinical appearance, distribution of the lesions, accompanying symptoms, and serological tests (see Table 1), discriminate between the different entities.
Scleroderma (systemic sclerosis and morphea) comprises a spectrum of disorders characterized by induration of the skin and increased collagen deposition in the involved tissues. Eosinophilic fasciitis is recently considered to be a variant of morphea. Scleredema can be considered as a separate entity. It is important to recognize these different disorders because the course and the prognosis of the diseases vary extremely.[1]

CASE REPORTS

Patient 1, a 52-year-old female, noticed swelling and stiffness of both hands. Since two years she complained of symptoms of Raynaud's phenomenon in both hands. Gradually, the skin of hands and face became tight. During the next years the tightness of the skin progressed to the arms, legs and upper part of the trunk. She noticed difficulty with swallowing solid food. At examination, there was sclerodactyly and digital scarring. The skin of the face, arms, legs and chest was shiny and sclerotically indurated (Fig. 1).

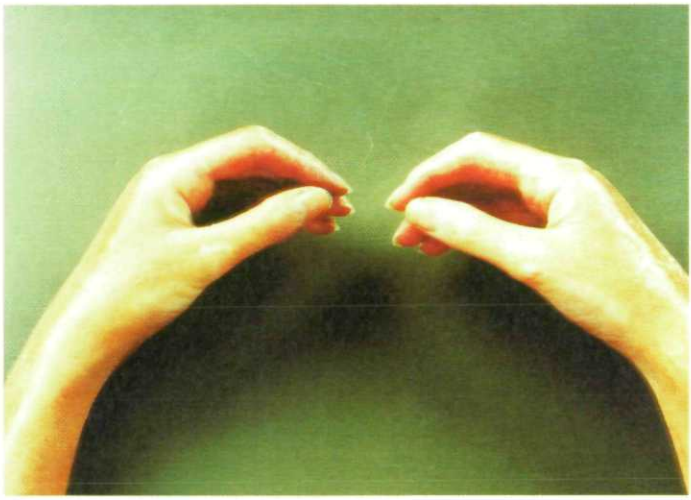

Figure 1. Shiny sclerotic appearance of the skin in systemic sclerosis.

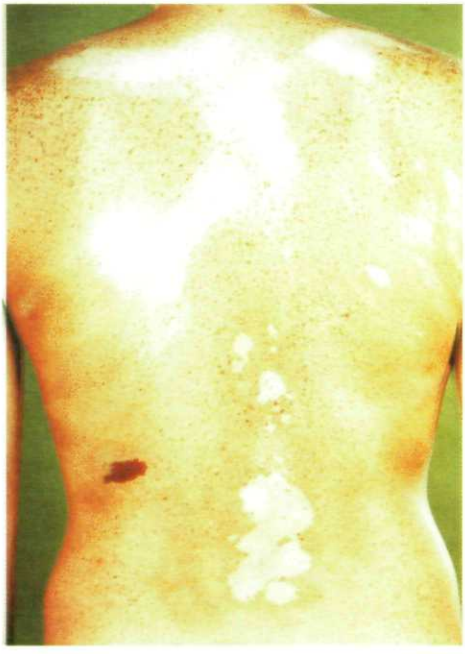

Figure 2. Ivory-white, well demarcated plaque of morphea.

Laboratory investigation revealed the presence of anti-nuclear antibodies and anti-topoisomerase I (Scl-70). Esophagus manometry disclosed dysmotility of the esophagus. Pulmonary function tests showed a carbon monoxide diffusion capacity of 38 % of the predicted value. Biopsy of the skin of her lower arm revealed a mild predominantly lymphocytic infiltrate, around blood vessels and collagen bundles.

Patient 2, a 28-year-old man, noticed an indurated white spot on his trunk. Apart from a slight itch, he had no complaints. During the next years he developed several more lesions on his trunk. At examination, Patient 2 had five asymmetrical ivory-white, well demarcated plaques on his trunk (Fig. 2). One was surrounded by erythema, the so-called lilac ring. The plaques were indurated. There were no complaints of Raynaud's phenomenon, the skin of his hands and face was normal. There were no signs of internal involvement. Histopathological examination showed thickened and closely-packed collagen bundles in the dermis. Newly formed collagen had replaced the subcutaneous fat around the sweat glands.

Patient 3, a 69-year-old woman, complained of swelling in both legs. Within a few weeks followed by pain and induration of the skin of the legs and the trunk. Another week later she noticed pain and induration of the skin of the forearms. There were no complaints suggestive for Raynaud's phenomenon nor systemic complaints such as shortness of breath or problems with swallowing.
At examination, the skin of both hands was normal, there was no digital ulceration. The skin of the arms, legs and trunk had a wood-like induration. The affected skin had a 'peau d'orange' appearance and was reddish-brown (Fig. 3). On the lower arms, the skin directly over the superficial veins showed no induration (groove sign, Fig. 4). Laboratory findings disclosed a raised ESR, 75 mm/h (normal 0-20 mm/h) and an increased number of eosinophils, 1040 x 10^6/L (normal < 200 x 10^6/L). Antinuclear antigen, Scl-70 and anti-centromere antibodies were absent. Histological examination of an excisional 'en bloc' biopsy of skin, fascia and muscle taken from the affected skin of the leg revealed a mild inflammatory infiltrate in the dermis, subcutis, fascia and muscle. The infiltrate consisted of lymphocytes, histiocytes and eosinophils. Patient 3 was treated with prednisone 40 mg/day. After 3 months, she noticed a gradual softening of the affected skin.

Table 1. Clinical differences between systemic sclerosis, morphea, eosinophilic fasciitis and scleredema

	Systemic scleroderma	Morphea	Eosinophilic fasciitis	Scleredema
Raynaud's phenomenon	+	-	-	-
Sclerodactyly	+	-	-	-
Aspect of the skin	diffuse, symmetrical, shiny	sharply demarcated, ivory-white	woody, orange-brown, peau d'orange	non-pitting edema
Persistent erythema	-	±	-	+
Pigment changes	+	±	-	-
Painful skin lesions	-	-	+	-
ANA	+	-	-	-
Visceral involvement	+	-	±	-
Arthralgias	+	-	±	±

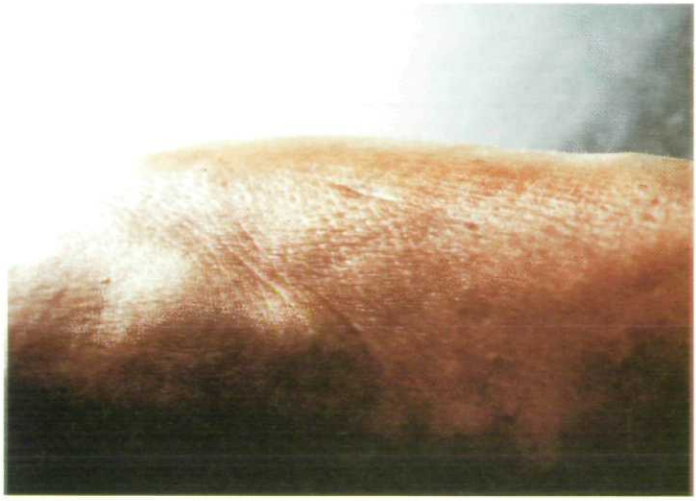

Figure 3. Orange-brown color and peau d'orange appearance of the skin in eosinophilic fasciitis.

Patient 4, a 56-year-old man, complained of hardness of the skin of the neck, back and ears. The first symptoms developed 10 years ago. He had insulin-dependent diabetes mellitus since 35 years. He suffered from complications of his diabetes, namely retinopathy and severe angiopathy. The hardness of the skin progressed very slowly, only recently the induration of his skin started to limit the mobility of neck and shoulders. Patient 4 never had Raynaud's phenomenon, and he had no complaints of shortness of breath or problems with swallowing.

At examination, the skin of neck, back and ears showed a waxy, non-pitting edema. There was a persistent erythema of the skin of the back (Fig. 5). The skin of the fingers was normal, there was no digital ulceration. There was no atrophy or depigmentation of the skin. There were no abnormal laboratory findings. Pulmonary function and esophagus manometry results were within normal ranges. Biopsy of the skin of the back showed a prominent thickening of the dermis. The collagen bundles appeared to be swollen and were separated from each other by clear spaces.

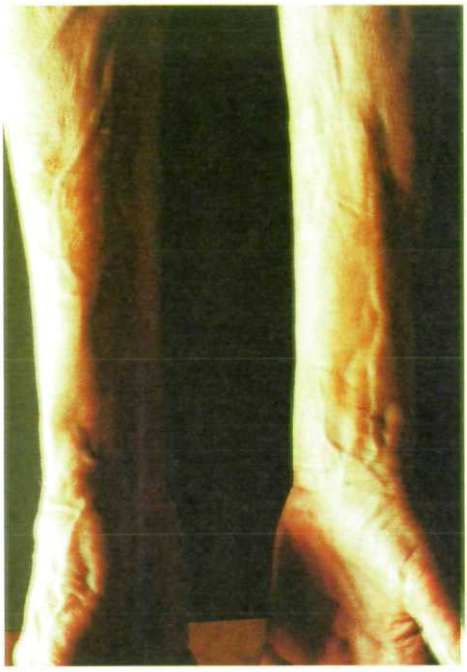

Figure 4. Groove sign in eosinophilic fasciitis

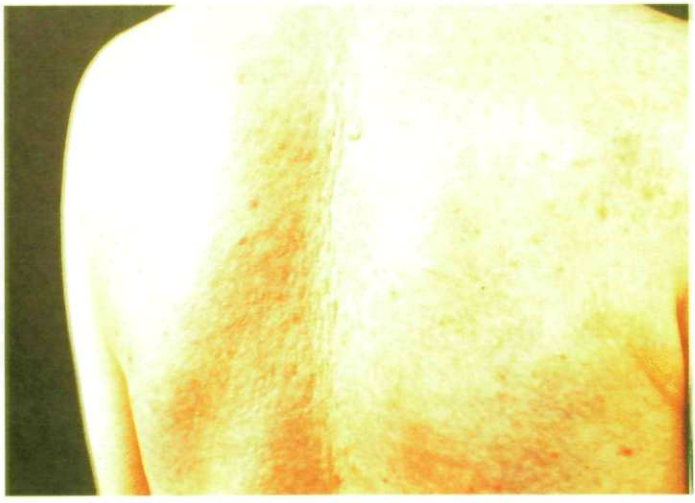

Figure 5. Erythema and waxy non-pitting edema of scleredema.

DISCUSSION

Systemic sclerosis

Scleroderma is a disease of unknown aetiopathogenesis.[2,3] The most striking symptom is the induration of the skin. The skin changes can be divided into three phases.[4] The first is edema and swelling of the skin, most often of the hands. The second phase is sclerosis and induration of the affected skin, which gives rise to the distinctive facial features and sclerodactyly. During the third and last phase there is atrophy of the skin. The sclerosis of the skin almost always starts at the hands and face and slowly progresses to the proximal parts of the body.[5] The skin indurations are symmetrical, and pigment changes are often observed. The skin has a shiny appearance. Scleroderma has a distinct histological picture. During the typical sclerotic phase, the dermis is thickened because of an increased number of compact collagen bundles. The epidermal appendages are obliterated or tightly packed in homogeneous collagen. As a rule, there are no mucin deposits in the dermis. The onset of systemic sclerosis is often preceded by Raynaud's phenomenon.[6] The fingertips of these patients characteristically show scars and ulcerations. The extent of visceral involvement due to sclerosis (fibrosis) of the affected organs is a major prognostic factor. Recently, patients with systemic sclerosis were subdivided into two major groups, limited cutaneous sclerosis and diffuse cutaneous sclerosis.[7] The first group has skin induration limited to the face and forearms, there is usually a long history of Raynaud' s phenomenon. Involvement of the viscera is usually restricted to the esophagus. The course of limited systemic sclerosis is usually benign. Diffuse systemic sclerosis belongs to the rapidly progressive end of the spectrum. Patients have widespread sclerosis of the skin, and more importantly regarding the prognosis of the disease, extensive involvement of their internal organs.

Morphea (en plaque, linear morphea and generalised morphea)

On histological grounds, no distinction can be made between systemic sclerosis and morphea (localized scleroderma). On clinical grounds this distinction can readily be made.[8,9,10] Patients with morphea often have one or more sharply demarcated indurated maculae or plaques on the trunk. The lesions vary from 1-30 centimeter and have a shiny, waxy and ivory-white appearance. Sclerotic plaques of recent onset are surrounded by a purple-red ring (lilac ring). The skin lesions are never painful and mostly asymmetrical. They appear predominantly on the trunk, sometimes the extremities are involved. The hands and especially the fingertips are never involved and appear completely normal. Patients never complain of symptoms of Raynaud's phenomenon, and there is

no visceral involvement. These last two features are very important in distinguishing between systemic sclerosis and generalised morphea. Spontaneous remission of the skin abnormalities often occurs, although (post inflammatory) hyperpigmentation may persist for years. There are no laboratory abnormalities. Linear morphea is a specific form of this entity, it consists of a linear, band-shaped lesion. This induration is asymmetrical and usually located on one of the extremities. Sometimes it causes atrophy of the underlying muscles, and normal growth of the bones may become impaired. If these anomalies occur at a young age, severe disfigurement may be the result.

Eosinofilic fasciitis (Shulman's disease).[11]

This disease is characterized by rapidly progressive and painful swelling of one or more extremities.[12,13] Within a few days to weeks followed by a orange-brown, woody induration of the involved skin. Very distinctive is the 'peau d'orange' aspect of the indurated skin. Often the so-called groove sign can be observed; this is the absence of fibrosis over the superficial veins. Eosinophilic fasciitis can be distinguished from systemic sclerosis by the above described clinical picture. Patients with this disease also show no sign of nailbed changes nor is there any evidence of Raynaud's phenomenon. The skin of the face and the hands is not involved, this is a major distinguishing feature of eosinophilic fasciitis compared to systemic sclerosis. There is often eosinophilia in the peripheral blood; a normal number of eosinophils does not rule out the diagnosis eosinophilic fasciitis. Antinuclear antibodies are absent. An excisional biopsy including fascia and muscle can be diagnostic. Typically, an infiltrate containing lymphocytes, histiocytes and eosinophils is found around the fascia and in the subcutis. Contractures of the joints are often seen. Synovitis and a symmetrical polyarthritis can develop. This disease is often preceded by a carpal tunnel syndrome.[13] Some patients react favourable on high dose steroids.

Scleredema (Buschke or diabeticorum)

Scleredema is a very rare collagen disorder.[15] The skin of the upper part of the back, the neck and the shoulders is most frequently involved. There are two types of scleredema, namely scleredema of Buschke[16] (adultorum), and scleredema diabeticorum.[17] The first is usually preceded by a febrile illness and regarded as a post-infectious condition. Scleredema diabeticorum is found in longstanding and often unsatisfactorily controlled diabetes.

Scleredema of Buschke starts often after a streptococcal infection. Days to weeks after the infection, a rapidly progressive, symmetrical and painless non-pitting induration of the skin of neck, back and shoulders is observed. Hands

and feet are seldom or never involved. A non-persistent erythema can be found. The non-pitting induration can resolve spontaneously in 6 to 24 months. Occasionally, these skin lesions persist for years. The skin shows no atrophy or pigment changes. There is no visceral involvement. No specific laboratory or immunological abnormalities have been noticed. In contrast, the histology is very specific, the epidermis is normal, the dermis is prominently thickened. This thickening is caused by swollen collagen bundles. These collagen bundles are separated from each other by clear spaces (fenestrations) which contain acid mucopolysaccharides. Scleredema diabeticorum shares the same clinical and histological features with exception of the infectious phase. The onset of the skin indurations is more gradually. The erythema is more often observed and seems to be persistent. Patients suffer almost always of complications of their disease, such as retinopathy, neuropathy or vascular problems. Better control of the diabetes mellitus has no effect on the clearing of the skin indurations. In contrast to scleredema of Buschke, spontaneous regression of the skin tightness is seldom seen. Other authors[18] proposed a subdivision into 3 groups. The first group the classical one as described by Buschke with a preceding febrile illness and spontaneous resolution. The second group are those patients who presented with scleredema but without a preceding febrile illness. These patients showed no spontaneous improvement. The third group are the patients with long-standing and poorly controlled diabetes with complications of their disease, especially vascular problems.

The name scleredema adultorum is a misnomer, it was introduced to distinguish this entity from scleredema neonatorum. Many children have been described with clinical features of scleredema (of Buschke), therefore the term scleredema is more suitable. Scleredema has been associated with paraproteinemia and multiple myeloma.[19,20]

CONCLUSION

In summary, sclerosis of the skin is not always scleroderma. A good knowledge of the different clinical presentations of systemic sclerosis, morphea, eosinophilic fasciitis and scleredema is necessary to discriminate between these closely resembling entities. Because the prognosis and therapeutic approach of each condition is completely different, it is important to make the proper diagnosis in an early stage of the disease.

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Chapter 3

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

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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 then 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.[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.[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.[6-9]

Photopheresis is the repeated extracorporeal exposure of peripheral blood lymphocytes to UVA (320-400 nm) in the presence of psoralens (8-methoxy-psoralen [8-MOP]). The lymphocytes are then reinfused into the patient. It has been recommended as an experimental treatment in patients with Ssc.[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.[12] Photopheresis was initially developed for treating CTCL patients.[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 [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[17] 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.[10,13] 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.[18] The psoralen levels in the treatment bag were monitored to make sure that they were above the recommended concentration of 60 ng/mL.[18,19]

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.[20-22] The sum of all skin scores obtained from 74 areas of the body (see Fig. 1) was used as outcome parameter.[23]

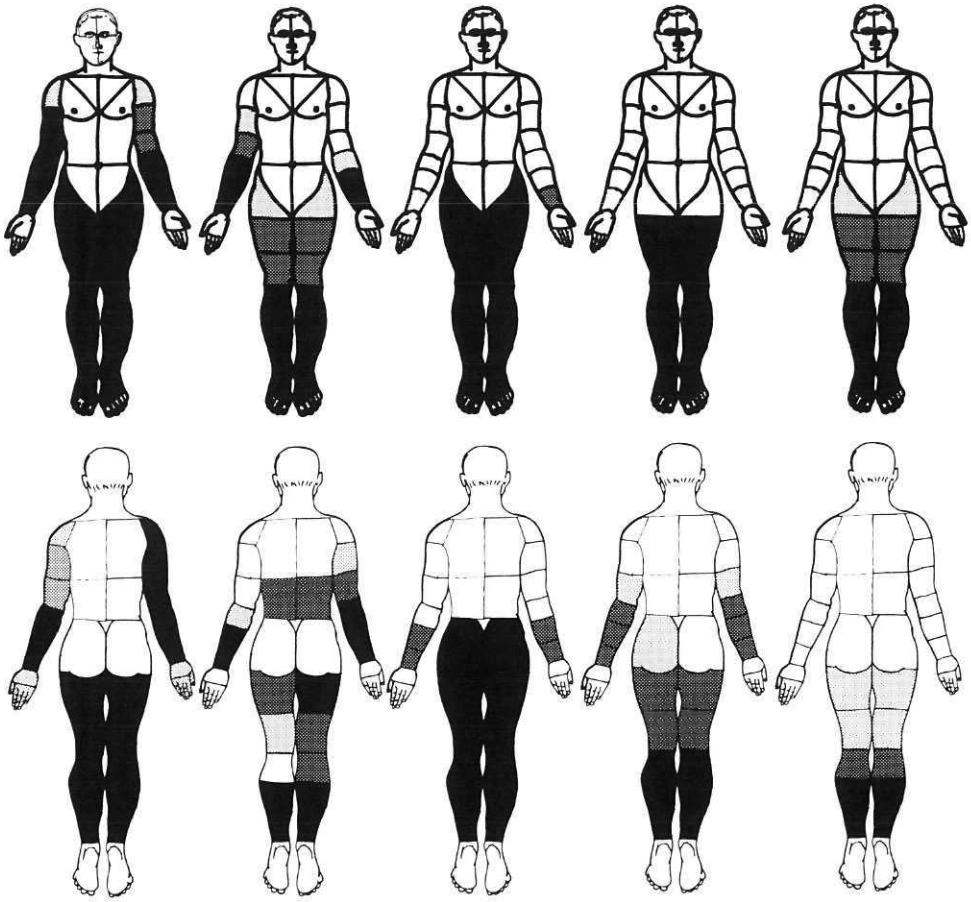

Figure 1. Improvement of skin score during photopheresis treatment. Skin score was assessed every 3 months, in 74 body areas. Black: severe induration (score 3), dark grey: moderate induration (score 2), light grey: mild induration (score 1), white: normal skin (score 0).

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.[29] 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

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

* s.e.: standard error

Table 2. Patient characteristics (individual parameters)

Patient number	Sex	Age (year)	Disease Duration (months)	Antibodies	Lung function (CO-diffusion) (%)	Esophagus Involvement
Group A						
1	m	85	2	ANA, RF, SS-A	64	-
2	f	54	48	ANA, Scl-70	35	+
3	f	30	9	-	81	-
4	m	44	51	ANA	86	+
5	f	29	15	ANA	44	+
6	f	67	35	ANA	47	+
7	f	55	58	ANA, Scl-70	35	+
8	f	19	2	ANA	82	-
9	m	27	8	ANA, RF, Scl-70	43	+
10	f	47	5	ANA	91	+
Group B						
11	m	41	9	-	99	-
12	f	40	7	ANA	77	+
13	f	69	23	ANA	43	+
14	f	28	9	ANA, Scl-70	79	+
15	f	51	15	ANA, a-Centr	86	-
16	f	52	10	ANA, RF, a-Centr	71	+
17	f	32	1	ANA, SS-A	100	+
18	f	44	1	ANA, Scl-70	49	+
19	f	45	19	ANA, RF	49	+

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

(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.

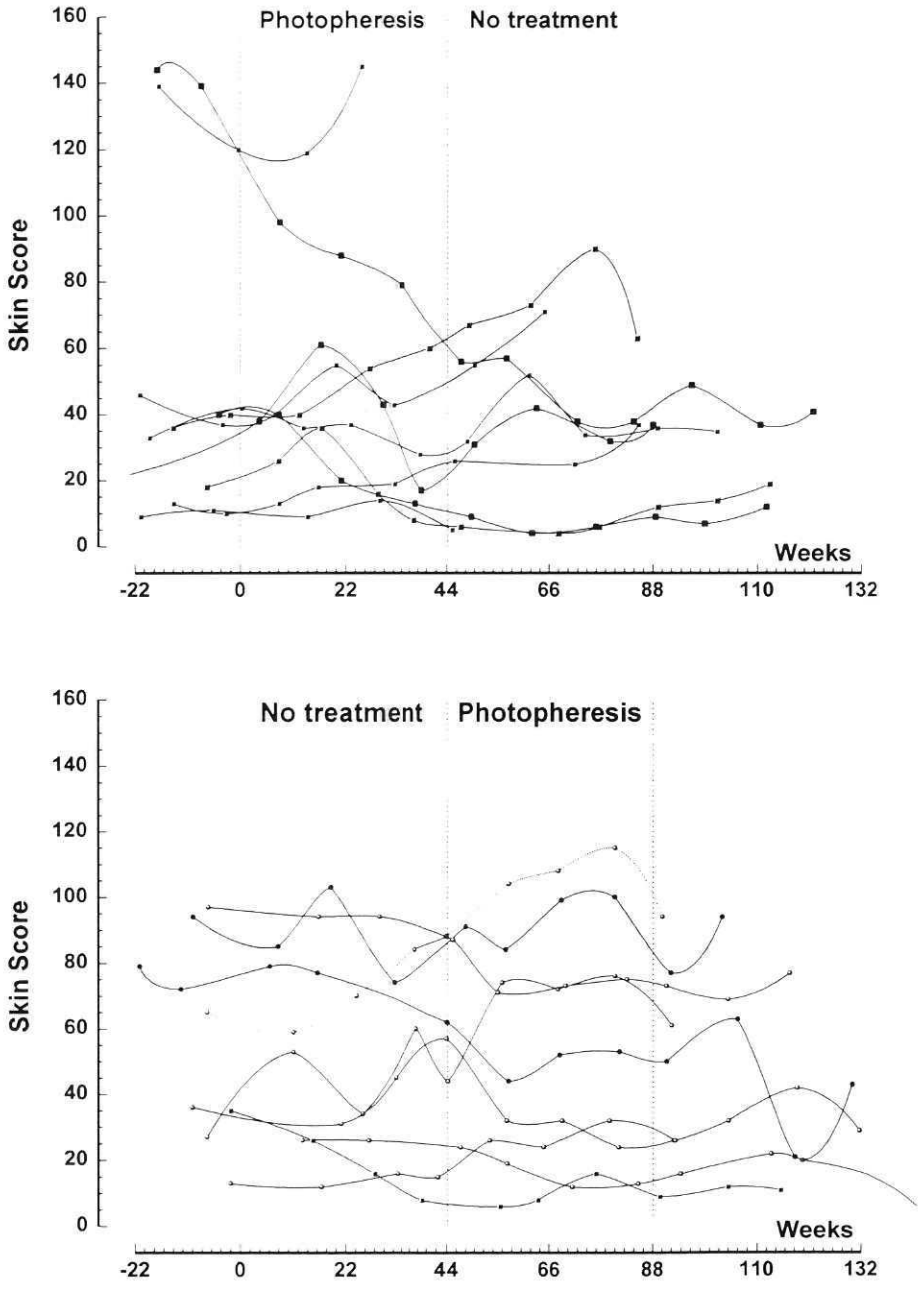

Fig 2. Individual curves reflecting the disease activity, measured with the skin score. In group A (upper graph), a slight average reduction (-5.4%) during the photopheresis period was observed. In group B (lower graph) where no treatment at all was given in this period, a slight average increase was 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.[6,32-38]

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.[39-44] 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).[45-48]

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.[49]

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.[50-56] It has been shown that collagen production by fibroblasts can be enhanced by cytokines such as transforming growth factor ß, released from inflammatory cells.[57]

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.[58] 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.[30] 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.

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Chapter 4

Extracorporeal photochemotherapy (photopheresis) induces apoptosis in lymphocytes: a possible mechanism of action of PUVA therapy

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SUMMARY

The mechanism of action of psoralen plus UVA (PUVA) and photopheresis is not entirely understood. These therapies are assumed to be immuno-modulating partly by gradually decreasing leukocyte viability. We investigated whether this delayed form of cell death was due to apoptosis. Untreated and treated (PUVA exposed) leukocytes obtained from six patients with systemic sclerosis and (untreated) leukocytes from healthy control individuals were studied. Qualitative gel electrophoresis and quantitative *in situ* nick translation analysis of DNA fragmentation was performed. Apoptosis of the treated cells did occur (gel electrophoresis) after 24 h. At t = 0 h, immediately after exposure to PUVA, there was no evidence of DNA fragmentation in the treated cells. The percentage of treated cells undergoing apoptosis was 20-55% at t = 24 h (*in situ* nick translation). The untreated leukocytes of the patients and the healthy individuals showed no distinctive rise in apoptotic cells. Apoptosis of the leukocytes after PUVA or photopheresis treatment might be a mechanism of action and might explain the therapeutic response.

INTRODUCTION

Photochemotherapy, the combination of psoralen and UVA (320-400 nm), is known as PUVA therapy. It is used in the treatment of a variety of skin disorders such as psoriasis,[1,2] vitiligo[3] and cutaneous T-cell lymphoma (CTCL).[4] Psoralens and its derivatives are naturally occurring tricyclic furocoumarins present in a large number of plants, but there also exist synthetic psoralen compounds. Psoralens are absorbed after oral administration and are distributed within all tissues and body fluids. Psoralens are known to be photosensitizing chemicals and in the absence of UV irradiation intercalate into DNA. When exposed to UVA irradiation, they form mono- and bifunctional adducts with pyrimidine bases in DNA, resulting in crosslinking by psoralen between base-paired strands of DNA.[5]

Psoralens plus UVA is known to decrease cell viability as well as to upregulate production of certain cytokines in epidermal cells.[6-8] Thus, its efficacy in various diseases may depend on its induction of loss of cellular functions (immunosuppression, decreased cell cycling: psoriasis and CTCL) or the induction of growth and differentiation of stimulating cytokines (vitiligo).

Although the effects of this treatment can be attributed to the formation of psoralen photoadducts in DNA,[9] other intracellular sites of action have been

identified.[10] An extracorporeal form of photochemotherapy, photopheresis, has been developed to treat patients with CTCL,[11] and is now an established therapy for this disease in the United States and several European countries. Recently, encouraging results with photopheresis in systemic sclerosis[12] and graft *versus* host disease[13] have been reported. Several theories have been put forward to explain the mechanism of action of photopheresis in CTCL and scleroderma, including the production of anti-idiotypic antibodies[14] and the generation of clone-specific suppressor T cells[15] after the reinfusion of leukocytes that have been exposed to PUVA extracorporeally.

In photopheresis peripheral blood leukocytes are collected in six cycles and exposed for 1.5 - 3.5 h to 8-methoxypsoralen (8-MOP) and UVA irradiation followed by the reinfusion of the treated cells into the (donor) patient. In a sample of these cells taken just prior to their reinfusion into the patient, we observed, *in vitro*, a decrease in viability of the irradiated cells over 48 h. In fact, these PUVA-treated mononuclear cells undergo a delayed form of cell death.[16] Because we did not find a substantial number of dead cells immediately after the photopheresis treatment, we investigated whether this form of cell death was due to apoptosis.[17] To support this hypothesis, qualitative (gel electrophoresis) and quantitative (*in situ* nick translation) analyses of DNA fragmentation were performed.

PATIENTS AND METHODS

Patients and healthy control individuals

This study was part of a development program investigating the value of extracorporeal photopheresis in patients with systemic sclerosis. All patients participating in this part of the program fulfilled the criteria of the American Rheumatism Association[18] and suffered from the diffuse form of progressive systemic sclerosis (type II and III).[19] They received no other immunomodulatory medication. Control cells were obtained from age- and sex-matched healthy individuals.

Photopheresis procedure

Patients were treated with the UVAR system (Therakos, Inc. West Chester, PA, USA). This machine collects the buffy coat leukocytes and exposes these cells to UVA irradiation. In six cycles, blood (125 mL per cycle) was withdrawn and centrifuged, the buffy coat of these six cycles were collected sequentially in the treatment bag. The erythrocytes and most of the plasma were directly reinfused

into the patient, before starting a new cycle. When the first buffy coat sample was collected (40 mL), 200 μg stabilized aqueous 8-MOP solution (EX-8-MOP, Gerot, Vienna, Austria), was administered directly into the treatment bag. The final concentration of 8-MOP in the treatment bag just prior to reinfusion into the patient was between 100-155 ng/mL. The treatment bag was connected to a disposable cassette where the leukocytes were exposed to UVA irradiation (2 J/cm^2). Collection of the buffy coat leukocytes (240 mL) was performed in approximately 1.5 h. The photoactivation of the leukocytes took another 1.5 h. Photopheresis therapy was given every four weeks on two consecutive days.

Leukocytes

In addition to the leukocytes obtained through photopheresis, we also studied peripheral blood-derived cells from patients as well as from healthy control individuals. Peripheral blood mononuclear cells (PBMNC) were isolated by Ficoll-Paque density gradient centrifugation from heparinized blood and directly used. Cells were also analyzed after stimulation by anti-CD28 (subclass IgG, CLB) and anti-CD3 monoclonal antibody (T3/4.E, subclass IgE, CLB). To determine the induction of apoptosis after 24 h, some of the cells were washed in phosphate-buffered saline (PBS), resuspended in Iscove's modified Dulbecco's medium (IMDM) supplemented with penicillin (100 U/mL) and streptomycin (100 μg/mL) and 10% heat-inactivated fetal calf serum (FCS) and incubated at 37°C for 24 hours. The cell density of the culture was 2 x 10^6/mL.

Gel electrophoresis

The fragmentation of DNA into subunits was visualized by gel electrophoresis. Cells (3 million) were collected by centrifugation at 8000 rpm for 7 min and the cells were lysed with 500 μL TTE solution (160 μL 0.5 *M* TRIS, 320 μL 5% Triton X-100, 16 μL 0.5 *M* EDTA). After vigorously vortexing, the lysates were centrifuged at 14000 rpm for 10 min at 4°C. The supernatant, containing fragmented DNA, was collected. One hundred microliters of ice-cold 5 *M* NaCl was added to the supernatant and after careful mixing, 700 μL iso-propanol (-20°C) was added to remove protein. The samples were placed at -20°C overnight to precipitate DNA. Samples were centrifuged at 14000 rpm for 10 min at 4°C, the supernatant was carefully removed, and 800 μL 70% ethanol (-20°C) was added and carefully mixed. The tubes were centrifuged at 14000 rpm for 10 min at 4°C, the supernatants were carefully removed. Pellets were air dried in a vacuumcentrifuge, 30 μL TBE (89 m*M* TRIS, 89 m*M* boric acid, 2 m*M* EDTA, pH 8) buffer was added to the pellet. After addition of 2 μL loading buffer (50% glycerol, 1 m*M* EDTA, 0.4% bromophenol blue, 0.4% xylene cyanol, 0.5% sodium dodecyl sulphate (SDS)) to 8 μL sample, electrophoresis

was performed in 1.5% agarose gel for 2 hours at 50 V and 38 mA. DNA was visualized by staining with ethidium bromide under UV irradiation.[20]

In situ nick translation

Cells undergoing apoptosis were labelled by *in situ* nick translation. Cells (2 x 10^6) were fixed in 1% formaldehyde and ethanol. After washing, they were incubated (90 min, 15°C) in a total volume of 10 µL with 55 µ*M* biotin-labelled dUTP, a mixture of 19 µ*M* dATP, dGTP and dCTP, and DNA polymerase (100 U/mL) in 50 m*M* TrisCl, pH 7.8, 5 m*M* $MgCl_2$, 10 m*M* 2-mercapto-ethanol and bovine serum albumin (10 µg/mL). Cells were washed in PBS containing 0.1% (vol/vol) Triton X-100 and incubated with 40 µL avidin-fluorescein-isothiocyanate (FITC, 2.5 µg/mL) and RNase (20 µg/mL) in 4 x standard saline citrate 0.1% (vol/vol) Triton X-100 and 5% (wt/vol) non-fat dry milk for 30 min at room temperature. Cells were then washed and resuspended in PBS containing 0.1% (vol/vol) Triton X-100 and propidium iodide (5 µg/mL) and analyzed on a FACSCAN cytofluometer. The percentage FITC-positive cells as determined by the propidium iodide staining represents the percentage of cells in apoptosis.[21]

RESULTS

Leukocytes were analyzed directly after their exposure to psoralen and UVA (t = 0) and at 18 h (t = 18) and 24 h (t = 24) after treatment. Untreated leukocytes from both patients and healthy control individuals were analyzed at the same time intervals. It appeared that the viability of the cells, determined by their ability to exclude trypan blue, gradually decreased over 24 h (Fig. 1).

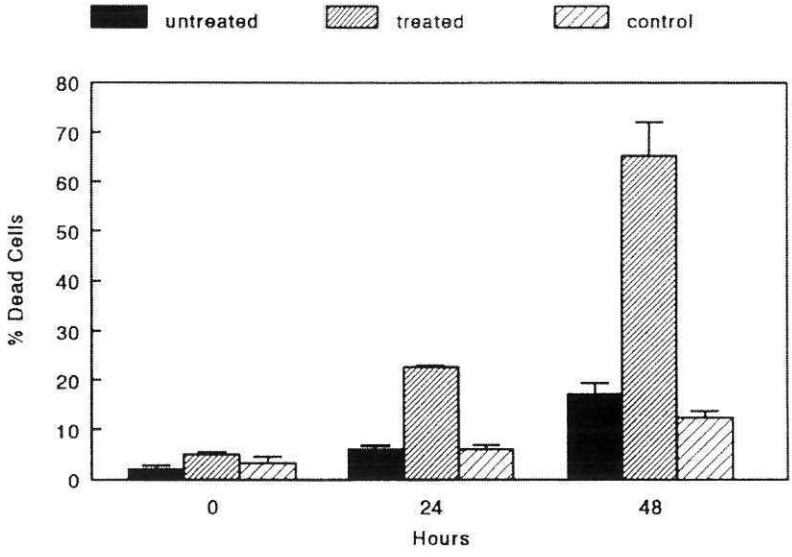

Figure 1. Loss of cell viability (% of dead cells), shown by trypan blue inclusion. Untreated cells: PBMNC obtained before photopheresis treatment. Treated cells: buffy coat cells from treatment bag after photopheresis. Control: PBMNC from healthy volunteers. The bars labeled untreated and treated display the mean of the analysis of the leukocytes from four different patients at the different time intervals. The bar labeled control shows the mean of the analysis of leukocytes from four age- and sex-matched healthy individuals. The error bars are the standard of the mean (SEM) of each analysis at the different time intervals.

There was no difference in viability between untreated patient cells and cells from healthy control individuals at t = 0 h; the viability of the treated cells however was slightly decreased. After 24 h there was an increase in the percentage of nonviable cells collected from the treatment bag compared to untreated cells of the same patients ($p = 0.0022$) and to cells of the healthy control individuals ($p = 0.0023$). Also, after 48 h there was an increase in the percentage of nonviable cells compared to the untreated cells of the patients ($p = 0.0005$) and compared to the cells of the healthy individuals ($p = 0.0003$). Apoptosis did occur as was evidenced by gel electrophoresis. *In vitro*, DNA fragmentation was indeed observed in cells from the treatment bag after exposure to psoralen and UVA at t = 24 h. At t = 0 h, immediately after their exposure to PUVA, there was no evidence of DNA fragmentation in the treated cells (Fig. 2). Cells from normal volunteers and untreated patient cells showed some apoptosis which is a normal finding in healthy individuals.

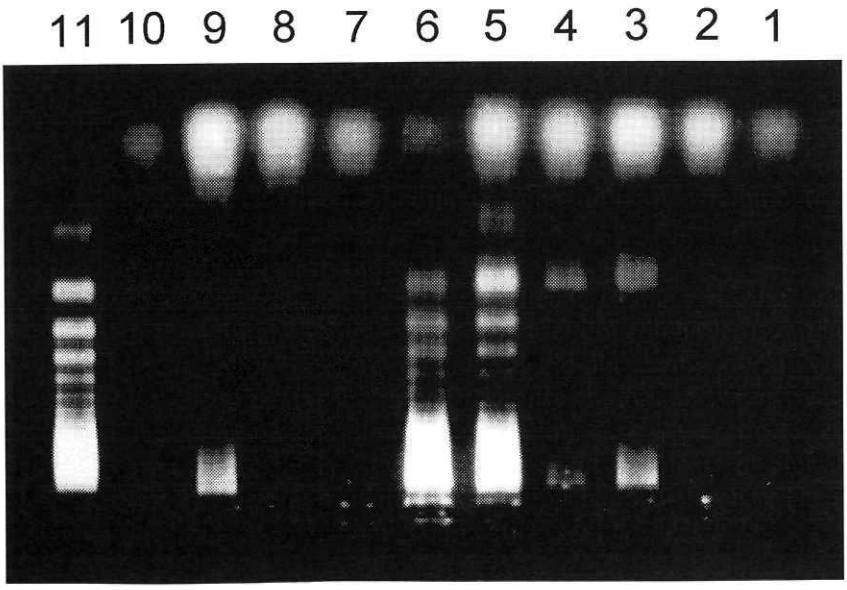

Figure 2. Lane 1, untreated cells from patient at t = 0 h; lane 2, untreated cells from patient at t = 18 h; lane 3, untreated cells from patient at t = 24 h; lane 4, treated cells at t = 0 h; lane 5, treated cells at t = 18 h; lane 6, treated cells at t = 24 h; lane 7, untreated cells from healthy individual at t = 0 h; lane 8, untreated cells from healthy individual at t = 18 h; lane 9, untreated cells from healthy individual at t = 24 h; lane 10, negative control; lane 11, positive control.

The percentage of cells in apoptosis was determined by *in situ* nick translation. The percentage of cells undergoing apoptosis was 1-5% at t = 0 h in treated and untreated leukocytes. After 24 h the percentage of cells undergoing apoptosis was 20-55% in the cells exposed to psoralen and UVA. The untreated leukocytes showed no distinctive rise in apoptotic cells (1-9%) after 24 h (Fig. 3) and this was significant (p = 0.0012).

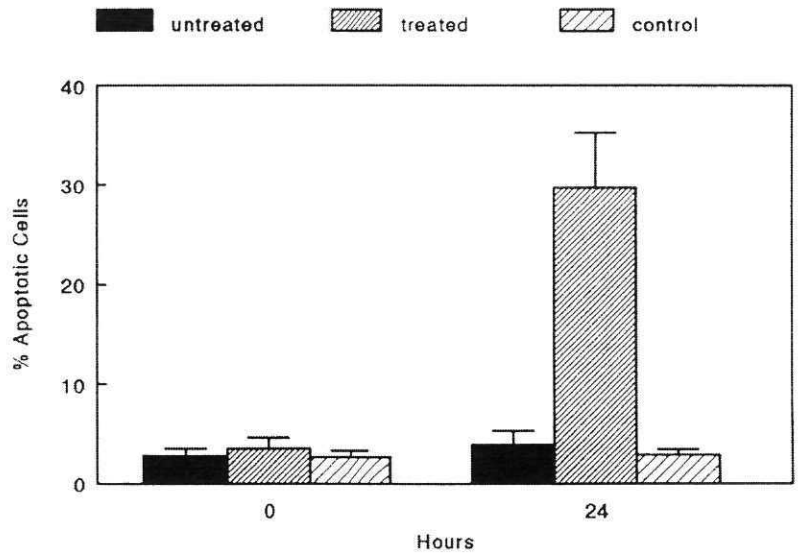

Figure 3. Percentage of cells in apoptosis, determined by in situ nick translation. Untreated cells: PBMNC obtained before the start of photopheresis treatment. Treated cells: buffy coat cells from treatment bag obtained after the start of photopheresis. Control: PBMNC from healthy volunteers. The bars labeled untreated and treated display the mean of the analysis of the leukocytes from four different patients at the different time intervals. The bar labeled control shows the mean of the analysis of leukocytes from four age- and sex-matched healthy individuals. The error bars are the standard of the mean (SEM) of each analysis at the different time intervals.

Stimulation of the isolated cells with a combination of two monoclonal antibodies directed against the CD3 T-cell receptor (TCR) complex and CD28 at t = 0 h, t = 18 h and t = 24 h in samples of treated and untreated cells did not alter the percentage of apoptotic cells mentioned above (results not shown). In general there is ample evidence that one of the triggers of apoptosis is activation of the TCR, for this reason we stimulated the cells with the monoclonal antibodies against the CD3 TCR.

DISCUSSION

Photopheresis has been reported to exert a beneficial effect on the cutaneous fibrosis in patients with systemic sclerosis.[12] The mechanism of action of extracorporeal photochemotherapy is not yet understood. It is important to know what happens to the peripheral blood lymphocytes after reinfusion. If they are severely damaged by photochemotherapy, they will be eliminated rapidly by the reticuloendothelial system and the chance that they will be able to interact with other immunomodulatory cells or exchange messages by cytokine release would be low. If they are still viable for some days, but modified to undergo irreversible programmed cell death, a highly regulated process, it is conceivable that they could release signalling proteins.[22] This is of course hypothetical and needs to be confirmed in the future. Our results support the hypothesis that after treatment these cells are still viable for a few days and can for instance respond by releasing immunomodulatory cytokines or for example through an altered expression of membrane receptors that could modulate immunoreactivity. Therefore we studied the fate of these cells after extracorporeal exposure to PUVA. The observation of gradual cell death over 48 h, as determined by trypan blue exclusion, raised the question whether this could be attributed to induction of apoptosis. Yoo et al[23] recently showed the induction of apoptosis in CTCL patients treated with photopheresis. Qualitative and quantitative analysis of DNA fragmentation by gel electrophoresis and *in situ* nick translation, respectively, indeed showed the occurrence of apoptosis of treated lymphocytes.

A rise in percentage of apoptotic cells was found 24 h after exposure to PUVA, whereas in the nonexposed peripheral cells there was not such a distinctive rise in apoptotic cells. Our results are in agreement with the earlier observations of Volc-Platzer *et al.*[13] and Marks and Fox,[24] although different methods to determine DNA fragmentation were used: morphology *versus in situ* nick translation.

It is possible that lymphocytes are altered or damaged after exposure to PUVA, which could lead for instance to a change in their functional capacity and the expression of membrane molecules. These altered cells may modu-late the immune response by either immune or nonimmune mechanisms. Induction of apoptotic cells as demonstrated in the present study could be part of the mechanism of action of photopheresis. This could be one of the explanations for the observed effects such as improvement of erythroderma in CTCL and the decrease of skin sclerosis in systemic sclerosis.

Photopheresis can be regarded as a modified (extracorporeal) PUVA therapy; the latter is extensively used in dermatology. Apoptosis of cells in the human skin after PUVA therapy might be a mechanism of action and might explain the therapeutic response in psoriasis, for instance.

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Chapter 5

Quantification of cutaneous sclerosis with a skin elasticity meter in patients with generalized scleroderma

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SUMMARY

Background: The skin score, a subjective assessment of skin elasticity, is widely used in patients with systemic sclerosis. Although this scoring method is regarded as a validated and accepted tool, the interobserver and intra-observer reproducibility is relatively poor.
Objective: Our purpose was to investigate whether the recently developed SEM 474 cutometer, which exerts a controlled vacuum force to the skin, can measure skin elasticity more objectively tha the skin score.
Methods: Skin elasticity was measured in 74 different body areas in patients with systemic sclerosis, and compared with the skin score obtained from the same areas.
Results: The cutometer produced quantitative and reproducible data. A large-diameter (8 mm) measuring probe was superior to a small probe. The interobserver intraclass correlation coefficient (ICC) was 0.92; the intraobserver ICC was 0.94. A linear correlation was found with the clinical skin score; the Spearman rank correlation test was 0.69.
Conclusion: The correlation with the skin score was reasonable, despite the observation that regional differences in skin elasticity were detected by the cutometer but not by the human observer, who automatically compensates for these factors and integrates them in the skin score. The high interobserver and intraobserver ICC makes the cutometer more suitable for quantifying changes in skin thickness than the subjective skin score.

INTRODUCTION

In patients with systemic sclerosis, the sclerotic skin changes correlate with the overall disease activity and prognosis.[1,2] The best accepted and most widely used evaluation method for skin thickness is the 'skin score', which is based on subjective examination of the skin by a trained observer.[1-11] Although the interobserver and intraobserver reliability of the skin score can be low,[12] it is still regarded as a suitable primary outcome variable in clinical trials because it is easy to use and clinically useful alternative methods are lacking.[11-14]
To reduce subjectivity and increase reliability, many investigators have tried to develop devices that can measure skin sclerosis objectively and quantitative-ly. Thickening of the skin in patients with scleroderma is caused by an increase in collagen formation in the dermis and possibly or temporarily by an increased amount of edema in the skin. The amount of collagen can be measured in

standard skin biopsy specimens by weighing, histometric methods, or biochemical assays.[8,15,16] The thickness of the dermis can be measured with high-frequency ultrasound[6,17-21] and possibly by nuclear magnetic resonance imaging.[22]

As a result of the accumulation of collagen and fluid, the skin develops its thickened appearance. It becomes impossible to pinch skin into a normal skinfold. This phenomenon, known as 'hidebinding' or 'tethering', is the most impressive change in sclerotic skin and is the basis of the skin score.[1] To quantify this fixation of the skin, several mechanical instruments have been developed that can exert a controlled physical force to the skin, such as impression by a durometer,[4,5] linear extension with an elastometer,[23] or rotation with a twistometer.[3,24,25] We tested a new skin elasticity meter, the SEM 474 cutometer,[26-29] which lifts the skin into a measurement chamber and therefore represents the closest possible mechanical imitation of pinching skin into a skinfold. The purpose of this study was to evaluate the interobserver and intraobserver reliability of the elasticity measurements and to assess the correlation between skin score and skin elasticity measurements.

PATIENTS AND METHODS

Patients

We evaluated the skin of 19 consecutive patients with systemic sclerosis. All patients fulfilled the criteria for the diagnosis of systemic sclerosis as defined by the American Rheumatism Association in 1980[30] and had the more severe type of scleroderma, described as type II or III scleroderma.[31-36] Type I is acroscleroderma, type II is acroscleroderma with progression of the sclerosis to proximal areas such as the arms and legs, and type III is diffuse scleroderma, usually starting on the trunk, with rapid progression to other areas including the extremities. In each patient 74 body areas were evaluated by means of the skin score and measured with the skin elasticity meter. In five of these patients, all women between 20 and 66 years of age (average, 45 years) with type III systemic sclerosis of recent onset (< 48 months; average duration, 26 months), the 74 areas were measured with 2 different probe sizes and by two independent observers.

Skin Score

Cutaneous sclerosis was assessed on a 0 to 3 scale by a trained observer who palpated the skin in 74 body areas (Fig 1). The modified Rodnan skin score was used (0 = normal skin thickness, 1 = mild skin thickness, 2 = moderate skin thickness, 3 = severe skin thickness with inability to pinch the skin into a fold).[1,8,11] To obtain a more refined score, the number of evaluated body areas was increased to 74 instead of the original 26 or 17 described by Rodnan. This score can be easily translated into the 26 or 17 areas skin score used in previous publications.

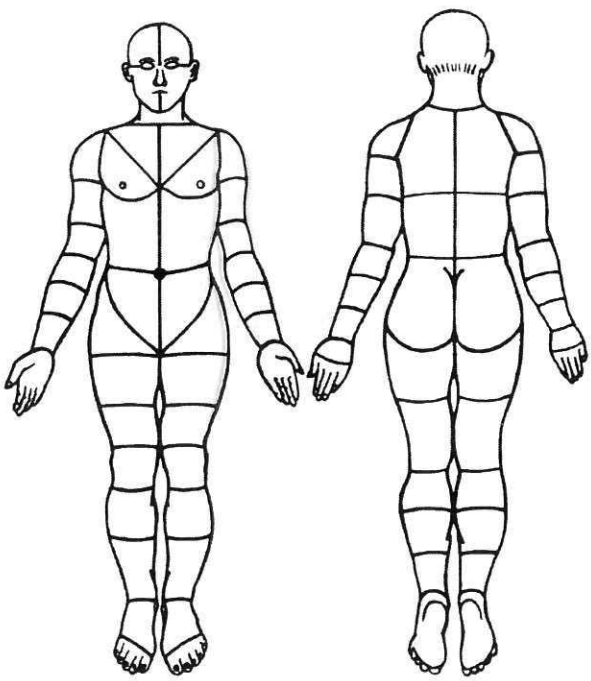

Figure 1. Skin score was assessed in these 74 body areas with a 0-3 scale known as the modified Rodnan score (see text).

The SEM 474 Cutometer

We used a specially developed skin elasticity meter, the SEM 474 cutometer (Courage + Khazaka Electronic GmbH, Cologne, Germany). This cutometer meter, equipped with an 8 mm measuring probe, must be connected to a personal computer (Fig. 2). For measurements the skin is drawn into a low-pressure (500 mBar) chamber. The depth of penetration in the low pressure chamber is determined by a noncontact optical measuring system (Fig 3).

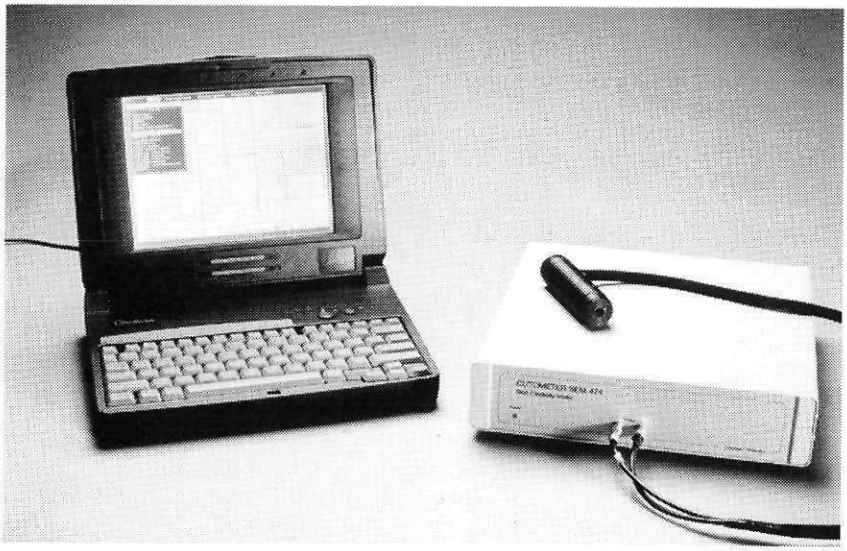

Figure 2. The SEM 474 cutometer system consists of a measurement unit containing a vacuum pump, a measurement probe with a fixed opening (2 to 10 mm), and a personal computer.

The SEM cutometer measurement protocols can be varied with the included software, which is flexible and easy to use. The pressure can be adjusted between 50 and 500 mBar, and can be built up immediately or gradually at a controlled rate. The suction time and relaxation time can be changed from 0.1 to 60 seconds, and the number of measurement cycles from 1-99. The software can deliver a graphic and numeric output in four different modes. Both skin elasticity and skin relaxation can be evaluated. Exchangeable measurement probes with different apertures (2, 4, 6, and 8 mm) are available. The measurement protocol consisted of suction at 500 mBar low pressure for 1 second, followed by 1 second of relaxation. This was repeated three times.

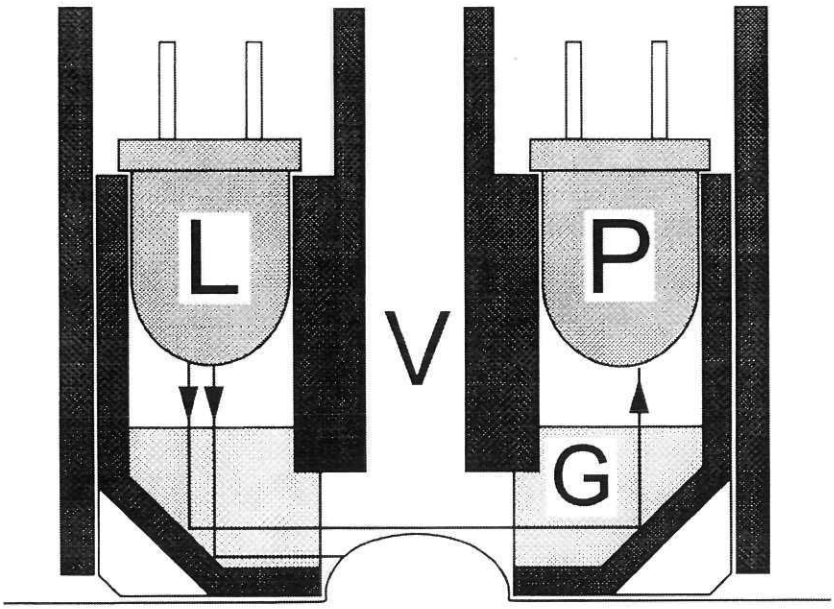

Figure 3. Schematic diagram of the measuring head of the SEM 474 cutometer. A spring ensures that the central part of the probe is pushed to the skin at constant pressure. The skin is drawn into a vacuum chamber (V) by a constant 500 mBar vacuum force. The extent at which the skin is drawn into the aperture is measured by the interruption of an infra-red light beam, which is emitted by a light-emitting diode (L), and guided through glass (G) and mirrors towards a photoelectric cell (P).

Fig. 4 shows two repeated measurements. The maximal or final deformation (called U_f according to the nomenclature used by Agache et al.[37]) depends on the skin thickness. It consists of a linear elastic part (U_e) and a nonlinear viscoelastic part (U_v). The presence of fluid in the skin reduces the skin's ability to recover to its initial position after deformation. As a result, the difference between the skin level after one suction cycle and the initial position is higher in edematous skin. This difference is called R_4 (result 4) in the graphic output from the cutometer (Fig. 4). On a theoretic basis, the final extension (U_f) after the first suction, as well as the maximum height after the second suction cycle (R_3) minus R_4 could be good indicators of skin sclerosis. Therefore both U_f and R_3-R_4 were used in the data analysis.

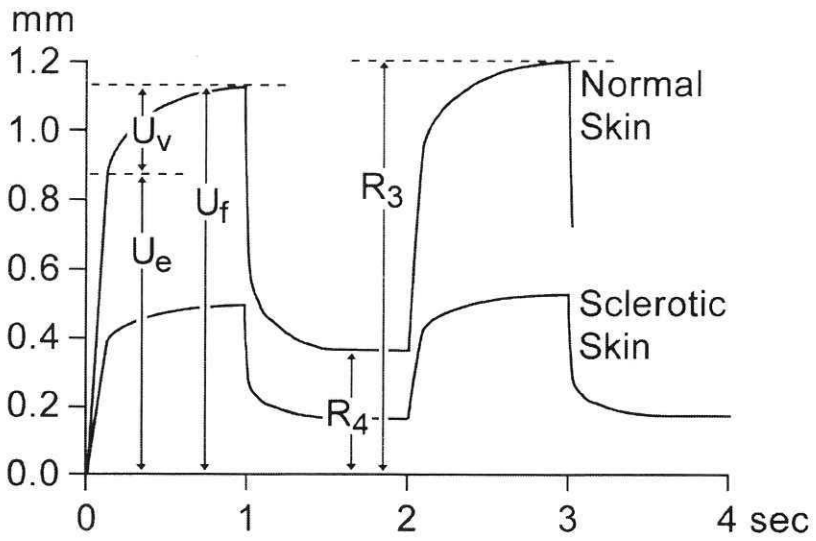

Figure 4. Graphic output from the SEM 474 cutometer. The lower line is obtained from a sclerotic skin area on the lower arm with skin score 3; the upper line is from a skin area on the upper arm with skin score 0 (normal skin). U_e represents the elastic trajectory of the final skin extension (U_f), and U_v the viscoelastic part. R_4 corresponds with the skin's inability to recover to its initial position.

To assess intraobserver reliability, 74 skin areas of a normal control subject were evaluated twice by the same observer (two repeated measurements within 1.5 to 2 hours). To assess the interobserver reliability, 74 areas of 5 patients were evaluated independently by two observers (within 1.5 to 2 hours). To obtain information about the intrinsic measurement errors of the equipment, 10 repeated measurements of the same skin area were done at a 5 minute interval. All measurements were performed with the patients in supine position, in an air conditioned room (temperature 20-21 °C, air humidity 55-60%). It takes about 45 minutes to measure 74 body areas.

Statistical analysis

Important questions for the overall judgement of the skin elasticity meter are whether the results are reliable (reproducible), valid, and accurate.[38,39] The intraobserver agreement was analyzed by calculating the intraclass correlation coefficient (ICC) of repeated measurements by the same observer.[40-42] The interobserver agreement was analyse by calculating the ICC of measurements by two independent observers. The ICC can be used to express levels of agreement between and within observers as a figure between 0 and 1. An ICC above 0.75 indicates an acceptable level of concordance.[42]

The validity was assessed by comparing cutometer measurements (the mean of two measurements performed by two independent observers) with the skin scores given to the same areas, by means of the Spearman rank correlation test. Student's *t* tests were used to compare results between normal skin and the three gradations of sclerotic skin. Statistical significance was defined as $p < 0.05$.

RESULTS

All patients were initially measured with the small 2 mm probe which is recommended for studies on epidermal elasticity.[27] After these data were analyzed, it appeared that the correlation between skin score and skin elasticity measured with the 2 mm probe was below 0.6 (Spearman correlation test), and that the correlation improved with the use of a larger probe. Therefore 5 patients with type III systemic sclerosis were measured again with the largest probe (8 mm). All further data in this report were obtained with the 8 mm probe in these five patients. Patient A had 36 body areas with skin score 0, 15 areas with score 1, 6 with score 2, and 17 with score 3. Patient B had 19 with score 0, 44 with score 1, 10 with score 2, and 1 with score 3. Patient C had 35 with score 0, 26 with score 1, and 13 with score 2. Patient D had 37 with score 0, 17 with score 1, 14 with score 2, and 6 with score 3, and patient E had 22 with score 0, 34 with score 1, 16 with score 2, and 2 with score 3.

The intraobserver agreement, expressed as the ICC calculated from two repeated measurements of 74 body areas by the same observer, was 0.94 for U_f, the 95% confidence interval (95% CI) was 0.92 to 1.00. The interobserver agreement, expressed as the ICC calculated from the skin elasticity measured by two independent observers in 74 different body areas of 5 patients with diffuse systemic sclerosis, was 0.92 (95% CI, 0.89 to 1.00) for U_f (Fig. 5) and 0.89 (95% CI, 0.84 to 1.00) for R_3-R_4.

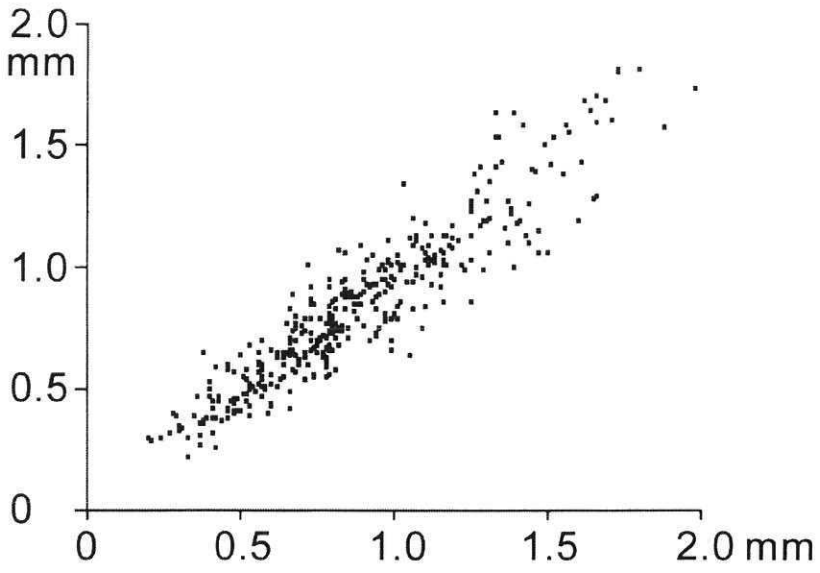

Figure 5. Correlation between cutometer measurements performed on the same day by two independent observers. The intraclass correlation coefficient between observers was 0.92.

Ten repeated measurements of the same skin area on the thigh of a normal subject (score 0), performed with a 5-minute interval to allow for skin relaxation, showed an average distension of 1.25 mm with a standard deviation of 0.029 mm (95% CI, 1.23 to 1.27 mm).

The cutometer measurements were also compared with the skin score of the same areas, assessed by an experienced observer. In general, the results were comparable. The Spearman rank correlation coefficient was 0.67 for the final deformation (U_f) and 0.63 for R_3-R_4. Fig. 6 shows the relation between the cutometer measurements and the different skin scores. As expected, low values for skin extensibility were found in skin with severe sclerosis, and high values were found in normal skin. The average skin extension was 1.29 (95% CI, 1.22 to 1.35 mm) in normal control skin, 1.11 mm (95% CI, 1.06 to 1.16 mm) for clinically normal skin (score 0) in the patients with scleroderma, 0.78 mm (95% CI, 0.75 to 0.82 mm) for score 1, 0.62 mm (95% CI, 0.57 to 0.66 mm) for score 2, and 0.41 mm (95% CI, 0.37 to 0.46 mm) for score 3. All differences between these scores were statistically significant (Student's *t* test, $p < 0.001$).

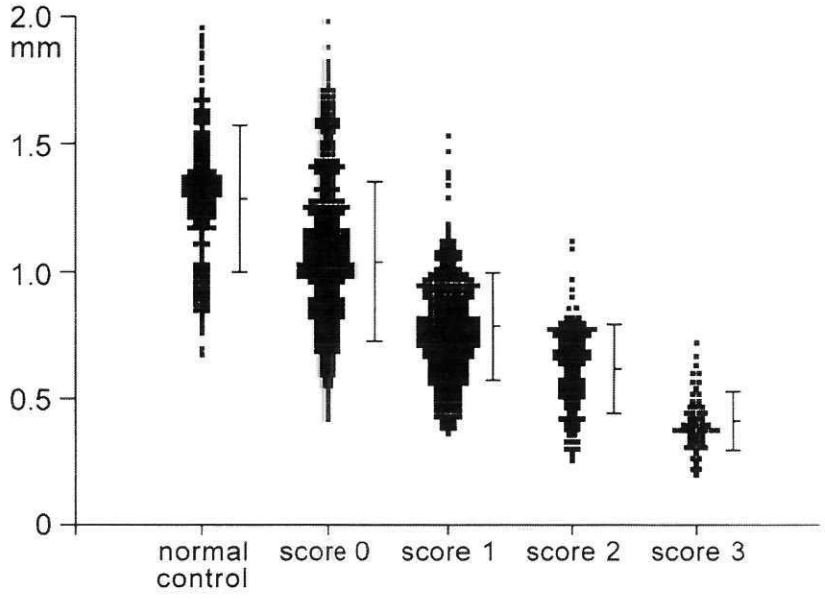

Figure 6. Correlation of skin elasticity measured by the SEM 474 cutometer with the clinical skin score. The extent at which the skin is pulled into a measurement chamber by a constant 500 mBar vacuum is plotted (in millimeters) at the Y-axis. Measurements were performed in healthy control persons (normal control), normal-appearing skin of patients with systemic sclerosis (score 0), and in sclerotic skin graded as score 1, 2, or 3. Bars indicate mean values ± the standard deviation.

Considerable differences in skin elasticity could be found between different anatomic regions, although these areas received the same skin score. For example, the mean distension (mean ± standard error of the mean) measured in skin areas judged as normal skin (score 0) by the human observer, was low at the dorsal side of the foot (0.83 ± 0.069 mm) and the leg (0.72 ± 0.023 mm), but high in the umbilical area (1.07 ± 0.033 mm), the gluteal region (1.35 ± 0.064 mm), and in the neck (1.44 ± 0.070 mm). Skin areas with skin score 1 in the face, showed lower distensions (0.68 ± 0.017 mm) than areas with the same score 1 at the inner arm (1.01 ± 0.037 mm).

DISCUSSION

The measurements with the SEM 474 cutometer were highly reliable. The standard deviation of repeated measurements was low and the intra- and interobserver agreement was good. These results were obtained despite the fact that the total number of patients was small. The reason is that the number of sample areas per patient was high (74), and all patients had type III scleroderma, therefore sufficient areas with skin scores of 0, 1, 2 and 3 were present. The reproducibility of the traditional skin score methods has been studied extensively.[12-14] These studies showed that the interobserver ICC of the Rodnan skin score (0.43) and the modified Rodnan skin score (0.53) are considerably lower than the interobserver ICC of the cutometer (0.92).[12] Also the intraobserver ICC of the Rodnan skin score is low (0.55 versus 0.94 with the cutometer).[12] Some studies suggest that the skin score can be simplified by reducing the number of measurement sites per patients, but this can result in the loss of the ability to detect change in a patient.[13] For this reason we prefer the combination of an increased number of body areas and a more accurate and reproducible measuring technique.

Despite the low inter- and intraobserver ICC of the traditional skin score, this method is still regarded as the best available tool until now to evaluate systemic sclerosis, because no other methods were available that are easy to use, reproducible, and clinically useful.[14] We believe that the cutometer method could be an alternative for the skin score, especially in multicenter trials, and we suspect that the value of this equipment has been under-estimated by investigators who have tried it because the instrument in its standard configuration is not suitable for measuring scleroderma.

In its standard configuration, the cutometer is usually equipped with a measurement probe with a small aperture (2 mm) because the device was originally designed to measure elastic properties of the epidermis. It has been used successfully in studies of regional differences in skin thickness and on aging and photoaging of the skin.[26-29] For the evaluation of scleroderma, however, the large probe (8 mm) gave the best results because with a smaller probe the contribution of the dermal component to the total measurement is relatively small.[27] Increasing the probe size further would make the probe unsuitable for certain convex areas such as the dorsal surfaces of the fingers and toes. The viscoelastic properties, determined by matrix proteins and the presence and properties of fluid in the skin, are also important in the overall evaluation of skin sclerosis. For this reason, the total skin deformation (U_f in Fig. 4) gives better results than U_e (the linear elastic part of the deformation) or

R_3-R_4 (the skin deformation after subtracting the part of skin deformation which is not immediately reversible because of tissue edema).

Accuracy is difficult to assess, because there are no other objective methods available that can serve as a standard, except perhaps quantification of the amount of collagen in skin biopsy specimens from all measurement sites. This is, of course, impossible for ethical and practical reasons. The second best standard to compare it with is the skin score.[11,14] However, there are several reasons that the skin score and the cutometer measurements may not be comparable. The skin score is subjective and therefore not free of observer bias. It is also not known whether the skin scores 0, 1, 2, and 3 are equidistant, or whether there is a linear relation between the skin score and the collagen content of the skin. Neither has it been shown that the scores are absolute, which means that score 2 in one patient is the same as score 2 in another patient. In fact, the skin score system could be partly a relational system, in which score 0 is normal skin, score 1 is not quite normal, score 3 is clearly abnormal, hidebound hard skin, and score 2 is somewhere in between. Furthermore, the human observer is capable of incorporating his or her knowledge about the normal regional differences in skin texture between, for instance, palm, inner arm, forehead, abdomen or back, into the overall judgement. Therefore, although the elasticity measured with the cutometer of normal abdominal skin is approximately twice as high as normal palm skin, both receive score 0 from the human observer. Finally, the palpating fingers of the human observer are not only assessing the inability to lift a skin fold, but also the induration of the skin, the resistance to pressure and lateral movement, and the presence of edema.

Our data indicate that the human observer using the subjective skin score is integrating all the information on the normal properties of skin in a certain area into the final score. This flexibility cannot be expected from a machine. The cutometer measures the absolute skin elasticity, which depends on the collagen amount but also on several other factors such as anatomical site, age, sex, genetic factors determining skin texture, habits (work, sun exposure), concomitant or previous skin diseases, or the presence of edema.[26-29,43] In theory there can be large differences between patients, which makes the method more suitable for intraindividual comparisons such as follow-up during treatment. In practice, the skin score and cutometer measurements correlated well. Because of the differences caused by the anatomic localization, it is not recommended to sum all individual measurements of the different body areas to one total score, because then the distribution of the sclerosis in the different body areas would influence the total score. We recommend that for follow-up purposes all data are recalculated to a percentage of the baseline measurement for each body region.

The induration of the skin can also be measured by other equipment such as the recently described durometer, a simple and easily used device.[4] The only disadvantage of the durometer appears to be that the method cannot be used over bony surfaces and that induration is not exactly the same as tethering.[5] The SEM 474 cutometer measurement method is the closest imitation of lifting the skin between the fingers to assess hidebinding.

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Chapter 6

Dermal organisation in scleroderma: the fast Fourier transform and the laser scatter method objectify fibrosis in nonlesional as well as lesional skin

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SUMMARY

Scleroderma, a chronic, progressive disorder, is characterized by dermal fibrosis with collagen bundles orientated parallel to the epidermis. Simple objective parameters to evaluate disease progression and therapies are needed. We describe two methods, the laser scatter method and the fast Fourier transform (FFT), to measure collagen bundle orientation and spacing. Lesional sclerodermic skin (LS), nonlesional sclerodermic skin (nonLS), and control skin (CS) sections were evaluated for orientation ratio using the laser scatter method. The FFT was used to calculate orientation ratio, variation and spacing of collagen bundles. Parameters were correlated with local and mean skin score measurements, on a scale of 0 (normal) to 3 (severely sclerotic). With both the laser scatter method and the FFT, orientation ratios of LS (respectively, 2.16 ± 0.33 and 1.83 ± 0.62) were significantly higher than CS (respectively, 1.70 ± 0.35 and 1.38 ± 0.15). NonLS ratios (respectively, 1.92 ± 0.15 and 1.48 ± 0.44) were between LS and CS ratios. Orientation variation and bundle spacing of LS (respectively, 57.3° ± 19.4° and 15.7 ± 5.6 μm) were significantly reduced compared to CS (respectively, 73.8° ± 15.0° and 18.9 ± 1.9 μm). NonLS values (respectively, 57.2° ± 29.0° and 15.6 ± 6.1 μm) were similar to LS. Bundles in LS are more parallel, show less variation in orientation, and are more densely packed than in CS. There was a linear correlation between mean skin score and orientation ratio. Local skin score was not linear correlated to orientation ratio. Our findings suggest that nonLS dermis without clinical sclerosis already shows fibrotic characteristics. Both techniques where easy to use and suitable for objectifying dermal fibrosis in scleroderma lesions. FFT is more accurate and reproducible than the laser scatter method and allows simultaneous pathological evaluation of the location of the analyzed tissue sections. Future studies will need to focus on the correlation between clinical disease severity and collagen bundle characteristics.

INTRODUCTION

Scleroderma forms a spectrum of connective tissue disorders ranging from systemic sclerosis at one end, to the so-called overlap syndromes in the middle, and localized scleroderma, also known as morphea, at the other end.[1] The pathogenesis is as yet unknown but the activation of T-lymphocytes, possibly through abnormal T cell receptors, super- and/or auto antigens, seems to play a major role in the increase of collagen and the vascular damage so typical of sclerodermic lesions.[2] Moreover, there is a genetic predisposition for

scleroderma in individuals with HLA-DR5, -DR8 and -DR11 genotypes.[3] Damage to genetic material might be another possible cause of scleroderma.[2] Toxic agents related to systemic sclerosis are aromatic hydrocarbons, silica and drugs like penicillamine and L-tryptophan. Recently, mast cells,[4] and fetal stem cells in the maternal circulation after childbirth have been addressed as possible determinants in the development of scleroderma.[5]

One of the main criteria of scleroderma is fibrosis of the dermal layer. Early clinical manifestations comprise ill-defined plaques of edema and erythema. Gradually, the distinct dermatological entity with sharply defined plaques of thickened, shiny, white, tanned, smooth centers arise. The margins can be characterized by a lilac ring which implies inflammation and probable progression of the disease.

Histologically, sclerosis is characterized in the early stages by an inflammatory cellular infiltrate composed of lymphocytes, macrophages, mast cells, eosinophils and plasma cells.[6] With time, collagen bundles become broadened and closely packed and the dermis as a whole thickens. In normal skin, collagen bundles show a three-dimensional basket-weave pattern, whereas in scleroderma lesions, the collagen bundles are oriented parallel to the epidermis.[6,7] The subcutaneous and peri-appendicular fat, as well as skin appendages, are progressively lost and replaced by collagen bundles.

To determine the severity of sclerodermic lesions, the skin score is a widely used subjective measure. It represents the induration or loss of elasticity assessed in a body area. The local skin score is estimated by pinching a skin fold in one area and scoring it on a scale of 0 to 3, indicating, respectively, no sclerosis to severe sclerosis.[8] The mean skin score is the average of the total body surface subdivided into 74 areas. Because the clinical course of scleroderma is unpredictable and the treatment is still the subject of clinical trials, objective parameters are needed for reporting disease progression and to evaluate therapies. Several devices to objectify skin elasticity in sclerodermic lesions have been developed. One of the devices used in our clinic, called the cutometer, exerts a controlled vacuum over the skin. The extent of deformation is determined by a noncontact optical measuring system.[9] Dermal thickness can be measured with high frequency ultrasound and nuclear magnetic resonance imaging.[10,11] Objective histologic evaluation can be performed by measuring the amount of collagen in standard skin biopsy specimens. These methods consist of weighing and histometric methods,[12,13] which are inaccurate, and biochemical assays, which are elaborate.[14]

In this paper we describe two techniques, the laser scatter method and the fast Fourier transform (FFT), for measuring the severity of dermal sclerosis based on the architecture of the collagen bundles. These two methods are compared

with the mean and local skin score. The laser scatter method was developed by Yannas and Ferdman to register the grade of organization in scars of deep dermal wounds.[15] The overall orientation of collagen bundles in fibrotic tissue was quantified using the laser light scatter characteristics of fibrotic tissue. In a previous study, we used this method to evaluate the effect of different dermal substitutes in wound healing.[16] Like mature scar tissue, sclerodermic lesions are also characterized by parallel collagen bundles.[3,17]

The FFT is a mathematical analogue of the laser scatter plot. It calculates orientation and spacing in an image. The periodicity and the orientation of structures, such as collagen bundles, can be represented as a power plot of the FFT of an image.[18] Interpretation of the power plot is much easier and more accurate than trying to extract the same information from the original image, because all the spacings and orientations are effectively averaged together in a frequency domain. The FFT allows measurement of spacing and orientation of collagen bundles as well as a histopathologic evaluation of the same location. This simultaneous evaluation is not possible with the laser scatter method. FFT has been used for the evaluation of dermal architecture in scar tissue,[19] and proves to be a simple, objective, and reproducible method for analyzing the severity of fibrosis in tissue sections from scleroderma lesions.

MATERIALS AND METHODS

Punch biopsies (4 mm) were collected from patients with a histologically and clinically confirmed systemic scleroderma diagnosis. Biopsies from patients without a fibrotic or sclerotic skin condition served as controls. Three groups were formed: 1) lesional scleroderma skin (LS), 2) nonlesional scleroderma skin (nonLS), both derived from scleroderma patients, and 3) control skin (CS) from patients without a fibrotic or sclerotic skin condition. Tissue sections (7 μm) were cut from formalin-fixed, paraffin-embedded biopsies and stained with hematoxylin-eosin.

The laser scatter method

Due to its wave characteristics, monochromatic light passing through a fine grid of parallel linear lines will be diffracted in preferential directions. When projected on a screen, a specific pattern of light peaks will occur perpendicular to the direction of the grid lines. A tissue section from dermal tissue can be interpreted as a grid. Normal dermis with its randomly oriented collagen bundles diffracts light in no preferential direction, causing a circular scatter pattern. The parallel collagen bundles in sclerotic tissue cause a light scatter

pattern with a preferential direction perpendicular to the direction of these fibers. As a result, an elongated oval-shaped scatter pattern will occur.
The laser scatter method was performed as previously described.[16] In short, a 3-mW helium/neon laser (Uniphase, Manteca, California) was mounted on an optical bench. To create a gaussian beam of suitable diameter (± 100 μm) a spatial filter was constructed from two achromatic convex lenses with different focal lengths, and a 100 μm pinhole. A slide section holder and a white paper screen were placed in line with the laser, and the scatter patterns were recorded with a black and white CCD camera (model VC-2512, Sanyo, Japan) connected to a computer image analyzer (Quantimet 500, Leica, Cambridge, United Kingdom). The light threshold of the image analyzer was standardized, and maximum length and maximum breadth of each scatter pattern were automatically calculated.

The fast Fourier transform (FFT)
Images (794 μm x 794 μm) were acquired with a confocal laser scanning microscope (Leica Micro Optics, Heidelberg, Germany) with a 6,3 x 0.2 objective and a 50 μm pinhole using the fluorescent properties of eosin (excitation wave length 488 nm, emission wave length >580 μm). This ensured a constant contrast-brightness index for all images independent of staining, and a constant optical section thickness (approximately 2 μm). This also guaranteed that the hematoxylin-stained nuclei, which dominate a conventional bright field image, were not imaged at all. The epidermis was always oriented parallel to the *x*-axis of the image.
Analysis of the images was performed with the FFT module of the Leica Qwin image analysis software (Leica). From each image, the FFT and its first-order power plots were calculated to provide an estimate of the orientation ratio in the image, the orientation variation and the bundle spacing (Fig. 1). The orientation ratio corresponds to the length to breadth ratio of the first-order maximum of the power plot. Randomly oriented collagen bundles will evoke an orientation ratio approaching 1, parallel collagen bundles a ratio greater than 1. The orientation variation corresponds to the angle over which the collagen bundles are oriented in the skin; in the power plot of an image, it is the angle between the tangent along the first order maximum and the center of the power plot (Fig. 1). Bundle spacing corresponds to the reciprocal of the distance from the center of the power plot to the center of the first-order maximum.

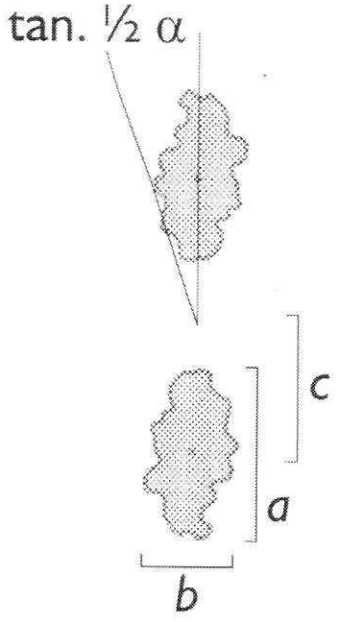

Figure 1. Interpretation of the Fourier power plots. Confocal scanning laser microscopy images of the dermis were used to calculate the FFT and corresponding first-order power plots. The orientation ratio corresponds to the ratio a/b. Orientation variation corresponds to the angle tan ½ α formed by the two lines, as shown. Bundle spacing corresponds to the distance c between the center of the power plot and the centroid of the first-order maximum.

Skin score

Results from the laser scatter method and FFT were compared with the local skin score obtained in the skin region where the punch biopsy was taken. Skin score was assessed by a trained observer by palpation using the Rodnan modification for assessing cutaneous sclerosis on a 0 to 3 scale (0 = no sclerosis, 1 = mild sclerosis, 2 = moderate sclerosis and 3 = severe sclerosis), as previously described.[9] The mean skin score is the sum of the 74 local skin scores devided by the number of regions (74). For comparison with collagen bundle orientation, the continuum of mean skin scores was divided into three groups: 1) mean skin score 0 (n = 16) , 2) 0,01 < mean skin score < 0,49 (n = 12) , 3) 0,50 < mean skin score < 2,00 (n = 7).

All statistical differences were tested with the unpaired Student's t test. The two-tailed Spearman's rank test was used to detect a correlation between two groups.

RESULTS

Patient population

Twenty patients, 17 woman and 3 men, were included in the study (Table 1). A total of 35 biopsies were collected, both lesional (n = 23) and nonlesional (n = 12). The mean age of the scleroderma patient group was 46.6 ± 16.2 years. Disease duration at the time of the first biopsy collection was 24.4 ± 23.6 months. Eleven biopsies, collected from persons without fibrotic or sclerotic skin conditions, served as control.

Histology

Lesional sclerodermic skin (LS) (Fig. 2a) showed the typical densely packed arrangement of collagen bundles parallel to the epidermis. Skin appendages, like hair follicles and glandular structures, were absent. Dermal capillaries were characterized by thickened capillary walls. The dermal compartment as a whole was increased and subcutaneous fat decreased compared with control skin (CS). In nonlesional skin (nonLS), the dermal thickness was increased to a lesser extent than in LS biopsies. Periappendicular fat was decreased, but skin appendages were present. It is difficult to evaluate the orientation of collagen bundles compared with LS and CS biopsies.

Unaffected CS (Fig. 2b) was characterized by dermal tissue with a randomly oriented, basket-weave network of collagen bundles. Skin appendages were abundantly present, surrounded with loosely arranged fat tissue.

The laser scatter method

The dermis of LS (Fig. 3a) showed a higher orientation ratio than CS (Fig. 3b), respectively, 2.16 ± 0.33 and 1.70 ± 0.35; p = 0.005 (Table 2). This indicates that the collagen bundles in sclerodermic skin show a more parallel alignment than do collagen bundles in normal dermis. No significant differences in orientation ratio were found between LS and nonLS (1.92 ± 0.15), nor between nonLS and CS.

Table 1. Patient and biopsy information

Patient number	Sex	Age (year)	Biopsy type	Disease Duration (months)	Perivascular inflammation
1	f	55	ls	58	absent
			nl	58	absent
2	f	29	ls	15	absent
			ls	48	trace
			nl	48	absent
3	f	30	ls	9	present
4	f	69	ls	23	trace
			ls	47	trace
			nl	47	trace
5	m	44	ls	51	absent
			ls	68	trace
			nl	68	present
6	f	51	ls	15	present
7	f	54	ls	48	absent
			ls	64	trace
			nl	64	trace
8	f	12	ls	74	present, eosinophils
9	f	12	nl	74	present
10	m	41	ls	9	present
11	f	32	nl	4	present
12	f	32	ls	4	present
13	f	44	ls	1	trace
			nl	1	trace
14	f	40	ls	7	present
			ls	29	absent
			nl	29	trace
15	m	85	ls	2	present
16	f	45	ls	19	trace
			nl	19	trace
17	f	67	ls	35	trace
			nl	35	trace
18	f	67	ls	35	absent
19	f	28	ls	9	present, eosinophils
			ls	31	trace
20	f	30	nl	31	present

f = female, m = male, ls = lesional sclerodermic skin, nl = nonlesional sclerodermic skin

Table 2. Orientation ratio calculated with the laser scatter method and Fast Fourier Transform (FFT)

technique		lesional (LS)	nonlesional (nonLS)	control (CS)
laser scatter	*n*	11	5	11
	x ± sd	2.16 ± 0.33#	1.92 ± 0.15	1.70 ± 0.35#
FFT	*n*	24	11	11
	x ± sd	1.83 ± 0.62*	1.48 ± 0.44	1.38 ± 0.15*

#$p = 0.005$, *$p = 0.002$

Table 2. With both techniques, the laser scatter method and FFT, the orientation ratio of LS is significantly higher than in CS. This indicates a higher degree of parallel orientation in lesional sclerodermic skin compared with control skin. The nonLS group shows orientation ratio's in between LS and CS.

The fast Fourier transform

Because of the fluorescent properties of eosin, which stains the collagen bundles, the confocal laser scanning microscope selectively images these bundles without interference of the hematoxylin-stained structures (ie, nuclei). The highly parallel orientation of collagen bundles in LS biopsies can be appreciated (Fig. 4a), as well as the basket-weave structure of collagen bundles in the CS biopsies (Fig. 4b).

The power plot of an FFT of the LS biopsies showed two first-order maxima oriented along a line perpendicular to the collagen bundle orientation (Fig. 5a). The power plot of the CS biopsies showed an oval without obvious maxima, indicating a random orientation (Fig. 5b).

Results similar to those found with the laser scatter method were obtained with the FFT. LS showed a significantly higher orientation ratio than CS (respectively, 1.83 ± 0.62 and 1.38 ± 0.15; $p = 0.002$; Table 2), and no significant difference was found in orientation ratio between LS and nonLS (1.48 ± 0.44), nor between nonLS and CS. With both techniques, the orientation ratio of the nonLS group was consistently lower than the LS and higher than the CS group.

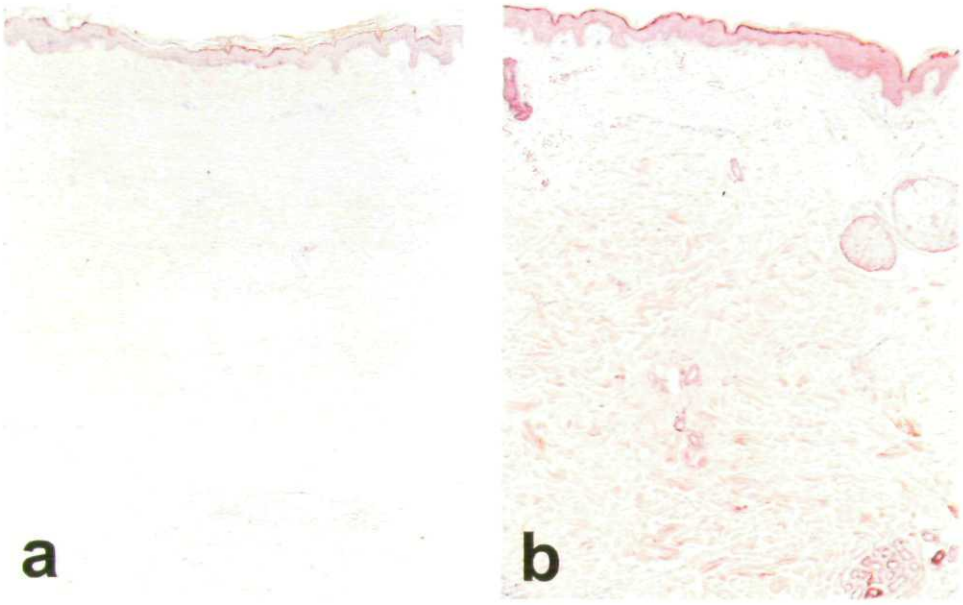

Figure 2. Histology. a) Lesional sclerodermic skin (LS) with parallel, densely packed collagen bundles. Skin appendages are absent. b) Unaffected control skin (CS) shows random oriented, more loosely arranged collagen bundles. Skin appendages are present (hematoxylin/eosin staining).

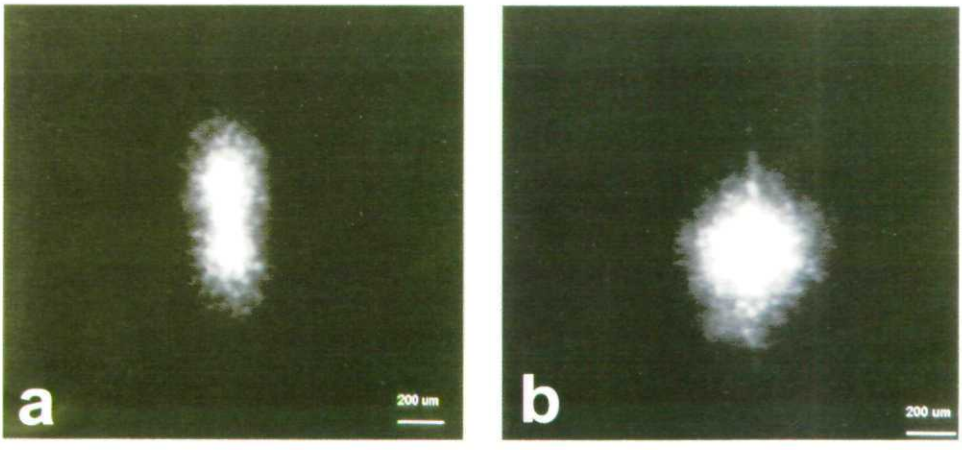

Figure 3. Laser scatter images. a) LS produces an elongated scatter plot, indicating a higher orientation of collagen bundles than CS. b) CS produces a circular scatter plot caused by the randomly oriented collagen bundles.

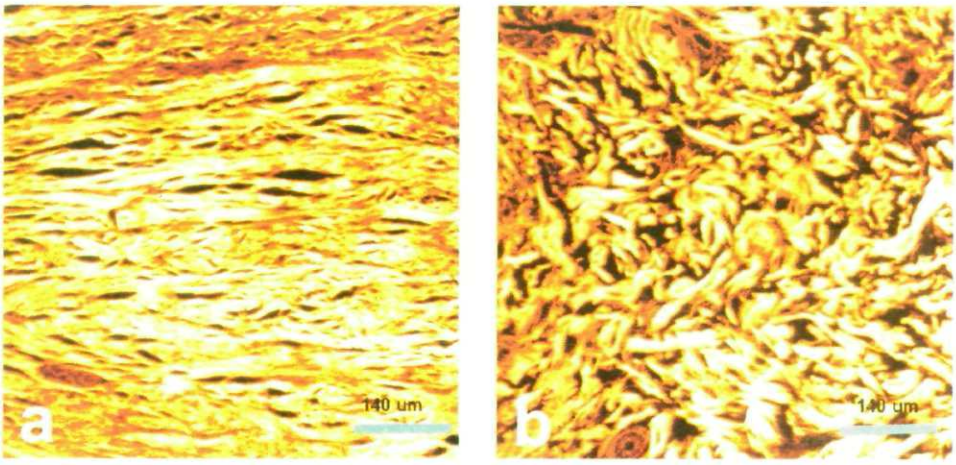

Figure 4. Confocal laser scanning microscope images. Because the fluorescent signal is from the eosin-stained collagen bundles exclusively, no hematoxylin stained (ie, cellular structures) are depicted. a) LS again shows parallel collagen bundles, in contrast to b) CS showing randomly oriented collagen bundles.

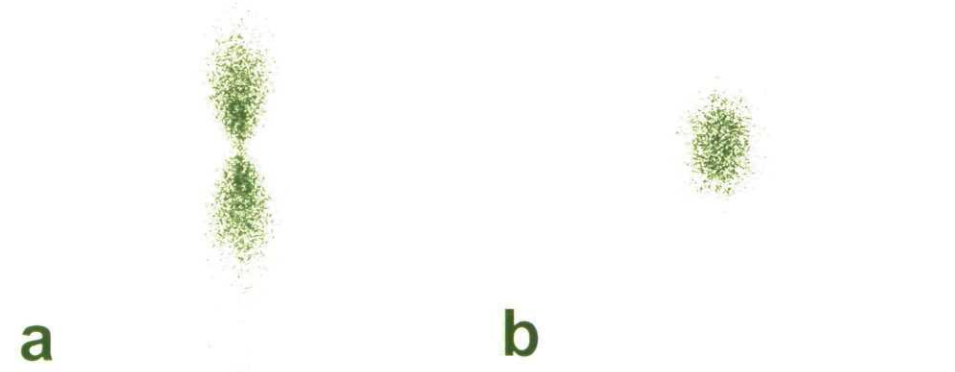

Figure 5. First-order power plots from the fast Fourier transform (FFT). a) LS creates a power plot with a preferential distribution in two maxima. b) CS is characterized by a power plot with a circular and no preferential distribution pattern.

Orientation variation and collagen bundle spacing

In both LS and nonLS biopsies, the orientation variation of the collagen bundles in relation to the epidermis was similar (respectively, 57.3 ± 19.4 and 57.2 ± 29.0; Table 3). In CS (73.8 ± 15.0), the orientation variation of collagen bundles was significantly larger than LS (p = 0.03). No significant difference was found between CS and nonLS (p = 0.15). These findings indicate that in LS and nonLS, the orientation of collagen bundles can be found within a smaller range than in unaffected control skin.

Bundle spacing represents the center-to-center distance between collagen bundles, and reflects the density in the dermal layer. A low spacing correlates with high bundle density and vice versa. Bundle spacing was comparable for the LS and nonLS group (respectively, 15.7 ± 5.6 and 15.6 ± 6.1) but was higher in the control group (18.9 ± 1.9). There was a significant difference between LS and CS (p = 0.03), but not between nonLS and CS (p = 0.14). These data prove that collagen bundles in LS are more densely packed than in unaffected skin, and that the bundle density in nonLS is increased.

Table 3. Orientation variation of collagen bundles in relation to the epidermis and spacing of collagen bundles as calculated with the FFT

parameter	lesional (LS)	nonlesional (nonLS)	control (CS)
n	24	10	11
orientation variation ($x \pm sd$ in °)	57.3 ± 19.4[#]	57.2 ± 29.0[+]	73.8 ± 15.0[#,+]
bundle spacing ($x \pm sd$ in μm)	15.7 ± 5.6*	15.6 ± 6.1[§]	18.9 ± 1.9*,[§]

[#] p = 0.03, * p = 0.03, [+] p = 0.15, [§] p = 0.14

Table 3. Orientation variation of the collagen bundles was decreased in LS compared with CS. This indicates a diminished variation of bundle orientation in sclerodermic lesions. The bundle spacing was also decreased in LS compared with CS, indicating more densely packed collagen bundles in sclerodermic lesions. NonLS shows similar orientation variation and spacing values as LS and not as CS.

Skin score

FFT data (orientation ratio, orientation variation and bundle spacing) were related to the mean skin score (Table 4) and to the local skin score results from the corresponding biopsy areas (Table 5). Mean skin scores were divided into 3 groups: skin score 0, skin scores 0 to 0.50, and skin scores higher than 0.50. There was a linear correlation between orientation ratio and mean skin score group (respectively, 1.41 ± 0.11, 1.82 ± 0.68, and 1.95 ± 0.74; Table 4). With Spearman's rank test, a correlation coefficient of 0.5 was found, significant at the 0.01 level (two-tailed). No significant relationships were found for orientation variation and bundle spacing.

The orientation ratio in the local skin score 0 group (1.36 ± 0.14) differed significantly from the local skin score 1 to 3 groups (respectively, 2.08 ± 0.83, $p = 0.02$; 1.70 ± 0.24, $p = 0.004$ and 1.95 ± 0.88, $p = 0.07$; Table 5). No linear correlation was observed between orientation ratio and the severity of skin scores, but the Spearman's rank correlation coefficient was 0.6 with a 0.01 significancy level (two-tailed). No significant relationships were found for orientation variation and bundle spacing.

Table 4. Mean skin score related to orientation ratio, orientation variation and bundle spacing as measured with the FFT

mean skin score	0 (n = 16)	0.01-0.49 (n = 12)	≥ 0.5 (n = 7)
orientation ratio ($x \pm sd$)	1.42±0.12#	1.82±0.68#*	1.95±0.74*
orientation variation ($x \pm sd$ in °)	63.0±25.8	54.8±23.5	57.8±19.0
bundle spacing ($x \pm sd$ in μm)	18.5±9.0	14.8±6.4	15.9±5.5

$p = 0.01$ #, $p = 0.2$*

Table 4. There is a positive correlation between the mean skin score severity and the orientation ratio, Spearman's rank correlation coefficient = 0.5 ($p < 0.01$, two-tailed). No significant differences were observed for orientation variation or bundle spacing in the different mean skin score groups.

Table 5. Local skin score related to orientation ratio, orientation variation and bundle spacing as measured with the FFT

local skin score	0 ($n = 10$)	1 ($n = 7$)	2 ($n = 6$)	3 ($n = 5$)
orientation ratio ($x \pm sd$)	$1.36 \pm 0.14^{\#*\S}$	$2.08 \pm 0.83^{\#}$	$1.70 \pm 0.24^{*}$	$1.95 \pm 0.88^{\S}$
orientation variation ($x \pm sd$ in °)	50.9±31.4	66.3±13.6	46.3±22.8	52.4±15.2
bundle spacing ($x \pm sd$ in μm)	16.8±13.0	16.5±2.2	14.0±7.6	16.1±6.4

$p = 0.02^{\#}$, $p = 0.004^{*}$, $p = 0.07^{\S}$

Table 5. The orientation ratio found in the local skin regions with a skin score 0 differed significantly from ratios found in regions with higher skin scores (1 to 3), Spearman's rank correlation coefficient = 0.6 ($p < 0.01$, two-tailed). No significant differences were observed for orientation variation or bundle spacing in the different local skin score groups.

DISCUSSION

In skin biology, processes like aging and scar formation alter the dermal organization. Skin diseases like scleroderma and lichen sclerosus affect the dermal collagen bundle architecture. The two analysis methods described in this article, the laser scatter method and the FFT, may provide information on the development, gradation and prognosis of sclerotic processes. The laser scatter method was used before to evaluate scar formation in experimental wounds in guinea pigs and in humans.[15,16] We demonstrated the use of the FFT to quantify two parameters of the dermal architecture in scleroderma, collagen bundle orientation and spacing.

The laser scatter method and the FFT proved to be easy-to-use tools to discriminate between normally organized and sclerotic dermal tissue. Tissue slides from routine formaldehyde-fixed skin biopsies suffice. For the FFT, a personal computer with commercially available image analysis software and a fluorescence microscope equipped with a digital camera with frame grabber are the main research tools. Normal bright field microscopic images of hematoxylin -eosin-stained material are not really suitable for FFT analysis because the hematoxylin-stained nuclei dominate the image and are consequently conspicuous in the power plot. Using the fluorescent properties of eosin eliminates this problem. We used a confocal microscope for data acquisition because it was available and convenient for digitizing the images, but a normal fluorescence microscope works just as well. The laser scatter method can be performed with a standard laser source, a simple optical bench arrangement plus a digital camera, a personal computer, and image analysis software.

The FFT proved to be a more accurate method for the evaluation of fibrosis than the laser scatter method. The latter does not allow exact positioning of the laser beam and simultaneous microscopic monitoring of the target area is not possible. The FFT is performed on digitized microscopic images that allow the investigator to accurately determine the position and size of the area to be analyzed.

In both the laser scatter method and the FFT, LS showed a more extensive parallel orientation of collagen bundles than unaffected CS. This was revealed by the orientation ratio observations (Table 2). The orientation variation, that is, the variation of the bundle orientation in relation to the epidermis, was significantly reduced in the LS group compared with the CS group (Table 3). This finding indicates that there is less variation in the organization of collagen bundles in LS. Bundle spacing, or center-to-center distance between the collagen bundles, was significantly lower in LS dermis compared with CS

dermis (Table 3). Thus it can be concluded that the collagen bundles in sclerotic skin are more densely packed than in unaffected CS.

Using FFT and the laser scatter method, we found indications that in the dermal layer of clinically unsuspected nonLS, the collagen bundle structure shows some alterations that indicate early fibrosis. Although the results are not significant because of the small number of samples, the orientation ratio found with both methods showed increased alignment of collagen bundles in nonLS biopsies compared with CS biopsies, but less alignment than in LS biopsies. More evidence of early changes in nonLS was supplied by the observations on orientation variation and bundle spacing. For non-LS as well as for LS, both parameters were of similar magnitude. The orientation variation and bundle spacing values of non-LS also differed from the CS group, although not significantly. It is assumed that fibrosis in scleroderma is a gradual process. Our findings support this assumption; nonlesional scleroderma skin without the clinical signs of sclerosis already shows some dermal characteristics similar to sclerodermic skin.

Orientation ratio, orientation variation, and bundle spacing as measured with the FFT were related to the skin score data. A positive linear correlation was found between mean skin score and the orientation ratio (Table 4). Although the correlation was not strong (Spearman's rank 0.5, $p < 0.01$, two-tailed), this finding supports our idea that the objective parameters as described in this article can be related to the overall clinical severity of sclerodermic skin. No correlation was found among orientation variation, bundle spacing and local or mean skin score. No linear relationship between local skin score and FFT orientation ratio was found, but Spearman's rank test detected a significant correlation coefficient of 0.6 ($p < 0.01$, two-tailed) (Table 5).

That only weak correlations where detected between skin score values and orientation ratio could be attributed to the small sample of the skin score groups, which would also explain the large standard deviation. It also seems that the clinical skin scores are not equidistant. In a previous study, skin elasticity values were compared to skin scores.[9] The difference in skin elasticity between skin score 0 (normal skin) and score 1 was larger than the difference between score 1 and 2, or between 2 and 3. The same phenomenon seems to take place in the observations on collagen bundle characteristics. In the skin score 1 group, collagen bundles show distinctive parallel orientation; in skin score groups 2 and 3, the parallel orientation is only slightly increased. The subjective discrimination made between skin scores 1 to 3 could be caused by other aspects than collagen bundle characteristics, for example, dermal edema. Using high frequency echography as a clinical parameter instead of the

subjective skin score method might reveal more appropriate correlations between the collagen bundle characteristics and disease severity.
For orientation variation or bundle spacing, no significant differences were found between the various skin score groups. These findings could be attributed to the substantial within- or between-observer variation which characterizes and hampers the skin score method.[20] For day-to-day clinical use, it will remain a first choice technique for assessing sclerosis, but when greater accuracy is required the skin score method does not suffice.

In conclusion, both methods are suitable for objectifying collagen bundle structure and for discrimination between fibrotic dermis and normal organized dermis, but the FFT proves to be more accurate and reproducible than the laser scatter method. Future studies will need to focus on the relationship between clinical disease severity and collagen bundle characteristics, possibly with more accurate methods to score disease severity like high frequency echography.

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Chapter 7

Medium-dose UVA1 phototherapy for localized scleroderma

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submitted for publication

SUMMARY

Background: Localized scleroderma is characterized by circumscribed induration of the skin. Underlying subcutaneous tissue can also be involved, this can cause significant disabilities. Recent studies showed that both high-dose and low-dose UVA1 phototherapy are effective in the treatment of sclerotic plaques.

Objective: The purpose of this study was to evaluate the efficacy of medium-dose UVA1 phototherapy in patients with localized scleroderma.

Methods: A controlled pilot study with medium-dose UVA1 (48 J/cm^2). The results were evaluated by means of skin score and two objective methods for quantifying sclerosis (cutometer and fast Fourier transform method). Patients were treated four times a week for 5 weeks. The follow up period was 12 weeks.

Results: All patients responded to therapy. Skin score and cutometer results showed improvement of skin elasticity of treated skin compared to control skin. Fast Fourier transform measurements showed no change in bundle orientation ratio and spacing.

Conclusion: Medium-dose UVA1 is a beneficial therapy and a well tolerated treatment modality for localized scleroderma (morphea).

INTRODUCTION

Localized scleroderma or morphea is characterized by circumscribed induration of the skin. Typically the skin alterations consist of ivory-white plaques surrounded by a lilac ring. Internal organs are not involved and the prognosis of this skin disease appears to be favorable because spontaneous remission frequently occurs. Sometimes the disease progresses, and not only the skin but also underlying subcutaneous tissue (fat and muscle) can be involved. This may lead to muscle atrophy and also flexion contractures if localized over the joints, which can cause significant disabilities.[1]

Many theories exist about the pathogenesis; one hypothesis is that endothelial cell damage leads to a cascade of events resulting in fibroblast stimulation and collagen production. Recent studies showed that in fibroblasts of lesional skin increased collagen expression was found together with decreased collagenase expression.[2,3] Ultraviolet A1 (UVA1; 340-400 nm) radiation is associated with induction of collagenase in fibroblasts of lesional skin.[4]

Several treatments have been tried such as topical and intralesional use of corticosteroids, systemic steroids, D-penicillamine, etretinate, interferon gamma, calcitriol, salazopyrine and penicillin. Until now no treatment has been proven to be beneficial in localized scleroderma. But recent studies showed that both high-dose (130 J/cm^2) and low dose UVA1 (20 J/cm^2) phototherapy is effective in the treatment of sclerotic plaques.[5,6]

The aim of our study was to evaluate the efficacy of medium-dose UVA1 (48 J/cm^2) phototherapy in patients with localized scleroderma. In this paper we describe the results of a controlled pilot study in which eight patients were treated with medium-dose UVA1, and were evaluated by means of two recently introduced objective methods for quantifying sclerosis (skin elasticity measurement and fast Fourier transform method).

PATIENTS AND METHODS

Patients

Eight patients (6 female and 2 male) with localized scleroderma participated in this study. All patients gave their informed consent. The diagnosis of localized scleroderma was made on clinical and histological evidence in all patients. Apart from localized scleroderma, all patients were healthy individuals. There was no evidence of systemic sclerosis or pseudoscleroderma. All patients had at least two clinically identical scleroderma lesions (mostly contralateral), in six patients the sclerotic lesions were located on the trunk, the other two patients had sclerotic skin on the legs. None of them had linear scleroderma. The surface of the treated and untreated individual scleroderma lesions varied between 20-80 cm^2.

Disease duration varied between 1-22 years (see table 1). No other treatment had been given for at least 3 months prior to the start with UVA1 radiation therapy. Before treatment skin biopsies were taken from lesional skin.

Table 1. Patient characteristics

Patient	Sex/Age	Disease dur. (y)	Localization	Treatment before UVA1
1	M/19	7	Trunk	Penicillin, steroids (topical)
2	F/34	22	Trunk, arms	Pentoxifylline, vitamin E
3	M/19	5	Trunk	Penicillin, steroids (topical)
4	F/56	2	Hips, back	Steroids (topical)
5	F/27	7	Leg	None
6	F/16	1	Leg	Calcitriol
7	F/56	4	Trunk	Steroids (topical)
8	F/25	9	Trunk	Steroids (topical)

Equipment

The UVA1 irradiation equipment consisted of a Waldmann 7001 K cabin with Waldmann TL10 R low pressure lamps. (Waldmann, GmbH, Schwenningen, Germany). These lamps generate UVA1 wavelengths in the 340-400 nm range. In addition, infrared irradiation is emitted. However these infrared wavelengths are filtered out by an acrylic glass screen. The UVA1 irradiation levels are approximately 35 mW/cm^2 and are measured by a standard intrinsic UV meter. A dose of 20 J/cm^2 is achieved in approximately 10 minutes.

Treatment

All patients were exposed to total body UVA1 irradiation four times a week for 5 weeks, resulting in a cumulative dose of 960 J/cm^2. Additional therapy was not allowed. In all patients one of two selected sclerotic plaques (contralateral) was randomly covered by cotton gauzes, to prevent UVA radiation underneath. These covered sites served as internal control. In 3 patients without two anatomical equally distributed lesions one half of one plaque with a square area of at least 12 cm^2 was covered with cotton gauze to serve as internal control lesion.

Clinical evaluation

Clinical examination was done by an independent observer. Skin score measurement[7] was assessed before, after 2.5 weeks, after 5 weeks, after 7 weeks and after 12 weeks. Cutaneous sclerosis was assessed on a 0 to 3 scale.

The modified Rodnan skin score was used (0 = normal skin, 1 = mild skin thickness, 2 = moderate skin thickness, 3 = severe skin thickness with inability to pinch the skin into a fold). All patients were interviewed at the same intervals and were asked about side effects and their subjective opinion about the efficacy of the therapy.
Cutaneous elasticity was also assessed at the previous mentioned intervals by means of the SEM 474 cutometer (Courage + Khazaka Electronic GmbH, Cologne, Germany). This equipment exerts a controlled vacuum force to the skin and can objectively measure skin elasticity.[8]

The fast Fourier transform (FFT)
Punch biopsies (4mm) from treated lesional skin and non-treated lesional skin were taken, before and after treatment. The FFT method can calculate orientation and spacing of collagen bundles.[9] Cutaneous sclerosis is characterized by parallel oriented collagen bundles. The periodicity and the orientation of structures, e.g. collagen bundles, can be represented as a so called power plot of the FFT of a digital image. Interpretation of the power plot is much easier and more accurate than trying to extract the same information from the original image, because all the spacings and orientations are effectively averaged together in a frequency domain. The FFT allows measurement of spacing- and orientation of collagen bundles as well as a histopathological evaluation of the same location in a section.

Statistical analysis
Statistical analysis of measurements obtained by skin score and cutometer was performed with Student t-test ($p < 0.05$, two-sided).

RESULTS

Skin score and cutometer measurements did not differ in treated and control skin before therapy. All patients responded to therapy, according to skin score results. Skin score measurements (table 2) showed a softening of the treated skin versus the control skin, resulting in a lower skin score result, 1,50 (±0,53) before therapy and 0,86 (±0,69) at follow up (2 weeks after cessation of therapy) versus 1,50 before and 1,43 after in unirradiated controls. None of the patients showed complete regression of the sclerotic plaques. During therapy cutaneous sclerosis responded well according to skin score results. No skin score evaluation was obtained after 12 weeks.

Table 2. Skin score values

	Control			UVA1		t-test
	n	value	sd	value	sd	
before	8	1,50	0,53	1,50	0,53	
after 2,5 weeks	8	1,50	0,53	1,13	0,35	0,080
after 5 weeks	8	1,38	0,52	1,25	0,71	0,598
after 7 weeks	7	1,43	0,79	0,86	0,69	0,030

UVA1 treatment improved skin elasticity as evaluated with the cutometer from 2 weeks on (table 3). Treated lesional skin showed a significantly increased elasticity at week 3, 5 and 7 when compared to control skin. At week 12, there was no significant difference in skin elasticity between treated and control skin. Fast Fourier transform technique did not reveal a change in bundle orientation ratio or bundle spacing during therapy, nor at follow up after 12 weeks (data not shown).

All patients completed therapy. UVA1 phototherapy was well tolerated and there were no serious side effects. All patients showed generalized hyperpigmentation of the skin due to phototherapy. At the end of therapy all patients had completed 20 treatments with doses of 48 J/cm^2 per session resulting in a total UVA1 dose of 960 J/cm^2.

Table 3. Absolute Cutometer values compared to baseline values

	Control			UVA1		t-test
	n	value	sd	value	sd	
before (baseline)	8	0,0	0,0	0,0	0,0	
after 3 weeks	8	-0,03	0,20	0,22	0,15	0,011
after 5 weeks	8	-0,06	0,12	0,14	0,22	0,015
after 7 weeks	7	-0,04	0,10	0,30	0,31	0,019
after 12 weeks	5	-0,06	0,42	0,01	0,29	0,538

DISCUSSION

Recent literature[5,6] showed a beneficial effect of treatment with both high-dose and low-dose UVA1 in clearing of sclerotic plaques in morphea. We investigated whether these results could also be obtained by medium-dose UVA1 therapy. In all patients softening of the sclerotic plaques was observed. This was not the case in the non-irradiated plaques. The observed beneficial effects can therefore not be attributed to spontaneous remission. The mechanism of action of UVA1 on sclerotic skin is still not elucidated, although previous reports[5] showed a dose-dependent up regulation of collagenase activity, which could be responsible for the softening of the skin.

Medium-dose UVA1 therapy induces indeed clinical improvement of lesional skin in patients with localized scleroderma when measured 2 weeks after the end of therapy. Longer follow up, revealed that the improvement of in elasticity attributed to UVA1 therapy did not last. After 3 months cutometer values did not differ from baseline value in the treated group.

We did not observe a significant change in collagen bundle orientation, as measured by fast Fourier transform. This can be attributed to the relatively short evaluation time (7 weeks). Apparently, transformation of collagen bundles from a parallel arrangement to a basket weave arrangement takes more time. This suggests that change in sclerosis of the treated skin, which can be detected by skin score and cutometer measurement (softening of the skin and more elasticity), but not by fast Fourier transform measurements, is not caused by a change in collagen bundle orientation.

We showed that medium-dose UVA1 induces softening of sclerotic skin, although in none of our patients there was complete clearing of sclerosis. Five weeks of therapy seems to be too short to induce complete remission of sclerosis. Since the initial beneficial effect of UVA therapy on elasticity was undone by 3 months we propose that UVA1 therapy should be given for a longer period of time, at least to induce complete clearing of sclerosis. According to our results maintenance therapy could be required to prevent disease relapse. Further studies should determine the optimal duration and frequency of treatment.

At present no proven beneficial therapy for morphea exists. UVA1 therapy seems to be an effective therapy for morphea. We evaluated the efficacy of medium-dose UVA1. Softening of the skin was observed in all of our patients after 20 treatments. UVA1 medium-dose long-term side effects (skin cancer due to DNA damage) are predicted to occur less frequently as compared to high-dose UVA1 therapy. Medium-dose UVA1 gives favorable results in a shorter period of time as compared to low-dose UVA1 therapy, where a minimum of 3 months exposure is needed to obtain clinical results. We concluded that medium-dose UVA1 therapy is a beneficial and well tolerated treatment modality for morphea. The use of medium-dose UVA1 is a valuable addition in the treatment of morphea, but long term studies are clearly needed.

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Chapter 8

SUMMARY

Diffuse systemic sclerosis is a rare but severe disease, with a high morbidity and mortality. Regrettably, the therapeutical options are limited. Around 1990, some preliminary case-reports were published suggesting that photopheresis might be effective in systemic sclerosis. Between 1992 and 1996, we were able to evaluate this new and expensive treatment modality, thanks to a research grant from the Dutch Health Care Insurance Council (ZFR). Primary outcome variables in this study were the effect of photopheresis on clinical parameters (skin score, internal organ function, immune system) and quality of life.

In the introductory ***chapter 1*** several aspects of systemic sclerosis are discussed, such as diagnostic criteria, classification, epidemiology, etiology, therapeutical options, and the working mechanism of photopheresis.

Diagnostic criteria and classification.
Systemic sclerosis is a heterogeneous disease with various clinical manifestations. The addition 'systemic' or 'generalized' sclerosis separates this disease from morphea (localized scleroderma). In morphea, the sclerosis of the skin is histologically indistinguishable, but there is no internal organ involvement.
There are many different classification systems for systemic sclerosis. In 1980, generally accepted preliminary diagnostic criteria for systemic sclerosis were formulated by the American Rheumatism Association. As major criterium, the presence of *proximal scleroderma* (sclerosis of the skin, proximal of the metacarpo- or metatarsophalangeal joints) was defined. Minor criteria were *sclerodactyly, digital pitting scars, loss of substance of the distal finger pad, and bibasilar pulmonary fibrosis*. The diagnosis systemic sclerosis required the major criterium or two minor criteria. Some regard the ARA criteria not precise enough and prefer the subdivision in a limited form (lSSc: limited systemic sclerosis) and a diffuse form (dSSc: diffuse systemic sclerosis). In lSSc the sclerosis of the skin is usually limited to the acral regions (hands, forearms,

face). The internal manifestations of the disease are mostly limited to esophageal dysmotility and sometimes pulmonal hypertension. The course of the disease tends to be more benign and has a rather favorable prognosis. The skin involvement in dSSc is usually rapidly progressive. The trunk is always involved. The internal involvement in dSSc can be variable. It is associated with a higher morbidity and mortality. The extent of skin sclerosis is of major importance for the classification in the different subgroups and for the prognosis of the disease. For the photopheresis study we subdivided the patients in *type I* (acrosclerosis, sclerodactyly only), *type II* (scleroderma skin changes proximal to the MP joints with sparing of the trunk) and *type III* (diffuse scleroderma skin changes including the trunk). This subdivision was in that period the most accepted classification system in Europe.

Epidemiology

Of these three subtypes, incidence, prevalence and mortality rates are known from different European countries and the United States. After extrapolation of these figures to the Dutch situation, it was estimated that approximately 25 new patients per year with type II or III sclerosis were available in the Netherlands.

Clinical features of systemic sclerosis

The skin changes in systemic sclerosis can be divided into 3 phases. The first phase is edema, on the hands noticed as stiff and swollen fingers. The second phase is the characteristic sclerosis. The skin is tight and shiny, causes restriction of movement nearby joints, and cannot be normally picked up or pinched because of the induration. There may be reduced hair growth, hypopigmentation or hyperpigmentation in the involved areas. Hypopigmentation is most easily noticed in dark skin and typically occurs in the neck area, in a perifollicular pattern. In the third phase the skin may become atrophic and soft.

The recognition of systemic sclerosis is relatively easy when the skin becomes tight and indurated. The changes always start at the hands and face; if not, another diagnosis should be considered. In the face, the skin around the eyes becomes tight, wrinkles disappear and the nose is small and pinched. The lips are thin, the opening of the mouth is restricted (microstomia) and there are radial furrows around the mouth. These facial changes are characteristic for systemic sclerosis. *Telangiectases* in the face, on the hands and the upper chest are seen in the whole spectrum of the disease; they cannot differentiate between lSSc, dSSc and the CREST-syndrome (calcinosis, Raynaud phenomenon, esophagus dysmotility, sclerodactyly, telangiectasia.) *Calcinosis cutis* is most often found in patients with the CREST syndrome, but can be found in the whole spectrum of the disease.

Raynaud's phenomenon is defined as episodic attacks of well-demarcated blanching or cyanosis of the fingers or toes, usually provoked by triggers such as changes in temperature and emotions. It occurs in 90-98% of patients with systemic sclerosis. In the limited form (lSSC), Raynaud's phenomenon is present for years without sclerosis of skin or viscera. In the diffuse form (dSSc), Raynaud's phenomenon is of recent onset or starts together with the skin changes. In all patients Raynaud's phenomenon causes serious morbidity, ranging from discomfort and pain to ulcerations and loss of digits.
Several *gastrointestinal symptoms* may occur, such as dysphagia, heartburn due to esophageal dysmotility and reflux, esophagitis, strictures of the esophagus, intestinal pseudo-obstruction, malabsorption, megacolon, transverse and sigmoid colon volvulus, stenosis and diverticular ulcerations. *Renal failure* is often associated with dSSc. Clinical symptoms are hypertension and proteinuria. The mortality has declined drastically after the introduction of haemodialysis and angiotensin converting enzyme inhibitors. Risk factors for the onset of renal crises are rapid progression of skin fibrosis, congestive heart failure, hypertension, and the use of high doses of corticosteroids. *Pulmonary disease* is a major cause of death in systemic sclerosis. The major manifestations are fibrosing alveolitis, mostly seen in dSSc, and probably caused by an inflammatory reaction leading to increased collagen deposition, and pulmonary hypertension, mostly seen in lSSc, caused by the vascular abnormalities. *Cardial* involvement may present as non-specific tiredness, dyspnea or palpitations, arrythmias due to fibrosis of the conduction system, pericarditis, and right-sided cardiac failure due to myocardial fibrosis or pulmonary hypertension. *The joints and muscles* may be affected. Symptoms are flexion contractures, arthralgias, disabling arthritis, and myositis.

Therapeutical options in systemic sclerosis
Raynaud's phenomenon causes a considerable morbidity. Avoidance of exposure to cold is very important. Vasodilating drugs such as nifedipine can reduce the frequency and severity of the attacks. *Gastric reflux* can be improved by practical measures such as elevation of the head of the bed and avoidance of food that lowers the gastroesophageal sphincter tone. Pharmacological treatment with proton pump inhibitors such as omeprazole is effective. Delayed gastric motility can be treated with cisapride. *Renal crisis* can be treated with angiotensin-converting enzyme inhibitors. *Pulmonal* hypertension can be treated with calcium-channel blockers or intravenous prostaglandins. *Alveolitis* can be treated with cyclophosphamide, sometimes combined with prednisone. Arrythmias can be treated with antiarythmic medication and pacemakers.

Most medications used in systemic sclerosis are only symptomatic; they do not prevent disease progression. In the past years, treatment was focused on the fibrotic component. *D-penicillamine*, believed to alter the process of fibrosis by inhibiting the cross-linking of collagen, was the drug of choice until a recently performed controlled trial could not confirm the effectivity of D-penicillamine. *Interferon-gamma* inhibits collagen synthesis but has no effect on established fibrosis and is associated with considerable side-effects. There is an increased use of *methotrexate*. A recent randomized, double-blind trial showed favorable outcomes in skin scores, general well-being and creatinine clearance, but studies with larger patient numbers and a longer follow-up period are required. *Corticosteroids* are still used in the inflammatory phase of systemic sclerosis, especially inflammatory myositis, pericarditis and alveolitis. The induction of scleroderma renal crises by high-dose corticosteroids remains a topic of discussion. Recently, procedures using purified *autologous stem cells* to eliminate potentially autoreactive T-cells, show promising results. This treatment modality is applied in auto-immune diseases with an unfavorable prognosis.

Photopheresis

Photopheresis, or extracorporeal photochemotherapy, is the extracorporeal exposition of the patient's lymphocytes to ultraviolet A (UVA), in the presence of psoralen. Blood is drawn from the patient, heparinized and separated in plasma, leucocyte fraction and erythrocytes by means of a continuous centrifugation process. The leucocytes are collected in a separate bag which also contains the psoralen. From this bag, the leucocytes continuously circulate through a thin photoceptor, positioned between UVA light bulbs. The leucocytes are irradiated for 1.5 hours and then reinfused into the patient.
Photopheresis was initially developed for the treatment of cutaneous T-cell lymphoma (CTCL). Psoralens, after activation by UVA bind to DNA and inhibit DNA replication. Based on the hypothesis that standard PUVA treatment works in CTCL through a direct cytotoxic effect on T-lymphocytes, a machine was developed to expose the lymphocytes extracorporeally to high-dose UVA and psoralen. This method was effective in the treatment of CTCL. Because per treatment only a small fraction of the total pool of lymphocytes is irradiated, the therapeutic effect could not be explained by the cytotoxic effect on the lymphocytes only. This led to several theories about the working mechanism of photopheresis. One idea was that photopheresis changes the T-lymphocytes in such a manner that their reinfusion elicits an immunological response, leading to inhibition of the malignant or disease specific T-cell clone. Until now, the working mechanism of photopheresis is still not elucidated.

In ***chapter 2*** some closely related clinical conditions are described: systemic sclerosis, morphea, eosinophilic fasciitis, and scleredema.

Morphea

On histological grounds, systemic sclerosis and morphea are indistinguishable. Clinically, the difference is clear. Patients with morphea often have one or more sharply demarcated indurated maculae or plaques on the trunk or extremities. The lesions vary from 1-30 centimeter and have a shiny, waxy and ivory-white appearance. Sclerotic plaques of recent onset are surrounded by a purple-red ring (lilac ring). The hands and especially the fingertips are not involved, Raynaud's phenomenon and visceral involvement are absent.

Eosinophilic fasciitis (Shulman's disease).

This disease is characterized by rapidly progressive and painful swelling of one or more extremities, followed by an orange-brown, woody induration and a 'peau d'orange' appearance of the involved skin, which distinguishes it from systemic sclerosis. Nailbed changes and Raynaud's phenomenon are absent. The face and the hands are not involved. Antinuclear antibodies are absent, eosinophilia is often seen. The diagnosis can be confirmed histologically.

Scleredema (Buschke or diabeticorum).

Scleredema is a very rare collagen disorder. The skin of the upper part of the back, the neck and shoulders is most frequently involved. Two types exist, scleredema of Buschke (adultorum) and scleredema diabeticorum. The first is usually preceded by a febrile illness and regarded as a post-infectious condition. Scleredema diabeticorum is found in longstanding and often unsatisfactorily controlled diabetes.

In scleredema of Buschke, a rapidly progressive, symmetrical and painless, non-pitting induration of the skin of neck, back and shoulders is observed. Hands and feet are seldom involved. It can resolve spontaneously in 6 to 24 months. Occasionally the skin lesions persist for years. The skin shows no atrophy or pigment changes. There is no visceral involvement. No specific laboratory or immunological anomalies have been noticed.

Scleredema diabeticorum shares the same clinical and histological features with exception of the infectious phase. The onset of the skin indurations is more gradually. Better control of the diabetes mellitus has no effect on the clearing of the skin indurations. In contrast to scleredema of Buschke, regression of the skin tightness is seldom seen.

In ***chapter 3*** the results of the photopheresis study are described, with the focus on clinical efficacy. Nineteen patients with progressive systemic sclerosis of less then 5 years' duration were randomized into two groups. The first group received photopheresis for 1 year, the other group served as a control and received no treatment. After 1 year, the groups switched (randomized controlled cross-over design). The main outcome parameter was the change in induration of the skin, measured by an independent observer, by using the skin score. The change in skin score after 1 year treatment was compared with the change in skin score in the control group.
Also the effect on esophageal motility, lung, kidney and cardiac function, routine blood parameters, and the immune system was measured. In addition, quality of life, general health, psychologic and physical distress, and general well being were assessed. All costs related to treatment were calculated.
The average skin score in the photopheresis group improved by 5.4%, in the control group it deteriorated by 4.5% ($p=0.7$). None of the other above mentioned parameters showed significant changes during photopheresis treatment.
We concluded that photopheresis, twice a month for 1 year, is not effective for the treatment of systemic sclerosis.

In ***chapter 4*** the working mechanism of photopheresis is studied. One of the hypotheses was that the lymphocytes are modified during PUVA-exposure (a change in cell membrane rigidity, modifications in cell surface receptors, or induction of the release of cytokines) and elicit an immune-response after reinfusion to the patient. One important issue in this respect was the viability of the PUVA exposed cells. This was investigated by determining whether photopheresis induces apoptosis (programmed cell death) in lymphocytes. Qualitative gel electrophoresis and quantitative *in situ* nick translation analysis of DNA fragmentation was performed. Apoptosis of the treated (photopheresis) cells did occur (20-55%). The untreated leucocytes of the patients and the healthy control individuals showed no distinctive rise in apoptotic cells. We concluded that apoptosis of the leucocytes after photopheresis treatment might be a mechanism of action.

In ***chapter 5*** we describe a new objective method to measure induration of the skin, using the SEM 474 Cutometer. Exact registration of the distribution and severity of the skin induration in systemic sclerosis is important for the classification and prognosis of the disease. The *skin score* is an internationally used and validated semi-quantitative measure of cutaneous involvement in systemic sclerosis. Cutaneous sclerosis is assessed on a 0 to 3 scale (0 = normal

skin thickness, 1 = mild skin thickness, 2 = moderate skin thickness, 3 = severe skin thickness with inability to pinch the skin into a fold). A drawback of the skin score is its subjectivity. An objective measuring device to quantify skin sclerosis could be the *SEM 474 Cutometer*. This device measures elasticity of the skin. The elasticity of the skin in systemic sclerosis is changed due to collagen accumulation in the dermis. As a result of the increase of collagen in the skin it becomes impossible to pinch the skin into a normal skin fold. This 'fixation' of the skin can be translated in 'less' elasticity. The elasticity meter consists of a unit containing a vacuum pump, a measurement probe with a defined diameter and a personal computer. The skin is lifted in the probe by vacuum. The depth of penetration into the probe is determined by a noncontact optical measuring system. This measurement is expressed in millimeters.
The cutometer produced quantitative and reproducible data. The purpose of this study was to investigate whether the cutometer could measure skin elasticity more objectively than the skin score. Skin elasticity was measured in patients with systemic sclerosis by cutometer and skin score. There was a reasonable correlation between both methods. The cutometer showed a high interobserver and intraobserver correlation coefficient, this makes the cutometer very suitable for quantifying skin sclerosis in an objective manner.

Chapter 6 describes 2 new methods to measure collagen bundle orientation and spacing. In normal skin, collagen bundles show a three dimensional basket weave pattern, whereas in scleroderma lesions the collagen bundles are oriented parallel to the epidermis, and the collagen bundles are tightly packed and broader. The two new methods, the laser scatter method and the fast Fourier transform method can measure the severity of dermal sclerosis based on the architecture of the collagen bundles. Both methods were compared with the skin score.
The laser scatter method quantifies the overall orientation of collagen bundles in fibrotic tissue. The fast Fourier transform method is a mathematical analogue of the laser scatter plot. It calculates orientation and spacing of the collagen bundles in an image (tissue slide). Both methods proved to be suitable tools for the evaluation of dermal structure of scleroderma lesions in a quantitative manner. They can objectively quantify collagen bundle structure and can discriminate between fibrotic dermis and normal organized dermis. The fast Fourier transform method seems to be very accurate and reproducible and it could be a valuable method to follow-up the course of fibrotic skin diseases.

Chapter 7 describes the results of a pilot study of the treatment of morphea (localized scleroderma) with UVA. The sclerosis in morphea is limited to the skin and the subcutaneous tissue and can cause significant morbidity. Recent studies showed that in fibroblasts of lesional skin increased collagen expression was found together with decreased collagenase expression. Ultraviolet A1 (UVA1) radiation is associated with induction of collagenase in fibroblasts of lesional skin. We treated 8 patients with morphea with medium-dose UVA1 (48J/cm^2) during 5 weeks, 4 times a week. The follow up period was 12 weeks. The results were evaluated by means of the skin score, the cutometer and the fast Fourier transform method. All patients responded to therapy. Skin score and cutometer results showed improvement of skin elasticity. The fast Fourier transform method measurements showed no change in bundle orientation ratio.

Chapter 9

SAMENVATTING

Systemische sclerodermie is een zeldzame maar zeer ernstige aandoening, die snel progressief kan verlopen, en gekenmerkt wordt door een hoge morbiditeit en mortaliteit. De therapeutische mogelijkheden zijn helaas beperkt.
Rond 1990 verschenen er enkele case reports over behandeling van systemische sclerodermie met fotoferese (extracorporele photochemotherapie), waarin een gunstig resultaat werd beschreven. Omdat het ging om een geheel nieuwe en kostbare medische technologie, leende dit onderwerp zich bij uitstek voor evaluatie in het kader van een ontwikkelingsgeneeskunde project.
In het onderzoek werd niet alleen gekeken naar klinische effectiviteit, maar ook naar effecten op het immuunsysteem, de kwaliteit van leven, en naar de kosten die implementatie van fotoferese behandeling voor de indicatie sclerodermie met zich mee zouden brengen.

In ***hoofdstuk 1*** van dit proefschrift wordt een aantal aspecten van systemische sclerodermie beschreven, o.a. andere diagnostische criteria, klinische subtypen, verschillende classificatie systemen, epidemiologie, etiologie, en therapeutische mogelijkheden. Verder wordt ingegaan op het principe van fotoferese.

Diagnostische criteria en classificatie
Systemische sclerodermie is een heterogeen ziektebeeld met verschillende klinische manifestaties die per patiënt kunnen verschillen. De toevoeging 'systemische', of, in andere omschrijvingen 'gegeneraliseerde' sclerodermie grenst het beeld af van gelokaliseerde sclerodermie (morfea), waarbij ook sclerose van de huid optreedt, histologisch niet te onderscheiden van sclerose bij systemische sclerodermie, maar zonder interne manifestaties.
Er worden voor systemische sclerodermie verschillende classificatie systemen gehanteerd. In de jaren 80 van de vorige eeuw is overeenstemming bereikt over de voorlopige criteria voor de diagnose systemische sclerodermie. De American Rheumatism Association definieerde 1 major criterium, proximale sclerodermie

(sclerose van de huid proximaal van de metacarpophalangeale respectievelijk metatarso-phalangeale gewrichten), en 4 minor criteria (sclerodactylie, acrale littekens, afname van het weefselvolume aan de vingertoppen, en dubbelzijdige longfibrose). De diagnose systemische sclerodermie wordt gesteld als er sprake is van aanwezigheid van het major criterium of als er twee minor criteria aanwezig zijn.
De ARA criteria zijn ruim, daarom wordt er door vele auteurs een onderverdeling gemaakt in twee subtypen; de gelimiteerde vorm (lSSc: limited systemic sclerosis) en de diffuse vorm (dSSc: diffuse systemic sclerosis). De huidsclerose beperkt zich bij lSSc tot handen, onderarmen en gelaat. De interne manifestaties van de ziekte beperken zich meestal tot oesophagus dysmotiliteit, soms is er sprake van pulmonale hypertensie. Deze vorm van systemische sclerodermie verloopt meestal gedurende jaren mild en heeft meestal een redelijke prognose. De huidafwijkingen bij dSSc zijn vaak snel progressief, de romp is altijd betrokken in het ziekteproces. De interne manifestaties van het ziektebeeld zijn divers, maar bijna altijd ernstig en uiteindelijk fataal. Het subtype CREST syndroom (*C*alcinosis cutis, *R*aynaud fenomeen, o*E*sophagus dysmotiliteit, *S*clerodactylie en *T*eleangiectasieën) kan het best ingedeeld worden bij de gelimiteerde variant van dit ziektebeeld. Voor de fotoferese studie is uitgegaan van de Europese indeling in 3 subtypen:

Type I: Acrosclerodermie. De huidafwijkingen blijven gedurende jaren beperkt tot de acrale gebieden.
Type II: Acrosclerodermie met uitbreiding van de sclerose naar proximaal (onder-armen, benen, bovenste deel van de romp en tenslotte de gehele huid).
Type III: Diffuse sclerodermie. De huidafwijkingen ontstaan snel na het begin van de ziekte aan de romp en breiden zich relatief snel uit. Dit type gaat vaak gepaard met ernstige interne afwijkingen en heeft de slechtste prognose.

Epidemiologie
Van deze 3 subtypen zijn incidentie, prevalentie en mortaliteitscijfers bekend, uit omringende Europese landen en de Verenigde Staten. Na extrapolatie van deze gegevens naar de Nederlandse situatie, bleek dat er per jaar naar schatting 25 nieuwe patiënten met type II of III sclerodermie bijkomen. Patiënten met type II of III sclerodermie komen in aanmerking voor fotoferese. Indien de incidentie-cijfers en sterftecijfers worden gecombineerd, blijkt dat er tijdens de inclusie fase naar schatting 90-118 patiënten aanwezig waren in Nederland met type II of III sclerodermie, korter dan 5 jaar bestaand.

Klinische kenmerken van systemische sclerodermie

Huidafwijkingen. De huidafwijkingen bij systemische sclerodermie ontwikkelen zich grofweg in 3 fasen. Tijdens de eerste fase is er sprake van oedeemvorming in de aangedane huid, aan de handen uit zich dat in stijve en gezwollen vingers. De tweede fase is de periode met de voor sclerodermie zo kenmerkende geïndureerde huidafwijkingen. De huid is strak en glanzend, en kan rond de gewrichten voor bewegingsbeperking zorgen. De huid kan niet normaal opgepakt en geplooid worden ('unable to pinch'). Het normale huidrelief verdwijnt en in de aangedane huid kan er verminderde haargroei optreden. Pigmentverschuivingen (zowel hypopigmentatie als hyperpigmentatie) kunnen nu al aanwezig zijn, deze vallen het meest op bij de donkere huid. Een typische lokalisatie is de nek. De pigmentverschuivingen kunnen zowel onregelmatig vlekkerig als diffuus zijn. Tenslotte kan de 3e fase optreden, de huid wordt atrofisch en zacht.

Het ziektebeeld wordt duidelijk wanneer de huid sclerotisch verhard raakt. De lokalisatie en manier van uitbreiden is diagnostisch. De huidafwijkingen bij systemische sclerodermie beginnen eigenlijk altijd aan de handen en in het gelaat. Indien er geen huidafwijkingen aan de handen bestaan, moet een andere diagnose overwogen worden. In het gelaat wordt de huid rond de ogen en van de neus strak, rimpels vervagen en de neus wordt puntig. De lippen worden zeer dun en rondom de mond ontstaan radiaire groeven. Het voor systemische sclerodermie zo typerende strakke gelaat ontstaat. Het progressief worden van de ziekte uit zich in het naar proximaal uitbreiden van de huidsclerose. Opvallend is het feit dat de huid laag lumbaal en de huid van de nates bijna altijd gespaard blijft.

Teleangiectasieën in het gelaat, op de romp en in de handpalmen kunnen in het gehele spectrum van systemische sclerodermie voorkomen en kunnen dus geen onderscheid maken tussen CREST-syndroom, lSSc en dSSc.

Calcinosis cutis komt met name voor bij patiënten met het CREST syndroom, maar kan in het hele spectrum van systemische sclerodermie voorkomen. Vooral aan de vingertoppen, maar ook boven de verschillende gewrichten kunnen kalkdepositie ontstaan. Dit kan variëren van een enkele subcutane nodus tot grote samengestelde pakketten op verschillende gedeelten van het lichaam.

Zoals blijkt uit de verschillende classificatiesystemen is de uitgebreidheid van de huidafwijkingen van groot belang voor de indeling in de verschillende subgroepen. De uitbreiding van de huid sclerose is ook bepalend voor de prognose. De betrokkenheid van de huid bij dit ziekteproces blijkt het meest betrouwbare identificerende criterium te zijn.

Raynaud fenomeen. Dit fenomeen bestaat uit trifasische episodisch optredende scherp begrensde verkleuringen van de acra. Het betreft meestal de vingers, maar tenen, neus, oren en tongpunt kunnen ook meedoen. Het Raynaud fenomeen wordt uitgelokt door temperatuurwisselingen en emoties. Het is aanwezig bij 90-98% van de patiënten met systemische sclerodermie. Dit fenomeen is verder van diagnostisch belang bij het differentiëren tussen de gelimiteerde en de diffuse vorm van systemische sclerodermie. Bij de gelimiteerde vorm is het Raynaud fenomeen vaak al jaren aanwezig, reeds voordat er sprake is van duidelijke huidafwijkingen. Daarentegen is het Raynaud fenomeen bij de diffuse vorm van systemische sclerodermie meestal pas recent ontstaan, vaak tegelijk met het begin van de huidveranderingen.
De vaatspasmen tengevolge van het Raynaud fenomeen worden bij patiënten met systemische sclerodermie gesuperponeerd op de daar al aanwezige vaatvernauwingen door intima proliferatie en fibrose. Dit heeft tot gevolg dat bij deze patiënten met name aan de vingertoppen chronische en vaak moeilijk te behandelen ulceraties ontstaan. Soms kan dit gangreen en (auto)amputatie van één of meerdere vingertoppen tot gevolg hebben.
De *acrale littekens* komen in het hele spectrum van systemische sclerodermie voor, vaak gecombineerd met het verschijnsel van verlies van weefselvolume aan de vingertoppen. De vingertoppen vlakken als het ware af, de vingers worden puntig en afgeplat.

Gastro-intestinale afwijkingen. Oesophagus dysmotiliteit komt het meest voor en uit zich in slikklachten, verslikken en zuurbranden, als gevolg van verminderde peristaltiek van de oesophagus door fibrose en oesophageale reflux. Dit laatste kan leiden tot oesophagitis en stricturen. Tengevolge van peristaltische afwijkingen kan vertraagde ontlediging van de maag optreden en veranderde motiliteit van zowel de dunne als de dikke darm. Daardoor kan pseudoobstructie en malabsorptie door bacteriële overgroei ontstaan. Verder kan een toxisch megacolon, volvulus en stenose optreden.

Renale afwijkingen. Nierafwijkingen ontstaan met name bij de patiënten met de diffuse, uitgebreide vorm van systemische sclerodermie. Klinische manifestaties zijn hypertensie en proteïnurie. Sinds het gebruik van ACE remmers en het ontwikkelen van hemodialyse is de mortaliteit tengevolge van renale crise bij systemische sclerodermie dramatisch afgenomen. Risicofactoren voor het uitlokken van een renale crise zijn: snelle progressie van de huidafwijkingen, congestief hartfalen, therapie resistente hypertensie en een significant verhoogde kreatininewaarde. Ook is beschreven dat hoge doseringen prednison een renale crise kunnen uitlokken.

Pulmonale afwijkingen. De laatste jaren zijn de pulmonale afwijkingen bij patiënten met systemische sclerodermie de voornaamste doodsoorzaak. Dit is mede het gevolg van de verbeterde therapeutische opties op renaal gebied. Een fibroserende alveolitis en pulmonale hypertensie zijn de meest voorkomende afwijkingen. Waarschijnlijk is de fibroserende alveolitis het gevolg van toegenomen collageen vorming en de daaraan voorafgaande ontstekings-reacties. De pulmonale hypertensie is een uiting van de vasculaire veranderingen bij dit ziektebeeld. De interstitiële afwijkingen komen vooral voor bij dSSc, de pulmonale hypertensie met name bij lSSc. Patiënten presenteren zich met klachten van moeheid, kortademigheid bij inspanning en droge hoest.

Cardiale afwijkingen. Cardiale klachten komen weinig voor, en de symptomen zijn vaak niet specifiek (moeheid, kortademigheid en hartkloppingen). Tengevolge van fibrose van het geleidingssysteem kunnen patiënten aritmieën krijgen. Pericarditis en myocard fibrose komt nog al eens voor. Rechtszijdige decompensatie door fibrose of pulmonale hypertensie kunnen een uiting zijn van progressie van het ziekteproces.

Gewrichts en spierafwijkingen. Flexiecontracturen, artralgie en arthritis kunnen voorkomen bij systemische sclerodermie. Soms kan het, samen met het Raynaud fenomeen een eerste uiting van de ziekte zijn. Flexiecontracturen kunnen het gevolg zijn van de bovenliggende sclerosering van de huid. Ook artritis en fibrose van de pezen komen voor. Hierdoor ontstaan de zogenaamde 'palpable tendon friction rubs', het duidelijk voelbare 'kraken' van pezen bij beweging. Dit laatste kan vaak gevoeld worden aan de pezen van de onderarmen. Een myositis, nauwelijks te onderscheiden van een polymyositis komt af en toe voor. Mogelijk wordt dit veroorzaakt door microvasculaire beschadiging. Proximale spierzwakte, een afwijkend EMG en een verhoogd CPK kunnen worden gevonden.

Neurologische afwijkingen. Trigeminus neuralgie komt bij 4% van de patiënten voor. De diagnose carpaal tunnel syndroom gaat soms vooraf aan de diagnose systemische sclerodermie. Dit is misschien een verkeerde diagnose, en in werkelijkheid het gevolg van een (nog) niet duidelijk Raynaud fenomeen, aangezien dit zich ook kan presenteren met pijnlijke en tintelende vingers.

Therapeutische opties bij sclerodermie

Het fenomeen van Raynaud veroorzaakt een hoge morbiditeit onder deze patiënten groep. Preventieve maatregelen in de zin van voorkomen van temperatuurverschillen (koude) zijn zeer belangrijk. Daarnaast kunnen vaatverwijders de frequentie en ernst van de klachten verminderen. Calcium antagonisten zoals nifedipine zijn qua werking superieur ten opzichte van de overigen.

Klachten van *de tractus digestivus* zoals gastro-oesofageale reflux en oesophagus dismotiliteit kunnen met praktische adviezen bestreden worden zoals het hoofdeinde van het bed verhogen en het nuttigen van frequente en kleine maaltijden. Pharmacologische behandeling bestaat uit het gebruik van protonpompremmers, met name omeprazol. Maagontledigingsstoornissen kunnen adequaat behandeld worden met cisapride.

De behandeling van de *renale crise* bij sclerodermie is verbeterd met de komst van de ACE (angiotensine converting enzyme) remmers.

Pulmonale hypertensie als complicatie bij lSSc kan soms reageren op behandeling met calciumantagonisten. In zeer ernstige gevallen kunnen intraveneus prostaglandines toegepast worden. Alveolitis wordt sinds enkele jaren met redelijk succes behandeld met cyclophosphamide, soms in combinatie met prednison.

Hartritmestoornissen en geleidingsstoornissen kunnen behandeld worden met de standaard anti-arythmica en het plaatsen van een pacemaker.

De meeste toegepaste therapeutica zijn slechts symptomatisch werkzaam of bestrijden complicaties zonder de progressie tot staan te kunnen brengen. In het verleden was behandeling voornamelijk gericht op de fibrotische component van sclerodermie. D-penicillamine is een middel dat het cross-linken van collageen verhindert en zodoende de uiteindelijke fibrose bestrijdt. Gedurende jaren werd dit geneesmiddel toegepast voor de behandeling van systemische sclerodermie, echter recente studies konden geen duidelijk gunstig effect aantonen. Interferon-gamma remt collageen synthese, maar gezien de forse bijwerkingen (nierfunctiestoornissen) en de matige effectiviteit, wordt dit middel nog nauwelijks toegepast.

De laatste jaren is er meer aandacht voor immunomodificerende therapie. Methotrexaat wordt in toenemende mate toegepast, dit middel zou een gunstig effect hebben op skin score, het algemeen welbevinden en kreatinineklaring. Studies met grotere patiënten aantallen en langere follow-up zijn echter nog nodig. Systemische corticosteroïden worden met name toegepast bij inflammatoire processen zoals myositis, pericarditis en alveolitis. Dit middel zou echter in hoge doseringen een renale crise uit kunnen lokken. Zeer recent

werd therapeutisch succes geboekt met een procedure waarbij autologe stamcel transplantatie gebruikt werd om de autoreactieve T-cellen te elimineren. Deze methode wordt toegepast bij autoimmuunziektes met een zeer slechte prognose. Voor al deze regulier toegepaste middelen geldt dat de bijwerkingen fors kunnen zijn, dat de progressie er niet mee tot staan kan worden gebracht, en dat voldoende evidence over de effectiviteit, met name de langere termijn prognose, ontbreekt. Door de zeldzaamheid van sclerodermie is het ook bijzonder moeilijk om klinische trials uit te voeren.

Principe van fotoferese

Fotoferese (extracorporele photochemotherapie) bestaat uit het buiten het lichaam bestralen van de leukocytenfractie (met daarin de lymfocyten) van de patiënt met UVA (ultraviolet A), in aanwezigheid van psoralenen (8-methoxypsoraleen). Daartoe wordt het bloed afgenomen van de patiënt en in het fotofereseapparaat d.m.v. een centrifugestap gescheiden in plasma, leukocytenfractie, en erytrocyten. Via een armvene of liescathether wordt bloed afgenomen van de patiënt. Dit wordt direct geheparinieerd en in een draaiende centrifuge gepompt. De lymfocyten met wat plasma worden opgevangen in een aparte kunststof zak ('treatment bag'), en hier worden ook de psoralenen aan toegevoegd. Vanuit deze zak worden de lymfocyten continu rondgepompt door een dunne plastic plaat, die zich tussen twee rijen UVA-lampen bevindt. De erytrocyten gaan weer terug naar de patiënt, en daarna wordt opnieuw een portie bloed afgenomen. In totaal wordt de cyclus 6 x herhaald. Het eindvolume wordt nog anderhalf uur bestraald, daarna wordt ook dit weer teruggegeven aan de patiënt. De patiënten krijgen 2 fotoferese behandelingen per 4 weken, op twee opeenvolgende dagen.

Fotoferese is circa 15 jaar geleden ontwikkeld door de Amerikaan Edelson, voor de indicatie T-cel lymfoom. Photochemotherapie (PUVA) wordt in de Dermatologie al jaren succesvol toegepast bij psoriasis, atopisch eczeem, en een groot aantal andere dermatosen, waaronder vitiligo, prurigo, en cutaan T-cel lymfoom. De door UVA geactiveerde psoralenen blokkeren DNA replicatie. Vanuit het idee dat de werkzaamheid van PUVA therapie berust op het rechtstreeks remmen van proliferatie van T-lymfocyten, heeft men overwogen of de PUVA bestraling ook extracorporeel kon plaatsvinden: dus niet door de epidermis heen de daaronder opgehoopte T-lymfocyten bestralen, maar direct het bloed. Deze extracorporele PUVA methode bleek effectief te zijn voor cutaan T-cel lymfoom, vooral de erytrodermische vormen, en verwierf FDA registratie. De doorslaggevende argumenten daarbij waren dat fotoferese zonder bijwerkingen is, dit in tegenstelling tot de alternatieve opties (chemotherapie). De overlevingsduur verdubbelde met fotoferese. Dit therapeutisch effect kon niet berusten op alleen het uitschakelen van de lymfocyten, omdat per

behandeling slechts een fractie van het totale aantal circulerende lymfocyten bestraald werd. Deze vaststelling leidde tot een aantal theorieën over het werkingsmechanisme van fotoferese, waaronder de hypothese dat fotoferese T-lymfocyten op een of andere manier wijzigt, waarna bij het opnieuw in de circulatie brengen van deze lymfocyten een immunologische reactie tegen deze lymfocyten op gang wordt gebracht, die uiteindelijk vooral de pathologische (geëxpandeerde T-cel kloon) lymfocyten treft. Tot op heden is het werkingsmechanisme van fotoferese echter nog steeds niet opgehelderd. Los van het effect van PUVA op DNA niveau zijn er ook rechtstreekse effecten van UVA op de keratinocyten (die na bestraling een scala aan cytokines produceren) en op Langerhans cellen (migratie), en zijn er mogelijk ook rechtstreekse effecten van psoralenen extracellulair en intracellulair.
In het werkingsmechanisme van fotoferese therapie is de nadruk tot nu toe vooral gelegd op het remmen van de T-lymfocyten. De meeste dermatosen waarbij fotoferese therapie wordt toegepast zijn dan ook T-cel gemedieerd. Naast cutaan T-cel lymfoom worden zeer recent ook graft versus host disease, rejectie reacties na harttransplantatie, reumatoïde artritis, pemphigus vulgaris, SLE, psoriasis arthropatica, en atopisch eczeem als mogelijke indicatie gebieden voor fotoferese beschouwd. Het onderzoek naar deze toepassingen is nog gaande. Omdat ook van sclerodermie, als waarschijnlijke immuunziekte, werd aangenomen dat het T-cel gemedieerd is, is sclerodermie op een gegeven moment als 'te onderzoeken indicatiegebied' in zicht gekomen.

In ***hoofdstuk 2*** wordt ingegaan op de differentiele diagnose van sclerodermie. Soms kost het moeite om te differentieren tussen systemische sclerodermie, morphea, eosinofiele fasciitis, en scleroedeem.

Morfea
Histologisch is er geen onderscheid mogelijk tussen systemische sclerodermie en morfea (lokale sclerodermie). Klinisch is dit onderscheid wel goed mogelijk. Patiënten hebben vaak een of meerdere, scherp begrensde geïndureerde maculae of plaques op de romp. De laesies variëren in grootte van 1-30 centimeter in doorsnede. Deze zijn meestal glanzend en was-achtig van kleur. In de actieve fase zijn de sclerotische huidgebieden nog omgeven door een erythemateuze rand (lilac ring). De huidafwijkingen zijn niet pijnlijk en meestal asymmetrisch. Het merendeel van de morfea laesies bevindt zich op de romp, soms kunnen ook de ledematen aangedaan zijn. De handen en vooral de vingertoppen zijn nooit afwijkend. Patiënten hebben geen Raynaud-achtige klachten, evenmin zijn er aanwijzingen voor viscerale afwijkingen.

Eosinofiele fasciitis (syndroom van Shulman)
Dit ziektebeeld wordt gekenmerkt door de snel ontstane, meestal pijnlijke zwelling van een of meer ledematen. Dit wordt binnen enkele dagen tot weken gevolgd door bruin-rode, houtachtige induratie van de huid ter plaatse. Typerend is het peau d'orange aspect van de geïndureerde huid. Naast deze genoemde klinische verschillen ten opzichte van systemische sclerodermie, ontbreken ook de nagelbed afwijkingen en het Raynaud fenomeen. Handen en gelaat doen nooit mee in dit ziekteproces, hierdoor kan eosinofiele fasciitis met name onderscheiden worden van systemische sclerodermie. In het perifere bloed wordt vaak een eosinofilie gevonden, het ontbreken hiervan sluit deze diagnose echter niet uit. Een hoge bezinking kan voorkomen. ANA's zijn negatief. Een spier-fascie biopt kan de diagnose rond maken, karakteristiek wordt een eosinofiel ontstekingsinfiltraat in de subcutis en rond de fascie gevonden. Contracturen van de gewrichten is een vaak optredend verschijnsel.

Scleroedeem (Buschke of diabeticorum)
Scleroedeem is een zeer zeldzame collageen afwijking. De huidafwijkingen bevinden zich voornamelijk op de rug en de schouders. Er bestaan twee vormen, namelijk Scleroedeem van Buschke (scleroedema adultorum) en scleroedema diabeticorum. De eerstgenoemde zou een post-infectieuze oorzaak hebben. Scleroedema diabeticorum wordt bij diabetici gezien. Scleroedeem van Buschke treedt vaak op na een streptococcen infectie. Enkele weken na de infectie kan een snel progressieve, symmetrische, pijnloze induratie van de huid van de nek, rug en schouders bemerkt worden. Handen en voeten zijn nooit afwijkend. Een voorbijgaand erytheem wordt nog al eens gezien. De induratie kan na 6 tot 24 maanden spontaan in regressie gaan. Soms echter persisteren deze gedurende jaren. Het laboratorium onderzoek laat geen specifieke afwijkingen zien. De histologie is wel specifiek, de epidermis is normaal, de dermis is echter sterk verbreed. Deze verbreding wordt veroorzaakt door een toename in grootte van de collageenbundels zelf. Deze bundels worden van elkaar gescheiden door heldere ruimtes die hyaluronzuur bevatten. Scleroedema diabeticorum vertoont hetzelfde klinische en histologische beeld met uitzondering van de infectieuze fase. De huidafwijkingen ontstaan meestal iets geleidelijker en het erytheem ter plaatse wordt vaker gezien en persisteert langer. De diabetici zijn vaak oudere patiënten met een slecht ingestelde diabetes, welke al jaren aanwezig is. Het goed instellen van de diabetes heeft geen effect op de huidafwijkingen. In tegenstelling tot scleroedeem van Buscke, wordt zelden een regressie van de huidafwijkingen gezien.

In ***hoofdstuk 3*** worden de resultaten van de fotoferese studie beschreven, met de nadruk op klinische effectiviteit. Voor het fotoferese onderzoek werden 19 patiënten met aangetoond progressieve systemische sclerodermie (type II of III), korter dan 5 jaar bestaand, in 2 groepen gerandomiseerd. De eerste groep (fotoferese groep, n = 10) kreeg direct fotoferese, gedurende 1 jaar. De andere groep (controle groep, n = 9) werd een jaar poliklinisch, als controle groep vervolgd. In die periode werden geen immunosuppresiva gebruikt. Na een jaar kreeg de controle groep fotoferese en werd de fotoferese groep poliklinisch vervolgd (randomized controlled cross-over design).
De belangrijkste klinische uitkomstparameter was de verandering in induratie van de huid, door een onafhankelijk onderzoeker gemeten, m.b.v. de skin score. Voor de beoordeling van het effect werd de procentuele verandering in induratie t.o.v. de uitgangswaarde na 1 jaar behandelen vergeleken met die van de niet-behandelde controle groep. Voorts werd het effect op laboratorium-waarden, oesophagusmotiliteit, long-, nier- en hartfunctie gemeten.
Naast klinische effectiviteit werd gedetailleerd gekeken naar de kosten, bijwerkingen, belasting voor de patiënt, kwaliteit van leven, consumptie van zorg, en algemeen welbevinden. Verder werd uitgebreid laboratorium onderzoek verricht naar het effect op immunologische parameters, T-cel functie, toxiciteit, apoptose, en T-cel membraan-eigenschappen.
De skin score verbeterde in de fotoferese groep gemiddeld met 5.4% en verslechterde in de controle groep met 4.5% ($p = 0.70$). Er werden geen verschillen aangetoond tussen de fotoferese groep en de controle groep met betrekking tot lichamelijke beperkingen ($p = 0.75$), het dagelijks functioneren ($p = 0.27$) en de algemene gezondheidstoestand. Geen van de genoemde immunologische parameters veranderde tijdens de fotoferese behandeling.
Internationaal wordt fotoferese inmiddels in diverse centra toegepast bij sclerodermie. Desondanks zijn nog geen nieuwe gecontroleerde studies gepubliceerd die de effectiviteit overtuigend aantonen. De resultaten van een grote multicentre trial (70 patiënten) zijn nog niet gepubliceerd.
Omdat geen klinisch belangrijke en/of statistisch significante verschillen tussen behandelen en niet-behandelen werden aangetoond, noch enig effect op immunologische parameters of quality of life metingen, concluderen wij dat, bij deze behandelfrequentie en duur, niet is aangetoond dat fotoferese effectief is voor de indicatie sclerodermie.

In ***hoofdstuk 4*** wordt nader ingegaan op het werkingsmechanisme van fotoferese. Zoals reeds in de inleiding genoemd, wordt verondersteld dat de bestraalde lymfocyten bij terugkeer in de circulatie van de patient een immunologisch signaal afgeven, het zij door een verhoogde productie van cytokinen, hetzij doordat modificaties aan het celoppervlak een immuunrespons bij andere T-lymfocyten opwekken. Er wordt aangenomen dat deze therapie immunomodulerend is, deels door verminderde viabiliteit van de leucocyt. Wij hebben onderzocht of de veranderde levensduur van de cellen het gevolg was van apoptose. Hiertoe werd gebruik gemaakt van een qualitatieve methode door middel van gel electrophorese en een kwantitatieve methode door middel van *in situ* nick translatie. Er trad inderdaad apoptose op van de met fotoferese behandelde cellen (20-55%).

Hoofdstuk 5 beschrijft een nieuwe methode om de induratie van de huid bij sclerodermie objectief te meten.

Skin score

De huidafwijkingen bij systemische sclerodermie zijn het meest toegankelijk om in kaart te brengen. Nauwkeurige inventarisatie hiervan is belangrijk in verband met de classificatie en de prognose van het ziektebeeld. De skin score is een internationaal gebruikte en gevalideerde semi-kwantitatieve meet methode voor induratie van de huid bij systemische sclerodermie. Dit wordt gedaan volgens een 4 punts schaal, waarbij 0 normale huid is en 3 harde, niet plooibare geïndureerde huid. Een score 1 is een weinig geïndureerde huid, een score 2 is een matig geïndureerde huid. Deze semi-kwantitatieve meting is niet objectief, maar blijkt redelijk reproduceerbaar te zijn.

Skin Elasticity Measurement

Een mogelijke objectieve meetmethode om huidsclerose te kwantificeren zou de SEM 474 Cutometer kunnen zijn. Dit apparaat meet de elasticiteit van de huid, een directe afgeleide van de sclerose van de huid. Een probe met een bepaalde diameter wordt met constante druk op het te meten deel van de huid geplaatst. Het systeem bestaat verder uit een personal computer met speciale software en een computergestuurde vacuümpomp. De huid wordt opgetrokken door het vacuüm in de probe, in de meetkamer, alwaar de hoogte tot waar de huid op te rekken valt in millimeters wordt gemeten.

De cutometer produceert kwantitatieve en reproduceerbare gegevens. Het doel van dit onderzoek was om na te gaan of deze cutometer de huidelasticiteit objectief kan meten in vergelijking met de skin score. Daartoe werd de huid van patiënten met systemische sclerodermie met behulp van beide methoden gescoord. Er bleek een redelijke correlatie te bestaan tussen de beide meetmethoden. De interobserver en intraobserver ICC (intraclass correlation coefficient), een statistische maat voor de reproduceerbaarheid van de huid elasticiteit metingen, bleken zeer hoog te zijn. De cutometer blijkt dus zeer geschikt te zijn voor het objectief vastleggen en kwantificeren van huidsclerose.

Hoofdstuk 6 beschrijft twee nieuwe meetmethoden om de verandering in ligging van collageenbundels te bepalen. In de normale huid liggen de collageen bundels volgens een ongericht driedimensionaal vlechtwerk patroon ('basket weave' patroon), in de sclerotisch veranderde huid liggen de collageen bundels parallel aan de epidermis. Tevens zijn de collageen bundels dichter opeengepakt en breder. Met behulp van twee nieuwe meettechnieken, de laser scatter methode en de fast Fourier transformatie methode kan de mate van dermale sclerose worden beoordeeld, op grond van de architectuur van de collageen bundels. Deze twee methoden werden vergeleken met de skin score.
De laser scatter methode kan de gemiddelde oriëntatie van de collageen bundels in fibrotisch weefsel kwantificeren. De fast Fourier transformatie methode is een mathematische analoog van de laser scatter plot. Het berekent de oriëntatie en afstand tussen de verschillende elementen (collageenbundels) in een afbeelding (een in de computer ingescande histologische coupe). De twee genoemde methoden bleken goed bruikbaar te zijn voor de evaluatie van dermale structuren in sclerodermie laesies op een kwantitatieve manier. Een goed en objectief onderscheid kon gemaakt worden tussen fibrotisch/sclerotisch veranderde dermis en normale dermis. De fast Fourier transformatie bleek het meest accuraat en reproduceerbaar te zijn. Deze laatste methode zou goed bruikbaar kunnen zijn bij het vervolgen van huidsclerose ter evaluatie van een therapie.

Hoofdstuk 7 beschrijft de resultaten van een pilot studie voor de behandeling van morfea (lokale sclerodermie). De sclerose bij morfea beperkt zich tot de huid en het subcutane weefsel en kan ernstige morbiditeit veroorzaken. Enkele studies hebben aangetoond dat de fibroblasten van de aangedane huid bij

morfea een verhoogde expressie vertoonde van collageen en een verlaagde expressie van collagenase. Van Ultraviolet A1 (UVA1) is bekend dat het inductie van collagenase in de fibroblast van de aangedane huid kan veroorzaken. Wij hebben 8 patiënten met morfea gedurende 5 weken 4 keer per week behandeld met medium-dose UVA1 (48J/cm^2). Er was een follow up periode van 12 weken. De resultaten werden geëvalueerd door middel van de skin score, de cutometer en de fast Fourier transformatie methode. De huidafwijkingen van alle patiënten reageerden goed op de behandeling. Dit uitte zich in een verbeterde skin score en hogere cutometer waarden. De fast Fournier methode liet geen verandering zien wat betreft collageen bundel oriëntatie. Mogelijk is dit te wijten aan de relatief korte evaluatie tijd en heeft klaarblijkelijk de transformatie van collageen bundels van een parallelle schikking naar een driedimensionaal vlechtwerk patroon meer tijd nodig.

Chapter 10

DANKWOORD EN CURRICULUM VITAE.

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CURRICULUM VITAE

Dory Enomoto werd geboren op 2 maart 1962 te Amsterdam. Zij groeide op in de Amsterdamse binnenstad en doorliep aldaar de lagere school en de middelbare school. Na het behalen van het Gymnasium B diploma, begon zij in 1983 aan de studie Geneeskunde aan de Universiteit van Amsterdam. Op 27 november 1987 ontving zij haar doctoraalbul. Gedurende de lange wachttijd (ruim 18 maanden) voor de start van het co-assistentschap, heeft zij meegewerkt aan enkele lopende onderzoeken van de afdeling Dermatologie van het AMC. Begin 1991 behaalde zij het arts-examen en begon vrijwel direkt als arts-assistent Interne Geneeskunde in het Medisch Centrum Alkmaar (opleider: dr.W. Bronsveld). Vanaf 1 januari 1992 werkte zij mee aan diverse trials wondgenezing op de Kliniek van de afdeling Dermatologie onder leiding van dr. W. Westerhof. Vanaf 1 januari 1993 werd zij aangesteld op het Ontwikkelingsgeneeskunde project: *Behandeling van patiënten met chronische progressieve sclerodermie met behulp van fotoferese* en was zij belast met de uitvoering en coördinatie van dit onderzoek, haar direkte begeleider was dr. J.R. Mekkes. Op 1 juli 1995 begon zij met de opleiding Dermatologie in het AMC (opleider: prof. dr. J.D. Bos). Een deel van het werk beschreven in dit proefschrift werd uitgevoerd en opgeschreven gedurende deze periode. Op 1 augustus 2000 voltooide zij de opleiding Dermatologie. Per 1 januari 2001 zal zij toetreden tot de Maatschap Zwolse Dermatologen.

Scleroderma: Diagnosis and Experimental Therapy

Dory Enomoto