

**Diplomarbeit**

**ELEVATED IGF-1 SERUMLEVELS IN PATIENTS  
SUFFERING FROM SYSTEMIC SCLEROSIS WITH LUNG  
INVOLVEMENT**

**ERHÖHTE IGF-1 SERUMSPIEGEL BEI PATIENTEN MIT  
SYSTEMISCHER SKLEROSE MIT  
LUNGENBETEILIGUNG**

eingereicht von

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## List of abbreviations

ACA	anti-centromere antibodies
AFA	anti-fibrillar antibodies
ANA	anti nuclear antibodies
ATA	anti DNA topoisomerase I antibodies
ATG	antithymocyte globulin
AZA	Azathioprine
BAFF	B-Cell-Activating Factor
BLM	bleomycin induced mouse model
BP	bodily pain
CsA	Cyclosporine A
CT	Computerized Tomography
CXCL	chemokine ligand
CXCR	chemokine receptor
CYC	Cyclophosphamide
dcSSc	diffuse cutaneous disease
EBMT	European Group for Blood and Marrow Transplantation
ECM	extracellular matrix
ELISA	enzyme-linked immunosorbent assay
EULAR	European League Against Rheumatism
FVC	forced vital capacity
GH	General Health
HAQ-DI	Health Assessment Questionnaire disability index
HCMV	human cytomegalovirus
HRCT	high resolution CT
Ig	Immunoglobulins
IGF	insulin like growth factor
IGFBP	insulin like growth factor binding protein
IL	interleukin
ILD	interstitial lung disease
IMODH	inhibition of inosine 5 monophosphate dehydrogenase
IVIG	intravenous immunoglobulins

lcSSc	limited cutaneous disease in scleroderma
LES	low esophageal sphincter
MH	Mental Health
MHC	major histocompatibility complex
MMF	Mycophenolate mofetil
MTX	Methotrexate
PAH	pulmonary arterial hypertension
PBS	primary biliary cirrhosis
PCR	polymerase chain reaction
PF	Physical Functioning
RA	Rheumatoid Arthritis
RE	Role Emotional
RNP	Ribonucleoprotein
RP	Role-Physical
RTX	Rituximab
SF	Social Functioning
SRC	scleroderma renal crisis
SSc	systemic sclerosis
TGF	transforming growth factor
Th	T helper cells
VT	Vitality

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## Zusammenfassung in Deutsch

Die Sklerodermie (systemische Sklerose) ist eine unheilbare Autoimmunerkrankung, die mit einer Fibrosierung der inneren Organe und Haut einhergeht. Es wird schon seit längerem vermutet, dass verschiedene Wachstumsfaktoren, unter anderem der Insulin-like growth factor (IGF-1) eine wichtige Rolle für Entstehung und Verlauf der Sklerodermie spielen. In dieser Studie wurde der IGF-1 Serumspiegel von Patienten gemessen, die an Sklerodermie erkrankt sind und mit einer gesunden Kontrollgruppe verglichen. Die IGF-1 Werte der Sklerodermiepatienten wurden auch vor und nach der Therapie mit Rituximab verglichen. Die Serumspiegel von IGF waren bei Patienten mit Sklerodermie gegenüber der gesunden Kontrollgruppe nicht signifikant unterschiedlich. Patienten mit einer Rituximab-Therapie zeigten eine inhomogene Dynamik der IGF-1 Werte. Diese Patienten zeigten ein deutlich verbessertes klinisches Zustandsbild, sowie verringerte Zahlenwerte für die Krankheitsaktivität. Die Patienten wurden auch mittels Fragebögen zu ihrer Lebensqualität vor und nach einer Therapie mit RTX befragt. Die so beurteilte Lebensqualität besserte sich nach einer Rituximabtherapie deutlich.

In dieser Pilotuntersuchung erscheint der Wert der IGF-1 Bestimmung als Biomarker für die Krankheitsaktivität der systemischen Sklerose als gering.

## Abstract

Scleroderma (systemic sclerosis) is an autoimmune disease causing fibrotic changes within the skin and inner organs. Growth factors like IGF-1 may play an important role in the development of fibrosis in SSc.

This study found comparable IGF-1 serum levels from SSc patients and a healthy control group. We also undertook a comparison of the IGF-1 levels from SSc patients before and after treatment with rituximab. SSc patients showed inhomogeneous changes of IGF-1 levels after rituximab treatment. This therapy led to a better health status (as measured by questionnaire) and better SSc activity and SSc severity scores as well.

In this pilot investigation, IGF-1 serum levels seem not to be a suitable biomarker for the effectiveness of scleroderma therapies

# Systemic sclerosis

Systemic sclerosis (Scleroderma, SSc) is a chronic connective tissue disease. SSc is characterized by inflammation and autoimmunity, fibrosis and vascular insufficiency. Inflammation and autoimmunity are mainly found in the early-stage of the disease. Fibrosis and vascular insufficiency predominate the late-stage disease. (Hochberg, Marc C. et al 2011)

There are two main forms of scleroderma: localized scleroderma and systemic sclerosis. Localized scleroderma is usually divided in morphea, linear scleroderma, and a special form called scleroderma en coup de sabre. Systemic sclerosis is subdivided into limited cutaneous disease (lcSSc) and diffuse cutaneous disease (dsSSc). (Klippel, John H. MD et al 2007)

## Epidemiology

SSc has a female preponderance. The female-to-male ratios range from 4:1 to 6:1. The reasons for these ratios are not clear. There is much speculation around hormonal- and pregnancy-related events. (Hochberg, Marc C. et al 2011) SSc has a worldwide distribution and is found in all races. The onset of SSc seems to be earlier in African American than in white patients. The prognosis of African Americans is worse, caused by the common intestinal lung involvement. (Klippel, John H. MD et al 2007).

Familial aggregation occurs in SSc. This could be a result of genetic predisposition and/or environmental factors. (Hochberg, Marc C. et al 2011)

The prevalence is 16 to 25 cases per 100.000 inhabitants. Familial occurrence is the strongest risk factor for SSc. (Hochberg, Marc C. et al 2011) The survival rate of SSc is very low: 78% at 5 years and 55% at 10 years. (Leask 2010)

## Localized scleroderma

The main forms of localized dermal lesions are linear scleroderma and morphea and a special form scleroderma en coup de sabre. Typical for linear scleroderma are longitudinal bands of skin sclerosis. Patients with morphea show circumscribed oval plaques. Patients with Scleroderma en coup de sabre usually have linear scleroderma involving the face and head. Some of them also have neurologic abnormalities and severe facial and skull deformity. (Hochberg, Marc C. et al 2011)

Localized scleroderma has a female preponderance. The incidence is 2,7 per 100.000 and the prevalence is 50 per100.000. The onset occurs at a mean age of 46. (Hochberg, Marc C. et al 2011)

## Linear scleroderma and Scleroderma en coup de sabre

The epidemiology shows a higher prevalence of linear scleroderma in children than in adults. Linear scleroderma is different in comparison to linear morphea. Linear scleroderma shows hypopigmented or hyperpigmented skin areas on the limbs. The deeper part of the dermis and the subcutaneous tissue, the muscles, and the bones may show deformities. Linear scleroderma of the head (face and skull) is called scleroderma en coup de sabre. Scleroderma en coup de sabre has similarities to Parry Romberg syndrome. (Hochberg, Marc C. et al 2011)

13% of Patients with scleroderma en coup de sabre or Parry Romberg have seizures. The common treatments are methotrexate, cyclosporine, tissue allograft and also antimalarial drugs. (Hochberg, Marc C. et al 2011)

## Morphea

Morphea seems to be more common in adults than in children. Morphea has a wide spectrum of different subforms. Often found are deep morphea, plaque morphea,

pansclerotic morphea and diffuse morphea. There is also atrophoderma of Pasini and Pierini, lichen sclerosus et atrophicus, keloidal or nodular morphea.

Plaque morphea is the most common form: nearly 50% of all cases. Patients with plaque morphea first show hyperpigmented areas. In these areas, there is a lack of sweat glands and hair follicles. The typical presentation is a single or multiple circumscribed plaque usually on the trunk or extremities. The plaques are expanding. In biopsies lymphocytes, plasma cells, eosinophiles, macrophages and mast cells are present. The biopsy demonstrates an inflammatory event and also a deposition of collagen. (Hochberg, Marc C. et al 2011)

The typical histological findings for morphea profunda show inflammation and sclerosis in the deep dermis, fascia, panniculus and sometimes even in the superficial muscles. A subtype disables pansclerotic morphea of children. The most aggressive and severe forms involve fascia, muscles and bones. Significant deformity and disability and chronic ulcers are common complications. Pansclerotic scleroderma may develop into squamous cell carcinoma. (Hochberg, Marc C. et al 2011)

15 % of all morphea cases are generalized or diffuse morphea. Patients with generalized or diffuse morphea typically show multiple sclerotic plaques, hyperpigmentation and common muscle atrophy. (Hochberg, Marc C. et al 2011)

There are a wide variety of drugs for the treatment of morphea such as topical tacrolimus, cyclosporine, imatinib, mycophenolate mofetil, ultraviolet light and retinoic acid. The most common agent is methotrexate. Patients treated with methotrexate may show a regression of morphea. (Hochberg, Marc C. et al 2011)

## Types of Systemic sclerosis (SSc)

The main subtypes of SSc are diffuse cutaneous disease (dcSSc) and limited cutaneous disease (lcSSc). The differentiation of these two forms is important for the prognostic outcome. (Klippel, John H. MD et al 2007)

## Diffuse cutaneous disease (dcSSc)

Skin sclerosis proximal to the elbows and/or knees fulfills the definition of the diffuse cutaneous disease. Patients with dsSSc may also show truncal skin involvement. Facial involvement is found in both forms diffuse cutaneous disease (dcSSC) and limited cutaneous disease (lcSSc). The beginning of dsSSc is faster than the onset of lcSSc. The risk for scleroderma renal crisis (SRC) is higher in patients with early dcSSc. (Klippel, John H. MD et al 2007)

Anticentromere antibodies are not usually present in the serum of patients with dsSSc. The dcSSc can affect visceral organs such as lungs, heart, gut and kidneys. The survival rate is poor: 40% to 60% at 10 years. (Klippel, John H. MD et al 2007)

## Limited cutaneous disease (lcSSc)

At the onset of limited cutaneous disease (lcSSc) often there is the Raynaud's phenomenon. Patients with lcSSc further suffer from symptoms like tender pitting scars or ulcers on the fingers, thickening of the digital skin. The thickening of the skin may spread to the hands and forearms. Patients also show dyspnea caused by pulmonary fibrosis or pulmonary arterial hypertension. Telangiectasias on the hands and face are also found. Patients with lcSSc also have a visceral involvement but this occurs later and less severe than in patients with dcSSc. In contrast to dcSSc one can find anticentromere antibodies in patients with lcSSc. The survival rate is relatively good: >70% at 10 years. (Klippel, John H. MD et al 2007)

## Clinics

The typical pathologic criteria of SSc are vasculopathy and fibrosis. The earliest pathogenic change in SSc is vasculopathy. Particularly the small vessels and capillaries are affected. (Hochberg, Marc C. et al 2011)

## Raynaud phenomenon

Episodic peripheral vasospasm (also called Raynaud phenomenon) is a characteristic clinical vasculopathy sign at the beginning of SSc. It occurs up in 95% of SSc cases.

The cause for the Raynaud phenomenon is a vasospasm of the small vessels in response to cold changes. Emotional stress also operates as a trigger. There are three phases: pallor, cyanosis and rubor. Approximately 5-10 % or more of the general population observe episodic peripheral vasospasm sometimes during their life. This is called primary Raynaud's disease and is not necessarily indicating SSc.

Secondary Raynaud's disease results in tissue damage. Chronic ischemia may cause digital ulcers and even digital gangrene. One can also find a reduction of the finger pad substance. Secondary Raynaud's phenomenon is usually the first manifestation of SSc. (Klippel, John H. MD et al 2007)

The vascular damage is mainly caused by fibrosis. Characteristic histological changes are myointimal cell proliferation and migration, collagen accumulation and a reduplicated and thickened basement membrane. Cutaneous telangiectasia, microscopic vascular changes at the nail fold, pulmonary arterial hypertension, "watermelon stomach" (gastric antral vascular ectasia) and renal crisis are also typical signs of vascular transformation in SSc. (Hochberg, Marc C. et al 2011)

SSc is identified by thickened skin. More than 95% of the patients have evidence of skin thickening. But there are also patients with scleroderma sine sclerosis. Swollen hands (and sometimes feet), pruritus, hyper-and/or hypopigmentation, telangiectasias, calcinosis, dermal ulcers and digital gangrene are also common in SSc. The thickening of the skin starts distally and progresses proximally. Prevalent early symptoms are pruritus and skin pain. At first diffuse hyperpigmentation could be shown. Spotty hypopigmentation is called salt-and-pepper appearance. This is because the base of the hair follicles does not lose pigment. There are telangiectasias over the fingers, palms, dorsum of the hand and in the face. Telangiectasias do not lead to clinical problems, except they involve the GI-tract and cause significant blood loss. (Klippel, John H. MD et al 2007)

## Gastrointestinal System

The involvement of the GI system is common. Frequent heartburn, dysphagia, Barretts's esophagus (esophageal stricture formation mucosal dysplasia) gastritis, erosive esophagitis, postprandial bloating, weight loss, constipation, flatulence and malabsorptive diarrhoea are some of the symptoms. Muscle atrophy and fibrosis are important factors for dysmotility. (Klippel, John H. MD et al 2007)

SSc patients have reduced oral aperture caused by skin thickening. Mandibular atrophy and shortened frenulum are also found. Many SSc patients have abnormalities of the esophagus. Involvement of the esophagus can cause dysphasia. Gastroesophageal reflux and dyspepsia is the consequence of reduced lower esophageal sphincter pressure. More than one third of the patients suffer from Barrett's esophagus. The risk of esophageal cancer is increased. (Hochberg, Marc C. et al 2011)

Gastroparesis and small bowel dysmotility are related to early satiety, bloating and flatulence. Malabsorption and diarrhea are probably caused by bacterial growth in the small intestine. (Klippel, John H. MD et al 2007) While the liver usually is not involved in SSc, there are connections between primary biliary cirrhosis (PBC) and SSc. (Hochberg, Marc C. et al 2011)

## Pulmonary involvement

The most common cause of mortality among patients with SSc is pulmonary involvement. The lung diseases include pulmonary arterial hypertension (PAH), interstitial lung disease (ILD), aspiration pneumonia, pleural disease, obstructive airways disease, endobronchial telangiectasia with hemoptysis, malignancy and cryptogenic organizing pneumonia. Postmortem examinations or high resolution CT have shown that nearly 80% of patients with SSc have evidence of pulmonary fibrosis. In just 40% of the patients, one can find clinical evidence for pulmonary fibrosis. (Hochberg, Marc C. et al 2011) Many times, the early pulmonary involvement is asymptomatic. (Klippel, John H. MD et al 2007)

Clinical tests for lung disease include spirometry and diffusion capacity. Reduction of lung volumes and clinical symptoms should lead to the consideration of interstitial lung disease (ILD). Alveolitis may be the earliest stage of lung disease. Bronchoalveolar lavage or a high resolution CT (HRCT) will show evidence of alveolitis. HRCT is less invasive and commonly used. Patients with antitopoisomerase I (anti-Scl-70 antibodies) antibodies show more often pulmonary involvement. (Hochberg, Marc C. et al 2011)

There are no typical symptoms for early pulmonary arterial hypertension (PAH). For patients with suspected PAH an echocardiogram and a right heart catheterization is helpful. The right heart catheterization reveals an 8 -12% prevalence of PAH in SSc patients. Hypertension, older age, limited skin disease, and elevated echocardiographic pulmonary artery pressure are indicators for PAH. (Klippel, John H. MD et al 2007)

## Cardiac involvement

Postmortem examinations have found cardiac involvement in 80% of the patients with SSc, notably even in patients without clinical symptoms. Cardiac manifestations impair the prognosis. The most common symptoms for cardiac involvement in SSc are dizziness, chest pain, palpitations, and dyspnea on exertion. A late occurrence of SSc is a left or right ventricular systolic dysfunction. The pericardium could also be involved. Pericarditis or a pericardial effusion may present. Patients with transient palpitations should be examined for arrhythmias. (Hochberg, Marc C. et al 2011)

The histology shows myocardial contraction band necrosis typically for ischemia/reperfusion injury. (Klippel, John H. MD et al 2007)

## Scleroderma renal crisis

Scleroderma renal crisis (SCR) was the leading cause of mortality in patients with SSc before the use of angiotensin-converting enzyme (ACE) inhibitors. SCR now has a much better outcome. The outcome for patients with higher blood pressure is worse than in those

with lower blood pressure. Predictive factors for SCR are anti-RNA polymerase III antibodies, new-onset anemia, diffuse skin disease, tendon friction rubs, pericardial effusion (Hochberg, Marc C. et al 2011) and antecedent high dose corticosteroids (Klippel, John H. MD et al 2007). Approximately every 10<sup>th</sup> patient will have a SCR. (Hochberg, Marc C. et al 2011)

Other studies have shown that between 5 and 10 % of all SSc patients will develop a SCR: Nearly two thirds of patients with SCR need (transient) renal replacement therapy. The kidney function can improve up to 24 months after scleroderma renal crisis (Arad et al. 2011). With an aggressive antihypertensive treatment in case of a SCR the renal function improves and returns to normal or near normal function. The prognosis worsens in patients with creatinine level >3 mg/dL at the time of the diagnosis, delay in blood pressure normalization >3 days, of the male sex, older age and presence of congestive heart failure. (Klippel, John H. MD et al 2007)

## Joints and muscles

Arthralgia and myalgia are common and early symptoms in many SSc patients. Many patients with SSc suffer from musculoskeletal involvement like myopathy, myositis bone resorption, cutaneous calcifications, synovitis and compression neuropathies, (Klippel, John H. MD et al 2007) tendon friction rubs, generalized fatigue and muscle weakness caused by deconditioning and joint flexion contractures. Joint flexion contractures are often caused by progressive skin changes. (Hochberg, Marc C. et al 2011)

An osteolysis or bone resorption of the digital tuft can be found in 40 to 80% of the patients. Patients with SSc often show carpal tunnel syndrome. Ulnar neuropathy caused by thickened skin also occurs. Nearly every joint can be affected by contractures but the most common are the hands, the wrists and the elbows. An increased CK can be a sign for an inflammatory myopathy. An inflammatory myopathy also shows inflammatory changes on the muscle biopsy. (Hochberg, Marc C. et al 2011)

## Depression and sexual dysfunction

Depressions are common in patients with SSc. Approximately 50% suffer from mild depressive symptoms and nearly 17% have moderate or severe depression. (Hochberg, Marc C. et al 2011)

A typical symptom for SSc is sexual dysfunction. Both men and women are affected. Up to 80% of the patients suffer from sexual dysfunction. The beginning of the erectile dysfunction commonly occurs at 3 years after disease onset. Typical symptoms from women are vaginal dryness, dyspareunia, vaginal ulcerations, decreases in libido and orgasms. There is a connection between sexual dysfunction, skin tightness and gastroesophageal reflux in women. (Hochberg, Marc C. et al 2011)

## Diagnostic Autoantibodies

Several autoantibodies are found in SSc. Anti-centromere antibodies, anti-Scl 70 antibodies or/and anti-RNA polymerase III antibodies and antiU3-fibrillarlin are found in patients with SSc. (Bosello et al. 2011)

There are also antiendothelial cell antibodies, antifibrillin-1, anti-matrix metalloproteinase1 (interstitial collagenase) and 3 (stromelysin), and anti-platelet derived growth factor (PDGF) receptor antibodies. (Bosello et al. 2011)

### Overview of the common autoantibodies in scleroderma

Anti-nuclear antibodies	Anti-ribonuclear protein (U1-RNP)	Anti-Scl 70 antibodies (Anti-DNA-topoisomerase)	Anti-centromere antibodies
> 70%	15 %	30 – 70%	20-40%

Table 1(Böcker W. et al 2008)

Approximately 75% of SSc patients possess at least one of the antibodies alluded to above.(Leask 2010)

Antifibrillin-1 antibodies were found in 34-80% of SSc patients. These antibodies can cause an activation of fibroblasts. Profibrotic effects involving TGF- $\beta$ -mediated mechanism are discussed. (Manno, Boin 2010)

Antiendothelial cell antibodies are suspected to cause apoptosis in dermal microvascular endothelial cells together with the activated NK cells via the Fas pathway. The presence of these antibodies can lead to endothelial cell damage or cell death. Lunardi et al identified patients with SSc antibodies associated with the human cytomegalovirus (HCMV) late protein UL94. The group found that these antibodies could cause the death of endothelial cells in vitro.

Antibodies which initiate a reaction at the PDGF receptor were found. These antibodies are suspected to induce the production of collagen and reactive oxygen species. This reactive oxygen species could be also responsible for the inflammation of the vessels and the activation of the fibroblast. (Bosello et al. 2011)

## Etiology

The etiology of SSc is still unknown. Small studies have shown that infections, working conditions and ingestion of drugs maybe be a trigger factor for SSc. A bacterial infection as a trigger for SSc could not be detected so far. UL83 and UL94 protein epitopes of human cytomegalovirus (hCMV) were found in some patients with SSc. There is the possibility of a cross reaction between hCMV-derived proteins and anti-topoisomerase-I antibodies. The activation of fibroblasts and the induction of endothelial cell apoptosis in vitro could be explained by UL94. (Hochberg, Marc C. et al 2011)There are some assumptions about a contribution of human parvovirus B19 infection in some cases of SSc. (Ferri et al. 1999)

Epidemiologic investigations have never shown clusters of SSc. However there are well-documented clustered cases of SSc-like illnesses. One example is the toxic oil syndrome in Spain. There is a link to the ingestion of contaminated rapeseed cooking oils. Workers with the exposition of silica or similar substances have an increases incidence of SSc. Polyvinyl chloride; trichloroethylene and organic solvents are also suspected to trigger SSc. In literature surrounding the subject, one can find an association between exposure to pesticides, hair dyes and industrial fumes. (Hochberg, Marc C. et al 2011)

Epidemiologic surveys confirmed no higher risk for connective tissue diseases and silicone breast implants. Potentially associated drugs with SSc are bleomycin, penatzocine, taxol, and cocaine. The effect of genetic predisposition in SSc is rather unclear. A study with a small number of twin pairs (16 and 22, respectively) show that there is a higher rate in dizygotic twins (5,6%) than in monozygotic twins (4,2%).(Hochberg, Marc C. et al 2011)

Weak links with DRB1\*11 and DRB1\*0301 were known from earlier studies. Recently the genome-wide associations studies (GWAS) of a European-ancestry population involved nearly 3000 patients with SSc and over 5000 controls. It was shown that MHC, IRF5 and STAT4 are genetic risk factors for SSc. GWAS also detected IRF4, CDH1 and CD247. (Radstake et al. 2010)

CD247 could play an important role in the pathogenesis of SSc. The CD 247 locus encodes a T cell receptor subunit. This subunit could be important in the regulation of the immune response. HLA region, STAT4 and IRF5 have also a link to other autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. (Hochberg, Marc C. et al 2011)

## Fibrosis

Tissue fibrosis is one of the most important symptoms of scleroderma. Fibrosis in SSc is defined as an excessive extracellular matrix deposition in the skin and visceral organs. Fibroblasts cause a synthesis of extracellular matrix. The effect between the tissue fibrosis and autoimmunity is not yet clarified. (Hasegawa 2010)

There are different models to explain the fibrosis in SSc. One is the theory of an imbalance between T helper cell (Th) 1 and Th2. The chronically activated B cells could increase the

activation of the Th2 cells. This may cause an over expression of IL-4, IL-6, IL-10 and IL-13. TGF- $\beta$  and CTGF may also play an important role by causing the fibrosis. Th1 cells produce IFN- $\gamma$ , TNF- $\alpha$ , IL-2 and IL-12.

An interaction between TGF- $\beta$  and IL-4 was found in vitro. TGF- $\beta$  and IL-4 are cofactors in the regulation of collagen gene expression in vitro. TGF- $\beta$  can boost the increase of antigen-specific Th2 cells. TGF- $\beta$  and IL-4 cause a higher CTGF gene expression in skin and lung fibroblasts. (Hasegawa 2010)

Activated B cells are also able to produce TGF- $\beta$  in vitro. There are many activated B cells found in patients with SSc. The higher levels of activated B cells and the increased TGF- $\beta$  might be associated with the excessive collagen synthesis by fibroblasts in SSc. An interaction between TGF- $\beta$  and other cytokines for example CTGF is very likely. (Hasegawa 2010)

## IGF-1

IGF-1 (insulin-like growth factor 1) is mostly synthesized in the liver. *Insulin, like growth factor -1 (IGF-1), is a single chain polypeptide consisting of 70 amino acids and it shares an approximate 50 % homology with insulin* (Guevara-Aguirre 1996).

Among many other cells, fibroblasts produce this growth factor. IGF-1 receptor (IGF-1R) plays an important role in the function of IGF-1. Up to 90% of IGF-1 is bound to IGFBP-3 in a ternary complex. (Hamaguchi et al. 2008)

Growth factors and cytokines like tumor necrosis factor  $\alpha$ , interferon  $\gamma$  and colony stimulating factors enhance the synthesis of IGF-1. (Fawzi et al. 2008)

IGF-1 is an important factor during development. The activity of IGF-1 and IGF-2 range from endocrine, up to paracrine and also up to autocrine. Already in the eight-cell stage one can find receptors for IGF-1 and insulin. During the pre-implantation period both IGF-1 and IGF-2 play an important role. Later IGF-1 is predominant. It seems that IGF-1 is necessary for the development of the early nervous system. (Guevara-Aguirre 1996)

IGF-1 has an effect on immune cells like T cells, B cells and natural killer cells. (Hamaguchi et al. 2008)

IGF-1 stimulates the proliferation of chondrocytes, it also raises the production of proteoglycan, glycosaminoglycan and collagen. IGF-1 plays an important role for the growth of thymic epithelial cells in vitro. (Fawzi et al. 2008)

IGF-1 is an inducer of an immediate early genetic response in fibroblasts. (Harrington et al. 1994)

A study with 13 SSc patients controlled the IGF-1 serum level. The IGF-1 serum level was normal and there was no connection between IGF-1 and clinical features. However, in another study significant elevated serum IGF-1 and IGFBP-3 in patients with SSc were found. This study also shows an increased IGF-1 level in patients with dcSSc compared with lcSSc patients. Patients with higher IGF-1 levels more likely have pulmonary fibrosis. This study also showed no correlation between IGF-1 levels and IGFBP-3 levels. It seems that they are independently involved in SSc. The authors found no correlation between higher IGFBP-3 level and the duration of the disease, gender and Rodnan TSS (modified Rodnan skin score) in this study. (Hamaguchi et al. 2008)

IGF-1 serum levels were claimed to be associated to the severity of the pulmonary fibrosis and skin thickness. IGF-1, not bound to IGFBP-3, is suspected to cause fibrosis. This is only a hypothesis because total IGF-1 was measured and not free IGF-1. Patients with higher IGF-1 levels have a similar Rodnan TSS. More patients with dcSSc showed an elevated IGF-1 serum level compared to patients with skin sclerosis and pulmonary fibrosis in SSc. In the skin of healthy patients the IGF-1 mRNA is much lower than in patients with SSc. IGF-1 and IGFBP-3 are playing an important role in patients with SSc but this role seems to be different. (Hamaguchi et al. 2008)

Another study with 15 patients and a control group of nine healthy people showed that IGF-1 is found in patients with circumscribed plaques. The tissue level of IGF-1 in the skin biopsy of the lesional skin was significantly higher than the skin biopsy of the non-lesional skin and the control skin biopsy. There were no meaningful differences between the non-lesional skin biopsy and the control group. The study showed a correlation between the IGF-1 tissue level and the Rodnan score. Although there was no correlation between the

IGF-1 serum level in the patient group and the Rodnan score, IGF-1 was seen as a relevant factor in the pathogenesis of fibrosis and the characteristic of morphea. (Fawzi et al. 2008)

Krein and Winston reported that a higher IGF-1 level led to fibrotic lung disease like idiopathic pulmonary fibrosis, while another study demonstrated a dysregulation of IGF-1 in patients with oral submucous fibrosis. (Fawzi et al. 2008)

## B cells

B cells belong to the group of lymphocytes. They play an important role in the immune system and (Murphy, Travers & Walport 2008) also in systemic autoimmunity diseases. The functions of B cells are multifaceted. It includes antibody production, co-stimulation of T cells, antigen presentations, dendritic cell regulation, lymphoid organogenesis and production of cytokines. (Hasegawa 2010)

## Development and regulation

The development of B cells occurs in the fetal liver and the bone marrow.(Male, David K. et al 2007) It starts with pro B cells then pre B finally maturing at the end of the development they develop into memory and plasma cells. The expression of CD19, CD20 and CD22 and the BAFF receptor are in direct connection with this development. (Hasegawa 2010) More than 75% of the B cells must die at the pre-B cell stage. The reasons are non-productive rearrangements of immunoglobulin genes or the illicit expression of autoantibodies. (Male, David K. et al 2007)

B cells are able to proliferate and differentiate into plasma cells and some of them differentiate into memory cells. The precondition for this transformation is a contact and binding with an antigen. After this activation the B cells start the transformation into plasma cells. Plasma cells produce antibodies. (Murphy, Travers & Walport 2008)

B cells also are antigen-presenting cells. That means specific antigens are processed and presented by B cells. (Murphy, Travers & Walport 2008)

The activation of B cells is a complex reaction. After the connection with an antigen a kinase cascade and translocation of nuclear transcription factors starts. There are two options of activation. An antigen becomes cross-linked with membrane immunoglobulin or an antigen is cross-linked with a membrane immunoglobulin and the co-receptor complex. The co-receptor complex consists of CD21, CD19 and CD 81. Cytokines are important for the activation, division and differentiation of B cells. After the activation of B cells antigen presenting cells (APCs) secrete IL-1. Important is the direct connection of B cells with TH2 cells after the presenting of antigens. TH2 cells influence the division and differentiation of B cells with IL-4, IL-5, IL-6, IL-10 and IL-13. Th1 cells are also involved by secreting IFN $\gamma$ . (Male, David K. et al 2007)

## B cells and systemic sclerosis

Daoussis, et. al. showed that B cells of patient with SSc have a 20% higher expression of CD19 than healthy controls. These patients also have a higher level of peripheral blood B cells. In particular, the level of native B cells is higher. Patients with SSc show a lower level of memory B cells and plasmablasts. The memory B cells also imitate more often an apoptosis. (Daoussis et al. 2011)

B cell infiltration is common in the involved skin and in the lungs of patients with SSc and interstitial lung disease. An over-expression of CD19 and a polymorphism of the CD19 promoter region were found in SSc patients. This could be an explanation for the chronical activation of memory B cells in vivo in SSc patients (Hasegawa 2010). At the moment it is not exactly clear why patients with SSc have an “over-activated” phenotype of B cells. Maybe B cell survival factors are an important key. (Daoussis et al. 2011)

BAFF, also known as BLys, a member of the tumor necrosis factor family may play an important role in the abnormalities of B cells in SSc. A positive correlation was found between BAFF serum levels and the severity of SSc. (Hasegawa 2010)

Daoussis and colleagues found a higher serum level of BAFF in patients with SSc compared to health groups. Patients with diffuse SSc showed higher levels than patients with limited forms. An increased BAFF serum level could also be found in patients with other diseases such as SLE or DM. (Daoussis et al. 2011)

BAFF is an important activation factor for B cells. Its deregulation may cause an imbalance in cytokine production and is a contributing factor for autoimmunity. The focus is on IL-4, IL-6 and IL-13. These cytokines have a positive effect on the synthesis of collagen by human fibroblasts. (Bosello et al. 2011)

There is a correlation between B cells, BAFF stimulation and an increased production of IL-6 in patients with SSc. (Daoussis et al. 2011)

Not only B cells but also macrophages and stroma cells have the ability to produce IL-6. (Daoussis et al. 2011, Van Laar 2010, Van Laar 2010)

In B cells of SSc patient one can also find over-expression of CD23, CD80 and CD86. The increased production of IL-6 is accompanied by production of glycoaminoglycans by fibroblasts and antibody production. TGF- $\beta$  causes fibroblast activation and an extracellular matrix (ECM) deposition. Hyper- $\gamma$ -globulinemia, antinuclear antibodies and antitopoisomerase are produced by B cells in patients with SSc. (Bosello et al. 2011)

Thus B lymphocytes appear as an attractive therapeutic target in SSc. (Leask 2010)

## Mouse Model

A mouse model called TSK has shown an important connection between B cells and the development of fibrosis. In these mice one can find an increased synthesis of collagen and other extracellular matrix (ECM) proteins in the skin. The mice present pulmonary emphysema and cardiac hypertrophy similar to patients with SSc. Hyper- $\gamma$ -globulinemia and the antibodies against topoisomerase I (scl70), fibrillin 1, RNA-polymerase I and others were detected in TSK. They found an increased CD19 signaling in patients with SSc, there was no over-expression of CD19 in the mice. (Bosello et al. 2011)

The TSK mouse model may show us a connection between autoimmunity and fibrosis. Both patients with SSc and TSK + mice have chronically activated B cells in vivo. The reason may be an augmented B cell signaling. This B cell signaling can cause autoantibody production and a higher level of cytokines for example IL-2 and TGF- $\beta$ . Activated B cells may have a direct or indirect effect on T cells. The theory shows that especially Th2 cells are influenced and react with an increased cytokine production. The higher production of Th2 cytokines can be a reason for the tissue fibrosis. The deregulation of the B cells maybe is the missing link between the autoimmunity and the tissue fibrosis in patients with SSc. (Hasegawa 2010)

## B cells and their possible targets for therapy

In the recent years B cells moved more and more into the focus of therapy research. There are three main therapeutic strategies in the clinical trials. Blocking B cell activation pathways may reduce autoimmunity. There are two ways: blockade of the intrinsic or extrinsic regulation. The extrinsic regulation can be blocked in many different ways such as Anti-CD22, Anti-BAFF, Anti-APRIL, Anti-TNF; Anti-IL-6R, and/or blockade of co-stimulation. (Hasegawa 2010)

The intrinsic regulation blockade consists of a blockade of signaling and/or proteasome inhibition. A third possibility is the cell depletion. At the moment research are running for Anti-CD19, Anti-CD52 and Anti-CD20. Anti-CD20 Antibodies such as Rituximab come more and more into the main focus of the scientists. The treatment for SSc maybe change completely in the next years: B cell related therapy is a promising option for patients with SSc. (Hasegawa 2010)

# Treatment

## Biologic therapy for SSc

Biologic drugs are more and more common in the therapy of the different rheumatic disease. Many biologic agents were used experimentally in the treatment in patients with SSc such as antithymocyte globulin (ATG), imatinib mesylate, interferon- $\gamma$  (IFN- $\gamma$ ), IFN- $\alpha$ , recombinant human relaxin, delipidated, deglycolipidated Mycobacterium vaccae (PVAC), recombinant human anti-transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) antibody, type I collagen and rituximab. (Phumethum, Jamal & Johnson 2011)

There also was a trial with Anti-TNF therapy. SSc patients with Anti-TNF therapy had a decreased inflammation of the synovia and clearly reduced joint symptoms. (Phumethum, Jamal & Johnson 2011)

The trial with 5 mg/kg Infliximab given in patient with diffuse SSC in week 0, 2, 6, 14 and 22 showed an improvement of >10 % in skin score or an increase of 4 skin score units over a period of 3 months. (Phumethum, Jamal & Johnson 2011)

A randomized trial of IFN- $\alpha$  in 35 patients with diffuse SSc showed a better improvement of the skin score in the placebo group. Another randomized trial with relaxin had a better skin score but no effect on joint score or HAQ-DI. A randomized study with 168 patients with SSc and oral bovine type I collagen showed no difference in the change of MRSS after 12 months. (Phumethum, Jamal & Johnson 2011)

Other biological agents such as IFN- $\alpha$ , TGF- $\beta$ 1 or oral type I collagen did not show great promise for the treatment of SSc. Larger longitudinal studies are needed to find out more about biological agents and the treatment of SSc. (Phumethum, Jamal & Johnson 2011)

## Nonselective immunotherapy

The first line therapy for the treatment of autoimmune diseases such as systemic lupus erythematosus and similar disease is a nonselective immunotherapy. This general immunosuppressant in SSc should be helpful for the skin fibrosis, the inflammatory, the specific changes in the organs and the lung involvement such as ILD.

The “gold standard treatment” for Systemic sclerosis-associated Intestinal Lung Disease (SSc with ILD) is Cyclophosphamide (CYC). The anti-inflammatory function of CYC plays an important role in the treatment of SSc. CYC induces a reduction of the T and B cells caused by a direct cytotoxicity of bone marrow precursors and mature lymphocytes. In the Scleroderma Lung Study (SLS) patients treated with CYC showed a significant improvement (2,53%) of the forced vital capacity (FVC). Another study found out that patients with diffuse SSc and the treatment with CYC had a decreased modified Rodnan’s skin score compared to the placebo group. (Manno, Boin 2010)

However a relevant group of patients have no effect after the treatment with Cyclophosphamide. Some case reports showed that Rituximab (RTX) could play an important role for the treatment of SSc with ILD. There is a randomized controlled trial with 15 women suffering from SSc associated ILD. The criteria of this study were 1. Forced vital capacity (FVC) < 80% 2. Proven interstitial lung disease (with high-resolution computer tomography) and 3. Criterion of exclusion: other cause of ILD. The results of this study brought no evidence for a better response rate between the group with Cyclophosphamide monotherapy (83%) and the group with RTX and CYC (78%). (Tejeda-Andrade et al. 2011)

Mycophenolate mofetil (MMF) is an alternative nonselective immunotherapeutic agent. MMF affects the de novo syntheses of purines with the inhibition of inosine 5 monophosphate dehydrogenase (IMPDH). One reason for the use of MMF in rheumatic disease is the beneficial side-effect profile. Small retrospective studies showed an improvement of the vital capacity by 4,2% in patients treated with MMF for six or more months. Patients with a 12 months MMF therapy also had a stabilization of the FVC.

The important role of MMF in lung fibrosis and a reduction of skin fibrosis were demonstrated in other trials. (Manno, Boin 2010)

Case studies have shown that a treatment with MMF is able to stabilize the pulmonary function. Ongoing studies want to compare patients with Cyclophosphamide therapy and MMF treatment. (Ong, Denton 2010)

Another noteworthy drug is Azathioprine (AZA). AZA has active metabolites (6-mercaptopurine and 6-thioinosinic acid) which affect the purine synthesis and inhibit the proliferation of inflammatory cells specially mentioned T and B cells. A small study demonstrated a stabilization of the pulmonary function. However another trial did not verified the results. Although Paone, et. al. found out that there is no impairment in pulmonary function or the skin score in 13 patients with early dcSSc who got AZA for a year after a one-year treatment with intravenous CYC, patients treated for 6 months with CYC, and then predominantly treated with AZA for 18 months, showed a stable or increased pulmonary function. (Manno, Boin 2010)

Methotrexate (MTX), an antimetabolite agent, is also playing an important role in the nonselective immunotherapeutic treatment of SSc. With the inhibitory effect of the dihydrofolate reductase MTX induce a decreasing of the DNA and nucleotide synthesis. MTX often is used for the therapy of inflammatory autoimmune disease. The immunomodulatory effects of MTX reach from reducing of proinflammatory cytokines to the release of extracellular adenosine and impairment of an antigen-induced T cell activation. (Manno, Boin 2010)

MTX is used for SSc patients with arthritis and myositis. A study showed an improvement of the skin score in patients with a weekly treatment of MTX (15mg) compared to placebo group after the duration of 24 weeks, although this is not statistically significant. In this trial the number of patients was to low and also lcSSc were included. The other randomized study just found a small non-significant change in skin score between patients treated with MTX and the control group after a 12-month oral therapy. (Manno, Boin 2010)

## T cell targeted therapy

One important T cell targeted drug for patients with SSc is cyclosporine A (CsA). There is an antifibrotic effect and an inhibition of the main transcription factor for IL-2. IL-2 is produced by T cells. A 48-week open-label study with CsA showed a significant reduction of the skin score. A mentionable side effect is a raise of the serum creatinine in 4 of 5 patients at this study. A 12-month randomized trial confirmed the decrease of the skin score and demonstrated also a drop of the IL-6 levels. Many studies show the improvement of the skin score but the most common side effects of CsA are hypertension and renal toxicity indicated by high creatinine levels. (Manno, Boin 2010)

Sirolimus (rapamycin) is another possible agent for the therapy in SSc. Sirolimus effects the collagen production of dermal fibroblasts. A 48-week single-blind randomized Phase I study showed no significant improvement of the disease activity score between MTX and Sirolimus therapy in patients with early dcSSc. (Manno, Boin 2010)

Other possible agents for the treatment of SSc are antithymocyte globulin (ATG) Basiliximab, Abatacept, the small molecule Halofuginone and Alefacept. Smaller pilot studies demonstrated possible positive effects in the treatment of rheumatic disease with these drugs. (Manno, Boin 2010)

Basiliximab is a chimeric mAb and reacts with CD25 (IL-2 receptor). There are studies for patients with early dcSSc and a combined therapy of iv Cyclophosphamide, oral prednisolone and Basiliximab for 6 months. The patients got Basiliximab infusions first twice a week, and later, monthly. This treatment showed a decreased skin score and a possible positive effect in cardiac and pulmonary involvement. (Ong, Denton 2010)

Rodriguez-Reyna, et. al. found higher Th17 subpopulation levels in patients with SSc. There is evidence for a selective inhibition of Th17 caused by a treatment with Halofuginone. Halofuginone react with TGF- $\beta$  and demonstrated a reductive effect at the collagen production by fibroblasts. (Ong, Denton 2010)

## Hematopoietic stem cell transplantation (HSCT)

The European Group for Blood and Marrow Transplantation and the European League Against Rheumatism (EBMT/EULAR) collected data from 37 patients with SSc. These patients got a high-dose CYC therapy and later on autologous hematopoietic stem cell transplantation. 69% of the patients showed a decrease of the skin thickness (> 25% from baseline or 10% from maximum recorded). That enhancement was already significant after 30 days. The pulmonary function was constant and no aggravation of the other organs was reported, although there was a 17% mortality rate. Stricter exclusion criteria from the EBMT/EULAR were established due to this high mortality rate. (Binks et al. 2001)

Another report with 25 SSc patients showed a decrease in the mortality rate 8,7 % after BMT. 92% of the patients in this study had a prompt improvement of the skin score. Similar studies in the USA reported from a treatment-related mortality of 16%. These patients were also treated with total body radiation, CYC and ATG. These studies reported a 2-year survival rate of 78,9 %. Further multicenter, prospective and randomized controlled studies will give us more information for the decision high-dose immunosuppressive therapy and HSCT or just monthly CYC. Also more information about toxicity and effectively are needed. (Farge et al. 2004)

## Other experimental therapies

A small study of 7 SSc patients who were treated by intravenous immunoglobulin (IVIG) for 6 months showed an improvement in inflammatory and fibrosis. IVIG was used for other fibrotic disorders such as scleromyxedema. IVIG used in animal models had an antifibrotic effect. Patients with SSc from another study had an improvement of the skin score under the treatment with IVIG. The exact effect of IVIG is not yet clear. IVIG could determine a neutralization of antibodies and proinflammatory cytokines. IVIG may also have a modulating effect of the immune responses. (Nacci et al. 2007)

# Rituximab

Rituximab is a chimeric monoclonal antibody against human CD 20. (Bosello et al 2011) Hasegawa found out that there are promising results with Rituximab for the treatment for non-Hodgkin's lymphomas and autoimmune disease such as lupus nephritis, multiple sclerosis, thrombocytopenic purpura, as well as rheumatoid arthritis. (Hasegawa 2010) Rituximab induces the apoptosis of B cells and causes cellular cytotoxicity. (Manno, Boin 2010)

In an open label clinical and histopathological study with Rituximab, was shown that there was a significant change in skin score and also improvements in dermal hyalinized collagen content and dermal myofibroblast numbers in diffuse cutaneous systemic sclerosis. (Smith et al. 2010)

Rituximab in SSc is tolerated well compared to other therapies like the “gold standard” cyclophosphamide, imatinib, immunoablatives therapies or autologous stem cell transplantation. (Van Laar 2010)

Only a few events during the treatment with RTX were reported. In 3 out of 4 studies a treatment with RTX has shown an improvement of clinical skin thickening.

The study without clinical improvement shows histologic improvement. RTX plays an important role in skin fibrosis but so far there is no clinical evidence for an improvement in Patient with SSc-associated ILD. (Daoussis et al. 2011)

An open label and randomized controlled study with fourteen patients with SSc and SSc with ILD with eight patients grouped RTX and six patients in the control group were reported. The RTX group received two cycles of RTX at baseline and 24 weeks. The control group maintained standard treatment. At the 24 week evaluation the RTX group showed better results of lung function tests. The RTX group also had a better FVC (forced expiratory vital capacity) at the 1 year check. RTX may also have an effect of the reduction of collagen deposit in the papillary dermis, although there is no difference of collagen deposit in the reticular dermis between the RTX group and the control group. This study showed that B cells may play an important role in SSc and RTX may improve pulmonary function. (Daoussis et al. 2010)

RTX could be an alternative treatment for patients with diffuse cutaneous sclerosis nonresponsive to the gold standard medication Cyclophosphamide. RTX therapy shows decreasing of the skin fibrosis in patients with early onset disease. Patients with RTX therapy had lower levels of circulating B cells, BAFF and IL-6. Also the clinical conditions of the patients improved. (Van Laar 2010)

Another open label clinical and histopathological study included patients with dsSSc. Patients in this study got RTX (1000 mg) together with methylprednisolone (100mg) at day 1 and day 15. At the baseline and week 12, skin biopsies were taken. The hyalinised collagen content, the myofibroblast score and the lymphocyte numbers (CD20) decreased under the treatment with RTX. (Smith et al. 2010)

All these predominantly positive results indicate the necessity for research for the treatment of Rituximab in SSc. Multicenter studies will give us more information for the efficacy and safety of Rituximab. (Hasegawa 2010)

Not all studies are encouraging: Lafyatis, et. al. published a conflicting study. 15 patients with dsSSc showed no evidence of a significant skin improvement after 6 months. (Lafyatis et al. 2009)

## Rituximab in mouse models of scleroderma

A treatment with RTX in newborn TSK mice showed a 43% decreased skin fibrosis compared to the control mice. The newborn TSK mice treated with RTX also have a lower level of antibodies. But they were not able to found such improvement in 56-day-old mice with the same RTX treatment. The conclusion: RTX may play a more important role for skin fibrosis in the onset of SSc. (Daoussis et al. 2011)

## Hypothesis of this project

Systemic sclerosis seems to be caused by higher activity of fibroblasts. Fibroblasts are regulated by many different soluble proteins and growth factors. One of these growth factors is IGF-1. (Fawzi et al. 2008)

IGF-1 seems to play an important role in connective tissue diseases like lupus erythematosus, rheumatoid arthritis and fibrotic lung disease (Harrington et al. 1994)

Higher IGF-1 levels in lesional skin were found by Fawzi et al. But not only the IGF-1 levels in lesional skin are increased also IGF-1 serum levels were higher in patients with lesional skin than in control serum. IGF-1 serum levels seem to play an important role in the development of fibrosis and in the pathology of systemic sclerosis. (Fawzi et al. 2008)

Therefore we decided to determine the IGF-1 serum levels using ELISA.

The higher IGF-1 levels in serum and lesional skin could be an indicator for the activity of the disease. Patients with pulmonary involvement should have an increased serum level compared to patients with limited forms. The IGF-1 level of healthy persons compared to SSc patients should be decreased. There could be a reduction of the high IGF-1 level in the serum of SSc patients under sufficient therapy.

A part of our SSc patients is treated with Rituximab (RTX). For the IGF-1 ELISA we used the sera of patients before the treatment of Rituximab (Mabthera®) and afterward. Patients with an effective treatment should also have an improvement in their quality of life. It should be mentioned that 2 of the SSc patients also got 2,5 mg prednisone per day.

We measured their quality of life with the SF-36 questionnaire.

## Methods

### ELISA

*Enzyme-linked immunosorbent assay (ELISA) is a direct binding assay for the antibody (or antigen). ELISA assays are frequently used in viral diagnostics, for example in detecting cases of infection with human immunodeficiency virus (HIV), which is the cause of AIDS. For both these methods, one needs a pure preparation of a known antigen or antibody, or*

*both, in order to standardize the assay. For ELISA an enzyme is linked chemically to the antibody. The unlabeled component, which in this case would be antigen, is attached to a solid support, such as the wells of plastic multiwell plate, which will adsorb a certain amount of any protein.*

*The labeled antibody is allowed to bind to the unlabeled antigen, in conditions under which nonspecific adsorption is blocked, and any unbound antibody and other proteins are washed away. In ELISA the binding is detected by a reaction that converts a colorless substrate into a colored reaction product. The color change can be read directly in the reaction tray, making data collection very easy, and ELISA also avoids the hazards of radioactivity. This makes ELISA the preferred method the most direct-binding assays. (Murphy, Travers & Walport 2008)*

## IGF-1-ELISA

### General Information of the IGF-1 ELISA

AMP IGF-I-ELISA for 96 analysis from AMP (Asbach Medical Products GmbH)

Catalogue number: AMP 110-E38100

### Analysis

#### Standard curve

Blank value should not be higher than 0,25 optical unit and standard E should be higher than 1 unit.

Standard A: 2 ng/ml - 0,26 nmol/L

Standard B: 5 ng/ml – 0,66 nmol/L

Standard C: 15 ng/ml – 1,96 nmol/L

Standard D: 30 ng/ml – 3,93 nmol/L

Standard E: 50 ng/ml – 6,54 nmol/L

conversion factor: ng/mL – nmol/L:0,13074

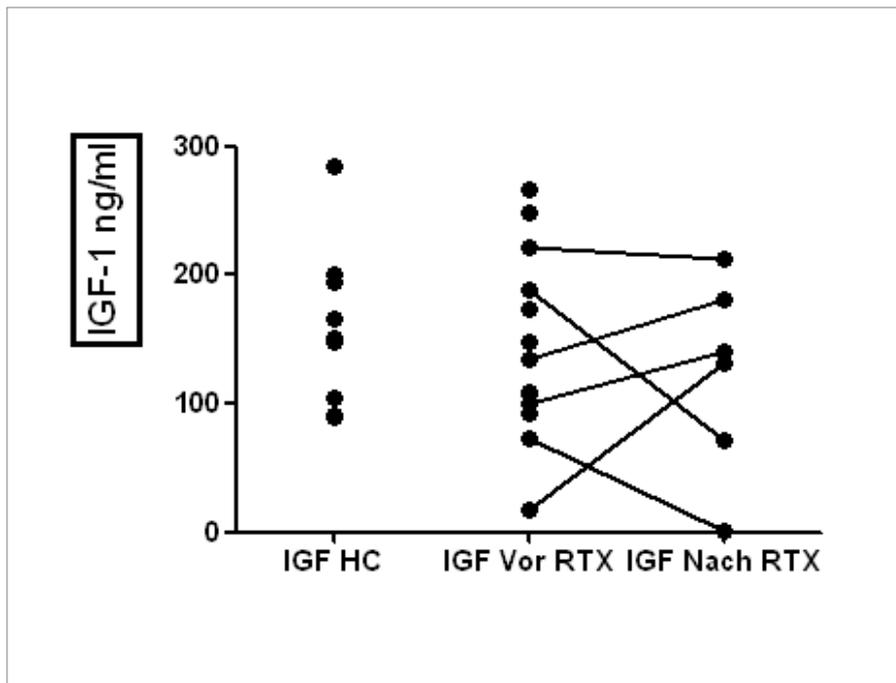
(Asbach Medical Product Katalognummer: AMP 110-E38100)

## Results

The IGF-1 ELISA was used on 26 blood sera samples from women. We included 7 samples of a healthy control group, 7 samples of patients with SSc without a treatment with RTX, and 6 paired samples before and after a treatment with RTX. The average age of the control group was  $42,3 \pm 6,2$ . The average age of the group without/before a treatment with RTX was  $57,1 \pm 12,5$  and the group after a treatment with RTX had an average age of  $54,0 \pm 12,6$ .

The IGF-1 level of our healthy control group in the blood sera was  $159,0 \pm 62,5$ . (mean/SD) There was no significant difference in comparison with the IGF-1 level of the SSc patients:  $169,3 \pm 66,0$ .

### Image 1



The study showed a change between the IGF-1 level of SSc patients before RTX treatment  $169,3 \pm 66,0$  and the IGF-1 level from SSc patients after a RTX treatment  $123,0 \pm 76,7$ . This change was not significant. (paired t-test).

## SSc Activity and SSc Severity

We obtained the SSc Activity and SSc Severity Score from 5 patients before and after the treatment with RTX. The 5 patients had an average SSc Activity Score of  $3,5 \pm 1,0$  before treatment and an average SSc Severity Score of  $7,8 \pm 4,1$  before their treatment with RTX. After their treatment with RTX, the Activity Score dwindled down to  $2,1 \pm 1,0$  and the SSc Severity Score dropped down to  $5,4 \pm 4,2$ . 4 of 5 patients had an improved Severity Score, and one patient's Score value was constant. The Activity Score from 4 patients dropped. There was just one patient with a slight deterioration in the Activity Score. It should be mentioned that this was not the same patient with the constant Severity Score.

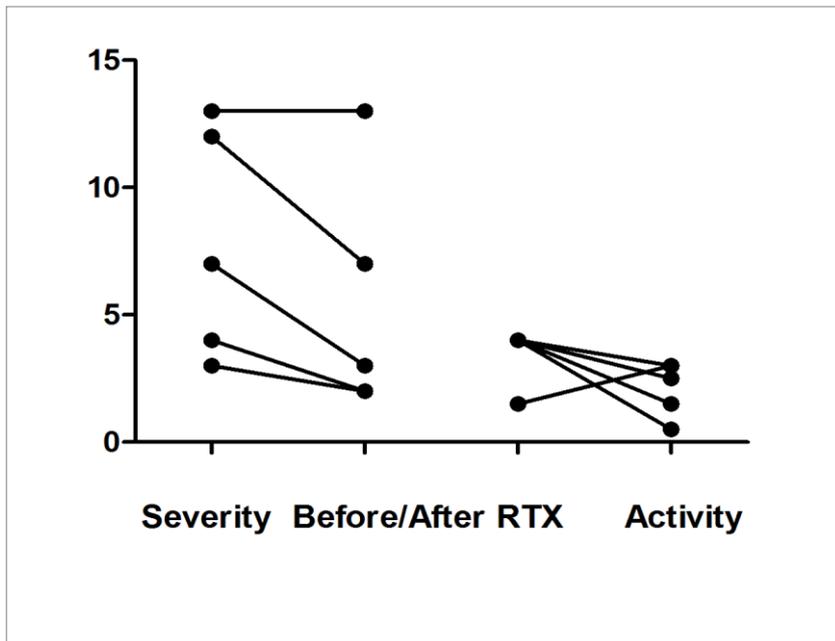


Image 2

## SF-36

### General information about the SF-36

The SF-36 is a questionnaire developed to evaluate the quality of life for patients with SSc. Cossutta, et. al. found a correlation between the SF-36 and the skin involvement. The SF-36 is able to document not only the quality of life also the disability of the patients. (Cossutta et al. 2002)

The SF-36 or Short Form 36 Health Survey is also used for patients with sarcoidosis and many other diseases to measure the quality of life. (Bourbonnais et al. 2012)

## Our survey with the SF-36

Four patients with scleroderma and a therapy with Rituximab answered the SF-36 two times. The answers of the first SF-36 were before their treatment with Rituximab and the second SF-36 after the therapy. The patients got the SF-36 by mail and brought it back to the scleroderma ambulance or send it back by mail.

The SF-36 consists of 8 domains. The questions cover Mental Health (MH), General Health (GH), Role-Emotional (RE), Social Functioning (SF), Vitality (VT), Physical Functioning (PF), Role-Physical (RP) and Bodily pain (BP). We compared the raw values in a pairwise manner.

## Results

The results of the SF-36 suggest an improvement of the nearly all raw data. The mental and the general health both increase. The patients have higher values in social and physical functioning. Their vitality is better and their physical and emotional well-being rise. There is only a slight deterioration in the bodily pain.

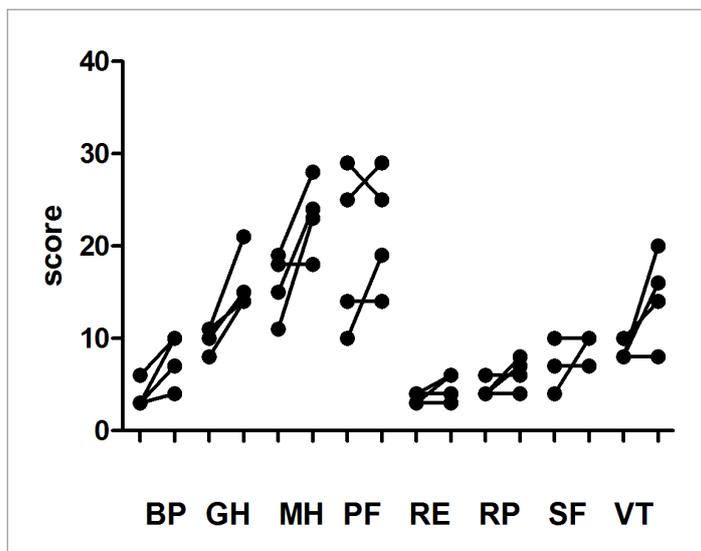


Image 3

## Discussion

In 2008, Hamaguchi reported that patients with SSc, and especially patients with dcSSc, have a higher serum level of IGF-1 compared to patients with systemic lupus erythematosus and a healthy control group (Hamaguchi et al. 2008).

Our results of the IGF-1 ELISA showed there is no significant difference between the IGF-1 serum level of patients with SSc and the healthy control group. The gender in both groups is the same and the age is rather concordant. The expectation of a significant higher or lower serum level of IGF-1 in patients with SSc in this study was rejected, although it should not be forgotten that this study includes only 13 patients with SSc. A following study with more patients would be necessary.

So far the results of the ELISA showed that the IGF-1 serum level is not attractive as biomarker for the effectivity of scleroderma therapies.

In an open label clinical and histopathological study was shown that patients treated with RTX have a significant change in skin score (Smith et al. 2010)

In our small observation it seems that RTX also improves the SSc Activity and SSc Severity Score. 3 of 5 patients had an improvement of the SSc Activity and SSc Severity Score. One patient had an improvement of the SSc Activity and a constant Severity Score. Just one patient had an impairment of the SSc Activity Score but also an improvement of the SSc Severity Score. These results show that RTX seems to have a positive effect for patients SSc Severity, although it should be considered that this study included merely 5 patients. For obtaining statistical significance, a following study with more patients is recommended to confirm the results.

Nearly all results of the SF-36 show an enhancement in the quality of life for the SSc patients. The mental and general health is better than before the therapy. The vitality, their physical and emotional well-being rise. There could be a connection between the quality of life and the Rituximab therapy. It should be mentioned that the SF-36 was evaluated in just 4 patients. For a better significance a study with more patients is recommended.

A connection between IGF-1 and SSc especially the Activity and Severity of SSc was expected. But the IGF-1 serum level seems to play no big role as a biomarker for the effectiveness for scleroderma therapies. Bigger multicenter studies are expected to follow. Our conclusion is that the role of IGF-1 serum level is not as big as expected.

## Literaturverzeichnis

- (1) Arad U, Balbir-Gurman A, Doenyas-Barak K, Amit-Vazina M, Caspi D, Elkayam O. Anti-neutrophil antibody associated vasculitis in systemic sclerosis. *Semin Arthritis Rheum* 2011 Oct;41(2):223-229.
- (2) Asbach Medical Product editor. Arbeitsanleitung IGF-I ELISA  
Enzymimmunoassay zur quantitativen Bestimmung von Insulin-like growth factor I.  
Obrigheim: Asbach Medical Product (AMP); Katalognummer: AMP 110-E38100.
- (3) Binks M, Passweg JR, Furst D, et al. Phase I/II trial of autologous stem cell transplantation in systemic sclerosis: procedure related mortality and impact on skin disease. *Ann. Rheum. Dis.* 2001;60(6):577–584.
- (4) Böcker W. et al. Pathologie.  
Elsevier, Urban und Fischer;4. Auflage 2008. Chapter 47, Page 1151.
- (5) Bosello S, De Luca G, Tolusso B, Lama G, Angelucci C, Sica G, et al. B cells in systemic sclerosis: a possible target for therapy. *Autoimmun Rev* 2011 Aug;10(10):624-630.
- (6) Bourbonnais JM, Subramanian M, Dalal BD, Samarakoon PC, Parikh SR, Samavati L. Distance Saturation Product Predicts Health -Related Quality of Life Among Sarcoidosis Patients. *Health Qual Life Outcomes* 2012 Jun 13;10(1):67.
- (7) Clements PJ, Wong WK, Hurwitz EL, Furst DE, Mayes M, White B, et al. The Disability Index of the Health Assessment Questionnaire is a predictor and correlate of outcome in the high-dose versus low-dose penicillamine in systemic sclerosis trial. *Arthritis & Rheumatism* 2001;44(3):653-661.
- (8) Cossutta R, Zeni S, Soldi A, Colombelli P, Belotti Masserini A, Fantini F. Evaluation of quality of life in patients with systemic sclerosis by administering the SF-36 questionnaire. *Reumatismo* 2002 Apr-Jun;54(2):122-127.
- (9) Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzi A, Sirinian C, et al. Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. *Rheumatology (Oxford)* 2010 Feb;49(2):271-280.
- (10) Daoussis D, Liossis SN, Yiannopoulos G, Andonopoulos AP. B-cell depletion therapy in systemic sclerosis: experimental rationale and update on clinical evidence. *Int J Rheumatol* 2011;2011:214013.
- (11) Farge D, Passweg J, van Laar JM, et al. Autologous stem cell transplantation in the treatment of systemic sclerosis: report from the EBMT/EULAR Registry. *Ann. Rheum. Dis.* 2004;63(8):974–981.

- (12) Fawzi MM, Tawfik SO, Eissa AM, El-Komy MH, Abdel-Halim MR, Shaker OG. Expression of insulin-like growth factor-I in lesional and non-lesional skin of patients with morphea. *Br J Dermatol* 2008 Jul;159(1):86-90.
- (13) Ferri C, Zakrzewska K, Longombardo G, Giuggioli D, Storino FA, Pasero G, et al. Parvovirus B19 infection of bone marrow in systemic sclerosis patients. *Clin Exp Rheumatol* 1999 Nov-Dec;17(6):718-720.
- (14) Guevara-Aguirre J. Insulin-like growth factor I--an important intrauterine growth factor. *N Engl J Med* 1996 Oct 31;335(18):1389-1391.
- (15) Hamaguchi Y, Fujimoto M, Matsushita T, Hasegawa M, Takehara K, Sato S. Elevated serum insulin-like growth factor (IGF-1) and IGF binding protein-3 levels in patients with systemic sclerosis: possible role in development of fibrosis. *J Rheumatol* 2008 Dec;35(12):2363-2371.
- (16) Harrington EA, Bennett MR, Fanidi A, Evan GI. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO J* 1994 Jul 15;13(14):3286-3295.
- (17) Hasegawa M. B lymphocytes: shedding new light on the pathogenesis of systemic sclerosis. *J Dermatol* 2010 Jan;37(1):3-10.
- (18) Hochberg, Marc C. et al. *RHEUMATOLOGY*. fifth ed. Philadelphia: Mosby Elsevier; 2011. (139):1387-1402; (140): 1403-1412; (141): 1413-1425; (142):1427-1432; (143): 1433-1437.
- (19) Johnson SR, Lee P. The HAQ disability index in scleroderma trials. *Rheumatology (Oxford)* 2004 Sep;43(9):1200-1201.
- (20) Klippel, John H. MD et al. *Primer on the rheumatic diseases*. 13th Edition ed. New York, NY: Springer; 2007.(17):343-362
- (21) Lafyatis R, Kissin E, York M, et al. B-cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheum*. 2009;60(2):578–583.
- (22) Leask A. B cell block: is rituximab a new possible treatment for systemic sclerosis? *J Cell Commun Signal* 2010 Dec;4(4):201-202.
- (23) Leroy Lda S, Lopes MH. Urinary incontinence in the puerperium and its impact on the health-related quality of life. *Rev Lat Am Enfermagem* 2012 Apr;20(2):346-353.
- (24) Male, David K. et al. *IMMUNOLOGY*. seventh Edition ed. Edinburgh: Mosby Elsevier; 2007.(19): 357-362; (20): 365-380.
- (25) Manno R, Boin F. Immunotherapy of systemic sclerosis. *Immunotherapy* 2010 Nov;2(6):863-878.
- (26) Murphy KP, Travers P, Walport M. *Janeway's Immunobiology*. 7. ed. ed. New York, NY [u.a.]: Garland; 2008. (8): 275-290,(10):387-395

- (27) Nacci F, Righi A, Conforti ML, et al. Intravenous immunoglobulins improve the function and ameliorate joint involvement in systemic sclerosis: a pilot study. *Ann Rheum Dis*. 2007;66(7):977-979.
- (28) Ong VH, Denton CP. Innovative therapies for systemic sclerosis. *Curr Opin Rheumatol* 2010 May;22(3):264-272.
- (29) Phumethum V, Jamal S, Johnson SR. Biologic therapy for systemic sclerosis: a systematic review. *J Rheumatol* 2011 Feb;38(2):289-296.
- (30) Simms RW, Lafyatis R. Rituximab: a potential therapeutic advance in scleroderma: what is the evidence? *Rheumatology (Oxford)* 2010 Feb;49(2):201-202.
- (31) Smith V, Van Praet JT, Vandooren B, Van der Cruyssen B, Naeyaert JM, Decuman S, et al. Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. *Ann Rheum Dis* 2010 Jan;69(1):193-197.
- (32) Sultan N, Pope JE, Clements PJ, Scleroderma Trials Study Group. The health assessment questionnaire (HAQ) is strongly predictive of good outcome in early diffuse scleroderma: results from an analysis of two randomized controlled trials in early diffuse scleroderma. *Rheumatology (Oxford)* 2004 Apr;43(4):472-478.
- (33) Tejeda-Andrade CF, Villalpando-Gomez Y, Gmez-Nava JI, Castro-Lizano N, Rocha-Muoz AD, Cardona Muoz EG, et al. Efficacy of the combined therapy with rituximab plus cyclophos - Phamide versus cyclophosphamide as monotherapy in systemic sclerosis-associated interstitial lung disease. *Journal of Rheumatology*. Conference: 2nd Mexican-Canadian Congress of Rheumatology Cancun Mexico. Conference Start: 20110210 Conference End: 20110215. Conference Publication: (var.pagings) 2011 June 2011;38(6):1193.
- (34) Tofighi S, Ahmad Kiadaliri A, Sadeghifar J, Raadabadi M, Mamikhani J. Health-Related Quality of Life among Patients with Coronary Artery Disease: A Post-Treatment Follow-Up Study in Iran. *Cardiol Res Pract* 2012;2012:973974.
- (35) Van Laar JM. B-cell depletion with rituximab: a promising treatment for diffuse cutaneous systemic sclerosis. *Arthritis Res Ther* 2010;12(2):112.
- (36) Waldburger JM, Firestein GS. Garden of therapeutic delights: new targets in rheumatic diseases. *Arthritis Res Ther* 2009;11(1):206.

Pat.-Initialen:  Rand.-Nr.:   
 Geb.-Dat.: ..19 Stud.-Nr.:  Datum: ..200  
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## SF-36

### Fragen zur allgemeinen Gesundheit (SF-36)

Bitte kreuzen Sie bei den Fragen 1, 2, 6, 8 und 10 jeweils eine Antwort an!

Bei den Fragen 3, 4, 5, 9 und 11 bitten wir Sie in jeder Zeile die zutreffende Zahl anzukreuzen. DANKE!

#### 1: Allgemeine Gesundheit: Wie

geht es Ihnen?		
Ausgezeichnet	1	<input type="radio"/>
Sehr gut	2	<input type="radio"/>
Gut	3	<input type="radio"/>
Weniger gut	4	<input type="radio"/>
Schlecht	5	<input type="radio"/>

#### 2: Gesundheitszustand im Vergleich zum letzten Jahr?

Viel besser	1	<input type="radio"/>
Etwas besser	2	<input type="radio"/>
Etwa gleich	3	<input type="radio"/>
Etwas schlechter	4	<input type="radio"/>
Viel schlechter	5	<input type="radio"/>

#### 3: Wie stark sind Ihre Tätigkeiten eingeschränkt? (1=stark; 2=etwas; 3=überhaupt nicht)

Anstrengende Tätigkeiten (Laufen, extremer Sport)	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Mittelschwere Tätigkeiten (leichter Sport)	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Einkaufstasche heben oder tragen:	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Mehrere Treppenabsätze (Stockwerke) steigen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Einen Treppenabsatz steigen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Sich beugen, knien, bücken	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>

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Mehr als 1 Kilometer zu Fuß gehen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Mehrere Straßenkreuzungen weit zu Fuß gehen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Eine Straßenkreuzung weit zu Fuß gehen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>
Sich baden oder anziehen	1	<input type="radio"/>	2	<input type="radio"/>	3	<input type="radio"/>

**4: Physische Schwierigkeiten bei der Arbeit, zu Hause ..... (1=ja; 2=nein)**

Kann nicht so lange wie üblich tätig sein	1	<input type="radio"/>	2	<input type="radio"/>
Habe viel weniger geschafft als ich wollte	1	<input type="radio"/>	2	<input type="radio"/>
Konnte nur bestimmte Dinge tun	1	<input type="radio"/>	2	<input type="radio"/>
Hatte Schwierigkeiten bei der Ausführung	1	<input type="radio"/>	2	<input type="radio"/>

**5: Psychische Schwierigkeiten bei der Arbeit, zu Hause ..... (1=ja; 2=nein)**

Kann nicht so lange wie üblich tätig sein	1	<input type="radio"/>	2	<input type="radio"/>
Habe viel weniger geschafft als ich wollte	1	<input type="radio"/>	2	<input type="radio"/>
Konnte nur bestimmte Dinge tun	1	<input type="radio"/>	2	<input type="radio"/>

**6: Beeinträchtigung der Kontakte**

Überhaupt nicht	1	<input type="radio"/>
Etwas	2	<input type="radio"/>
Mäßige	3	<input type="radio"/>
Ziemlich	4	<input type="radio"/>
Sehr	5	<input type="radio"/>

**7: Schmerzen**

Keine	1	<input type="radio"/>
Sehr leicht	2	<input type="radio"/>
Leicht	3	<input type="radio"/>

Pat.-Initialen:  Rand.-Nr.:

Geb.-Dat.: ..19 Stud.-Nr.:  Datum: ..200  
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- Mäßig 4
- Stark 5
- Sehr stark 6

**8: Tätigkeitsbeeinträchtigung**

**durch Schmerzen**

- Überhaupt nicht 1
- Etwas 2
- Mäßige 3
- Ziemlich 4
- Sehr 5

**9: Allgemeinbefinden (1=immer; 2=meistens; 3=oft; 4= manchmal; 5=selten; 6=nie)**

- |                    |                         |                         |                         |                         |                         |                         |
|--------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Voller Schwung     | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Sehr nervös        | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Niedergeschlagen   | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Ruhig und Gelassen | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Voller Energie     | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Entmutigt, traurig | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Erschöpft          | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Glücklich          | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |
| Müde               | 1 <input type="radio"/> | 2 <input type="radio"/> | 3 <input type="radio"/> | 4 <input type="radio"/> | 5 <input type="radio"/> | 6 <input type="radio"/> |

**10: Kontaktbeeinträchtigung**

**(Häufigkeit)**

- Immer 1
- Meistens 2
- Manchmal 3
- Selten 4
- Nie 5

