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Mapping of Disease Severity in Patients with Systemic Sclerosis

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Contents

List of Symbols and Abbreviations

The next list describes symbols and abbreviations that will be later used within the main text of the dissertation

$^{\circ}C$	Degree Celcius
μ	Greek symbol for 'mu' meaning 'mean-value'
σ	Greek symbol for 'sigma' meaning 'standard deviation'
®	Registered trademark symbol
$a.u.$	Arbitary units
ACA	Anticentromere antibodies
ACE	Angiotensin-converting enzyme
ACR	American College of Rheumatology
AE	Atopic eczema
AF	AquaFlux
ANA	Antinuclear antibodies
ATA	Antitopoisomerase I (Scl70) antibodies
AV	Acne vulgaris
CA	California
$CKD\text{-}EPI$	Chronic Kidney Disease Epidemiology Collaboration
CM	Corneometer
CMV	Cytomegalovirus
$COPD$	Chronic obstructive pulmonary disease
$CREST$	Acronym for the typical manifestations of SSc: calcinosis, Raynaud, esophageal dysmotility, sclerodactyly, and telangiectasia

CRP	C-reactive Protein
CTGF	Connective-tissue growth factor
CV	Coefficient of variation
dcSSc	Diffuse cutaneous systemic sclerosis
e.g.	Latin for *'exempli gratia'* meaning 'for example'
ECM	Extracellular matrix
ET-1	Endothelin-1
EULAR	European League against Rheumatism
EUSTAR	EULAR Scleroderma Trial and Research group
Fig.	Figure
g	Gram
GFR	Glomerular filtration rate
GmbH	Gesellschaft mit beschränkter Haftung
h	Hour
HR-CT	High-resolution computer tomography
HS	Healthy skin
i.e.	Latin for *'id est'* meaning 'that is'
IL	Interleukin
lcSSc	Limited cutaneous systemic sclerosis
Ltd.	Limited
m	Meter
MCP	Metacarpophalangeal joint
mRSS	Modified Rodnan skin score
n	Number
n.s.	Not significant

ND	Not determined
$NYHA$	New York Heart Association
PAH	Pulmonary arterial hypertension
PDE	Phosphodiesterase
$PDGFR$	Platelet-derived growth factor receptor
PGI_2	Prostacyclin
pH	Acronym for 'potential hydrogen'
PIP	Proximal interphalangeal joint
PPI	Proton pump inhibitors
r^2	R-squared meaning 'coefficient of determination'
RNA	Ribonucleic acid
RP	Raynaud's phenomenon
SRC	Scleroderma renal crisis
SSc	Systemic sclerosis
$SSRI$	Selective seretonin reuptake inhibitor
$Subj.$	Subjects
$TGF\text{-}\beta$	Transcription growth factor β
TUM	Technical University of Munich
UK	United Kingdom
USA	United States of America
$VEDOSS$	Very early disease onset of systemic sclerosis

Chapter 1

Introduction

In this introductory chapter we broadly discuss the epidemiology, pathology, classification and treatment of systemic sclerosis. It is not meant to be a review on all aspects of the disease, but rather to give the reader a concise overview of issues important to the understanding of the heterogeneity and complexity of this systemic disease. The focus will be on dermatologic aspects of the disease. For a more comprehensive overview see the standard references [Clements and Furst, 2004, Takehara et al., 2016]. For a compact and clear review of the pathology see the review article by Katsumoto et al. [2011].

1.1 Systemic Sclerosis: an Overview

The term 'scleroderma' originates from the Greek words *'skleros'*, meaning hard and *'derma'*, meaning skin. The name, "hard skin", describes the most visible attribute and hallmark of the disease. However, systemic sclerosis (SSc) is better described as a heterogeneous multi-systemic disease with vascular, immune, and fibrotic components [Varga and Abraham, 2007, Avouac et al., 2013, Gabrielli et al., 2009, Denton et al., 2006].

The most prominent pathological trait is a disorder of the connective tissue, showing fibrosis of skin, muscles, joints, and visceral organs such as the lungs, gastrointestinal tract, heart, and kidneys [Varga and Abraham, 2007]. The progression of systemic sclerosis is variable and largely unpredictable. Seldomly, patients experience spontaneous remission, often times the severity of the disease progresses and organ involvement finally leads to death [Steen and Medsger, 2007].

Clinically, systemic sclerosis presents itself through its distinct and universal attribute: skin thickening, tightening and hardening [van den Hoogen et al., 2013]. Skin fibrosis is caused by collagen-rich extracellular matrix formation, vascular damage, and inflammation [Varga and Abraham, 2007]. More than 90% of SSc patients show skin involvement [Knobler et al., 2017a]. Due to its heterogeneity, patients with systemic sclerosis are often medically supervised by an interdisciplinary team of doctors. In the case of the study in this doctoral thesis, the team consisted of dermatologists and rheumatologists.

1.1.1 Epidemiology

Systemic sclerosis is a rare disease which makes it difficult to conduct large population studies on the epidemiology. Comprehensive data in literature, indicating the precise prevalence and incidence of systemic sclerosis, are difficult to find. In addition, as discussed below, discrepancies in data are common in such rare diseases.

Observational studies have found variations in disease occurrence in different ethnicities and geographical areas [Mayes et al., 2003, Zhong et al., 2019, Bergamasco et al., 2019]. Bergamasco et al. performed a systematic review of publications between 2000 and 2016 in North America and Europe in which they searched for epidemiologic data on systemic sclerosis. The prevalance in Europe was found to be 7.2 to 33.9 per 100.000 individuals. The annual incidence was estimated at 0.6 to 2.3 per 100.000 individuals in Europe. In North America these numbers were slightly higher (for example: incidence 13.5 to 44.3 per 100.000 individuals). They also found a predominance of SSc in females (female:male ratio 4:1-15:1) [Bergamasco et al., 2019]. In a recent prospective study, Elhai et al. [2016] found that the female to male ratio is 6:1 and that men show a more severe phenotype than women.

Systemic sclerosis is typically a disease of middle-aged people. In an article about prevalence and incidence of systemic sclerosis in a large US population, Mayes et al. [2003] state that SSc typically first manifests itself in a mean age of 46 ($\pm$ 16) years, so in an age range of 30-60 years.

As a recent epidemiological study of death certificates of French patients with SSc showed, main causes of SSc death are heart diseases and lung complications [Elhai et al., 2017]. The 10-year survival rate of SSc is estimated 65-73% in Europe [Bergamasco et al., 2019].

1.1.2 Aetiology and Pathogenesis

The aetiology and pathogenesis of systemic sclerosis are not yet fully understood. The primary cause of systemic sclerosis has not yet been deliniated, yet recently, there have been some novel insights into the pathology, as discussed next.

Aetiology. Studies have tried to find causes of SSc due to viruses (such as cytomegalovirus, CMV), drugs, environmental or occupational exposures to toxic solvents, yet results have not been conclusive [Pandey and LeRoy, 1998, Nietert and Silver, 2000]. It is assumed that genetics play a role in aetiology, because one can see an accumulation in families. Arnett et al. [2001] found that the occurance of SSc is significantly higher in families (1,6%) than in the general population of the USA (0,026%). In contrast, monozygotic twin studies are not conclusive and do not support a hereditary aspect [McHugh et al., 1995]. This leads one to assume that other factors may play a more prominent role in aetiology. In a recent review, Mazzone et al. [2019] suggest that epigenetic mechanisms

such as DNA methylation could play a pivotal role in the development of autoimmune disorders like systemic sclerosis.

Pathogenesis. To this date, a clear, unifying hypothesis explaining the pathogenesis of SSc has not yet been established. In a review article, Katsumoto et al. [2011] provide an informative description of major factors contributing to the pathogenesis of systemic sclerosis [Katsumoto et al., 2011]. These factors include abnormalities in vascular, immunologic, and fibrotic pathways. They conclude that possibly the main events in the pathogenesis of SSc are complex interactions between the vascular and immune systems that finally lead to pathologic fibrosis.

Vasculopathy. Unique to SSc is that vasculopathy (vascular obliteration) and autoimmunity characteristically precede fibrosis [Prescott et al., 1992, Varga and Abraham, 2007, Gabrielli et al., 2009]. SSc-associated vascular abnormalities manifest as Raynaud's phenomenon, cutaneous telangiectasia, alterations in nailfold capillaries, pulmonary arterial hypertension (PAH), gastrointestinal dysfunction, and scleroderma renal crisis (SRC). Vascular changes (caused, for example, by autoantibodies, toxins, viruses, etc.) include endothelial cell injury and apoptosis, as well as blood vessel narrowing and impaired angiogenesis. These vascular changes in turn lead to hypoxia and degradation of connective tissue. [Wigley, 2009]

Autoimmune dysregulation is a further pathogenic process in systemic sclerosis. It involves lymphocyte activation, which leads to autoantibody production [Katsumoto et al., 2011]. SSc-specific autoantibodies are detected in almost all SSc patients [Mierau et al., 2011]. Their serum levels fluctuate with disease severity and activity [Hu et al., 2003]. A recent study by Baroni et al. [2006] discovered stimulatory autoantibodies against the platelet-derived growth factor receptor (PDGFR). PDGF is a growth factor that regulates blood vessel formation and proliferation of fibroblasts. Their findings strongly suggest that anti-PDGFR autoantibodies have a causal role in the pathogenesis of systemic sclerosis [Baroni et al., 2006]. In summary, while SSc-associated antibodies have an important clinical application as diagnostic markers [van den Hoogen et al., 2013], their contribution to disease pathogenesis remains uncertain [Fritzler and Choi, 2016].

Fibrosis. The pathological hallmark of systemic sclerosis is fibrosis. Pathogenic fibrosis progressively replaces normal connective tissue with collagen-rich extracellular matrix (ECM), causing functional impairment of affected organs. Fibrosis is most prominent in the following organs: skin, lungs, gastrointestinal tract, heart, tendons and ligaments, and in endocrine glads. Damage caused by fibrosis in organs leads to their dysfunction and is the main reason for death in SSc patients. It is widely accepted that fibroblasts and myofibroblasts play an important role in fibrosis [Ho et al., 2014, Varga and Abraham,

2007, Denton et al., 2006]. Fibroblasts are cells located in the ECM which secrete collagens and other ECM macromolecules, and thus remodel the connective tissue. Usually this remodelling process is initiated in would healing, however pathological fibrotic responses as in systemic sclerosis feature sustained and amplified fibroblast activation. [Varga and Abraham, 2007]

Growth factors in fibrosis. Recent mouse models show that the transforming growth factor, TGF-β, and excessive signalling of this factor on fibroblasts plays a fundamental role in pathogenesis [Varga and Pasche, 2009]. TGF-β has potent profibrotic activity [Roberts et al., 1985] and promotes collagen synthesis and secretion of matrix molecules [Roberts et al., 1992]. The factor is located in the ECM. Varga and Pasche [2009] show that especially in SSc, the link between TGF-β signalling and pathological fibrosis is particularly stringent. Milano et al. [2008] analyzed gene expression in lesional SSc skin and found a TGF-β responsive gene signature, which again underlines the important role of this growth factor in the pathogenesis of SSc. Other mediators of fibrosis are connective-tissue growth factor (CTGF), Endothelin-1 (ET-1) and various cytokines such as type I interferons [Sierra-Sepúlveda et al., 2019, Katsumoto et al., 2011, Gabrielli et al., 2009, Denton et al., 2006]. The detailed description of all pathogenic pathways would exceed the scope of this doctoral thesis. For a comprehensive and detailed review see, for example, [Katsumoto et al., 2011].

In addition, as part of its role as a pathogenetic factor, TGF-β has emerged as an attractive therapeutic target [Varga and Pasche, 2009]. The current therapy of SSc will be discussed in detail in Section 1.3.

Genome-wide expression profiling. DNA microarrays have become a powerful tool in analyzing heterogenous diseases such as systemic scleorsis. In a highly interesting study, Milano et al. [2008] took biopsies from 34 SSc patients and identified 177 genes which strongly correlate with the severity of skin disease. These gene expression profiles might lead to further insights into the pathology of systemic sclerosis.

1.2 Clinical Features and Diagnosis of SSc

Clinical features of systemic sclerosis include skin manifestations such as skin hardening, vascular dysfunctions such as Raynaud's phenomenon and organ dysfunctions. In this section, we will focus on the diagnostics of skin manifestations, particularly on classification criteria and division into cutaneous subtypes.

1.2.1 Skin and Vascular Involvement

First visible symptoms in SSc patients typically are skin thickening and hardening [Rodnan et al., 1979]. As described above, systemic sclerosis is characterized by inflammation

and fibrosis of the skin. Fibrosis is defined as the process of scar formation caused by the deposition of excessive collagen and other ECM proteins and is clinically visible in SSc as skin hardening, thickening and tightening. In addition to skin thickening, other cutaneous signs are nailfold and fingernail alterations, digital ulcerations, telangiectasia, hyperpigmentation of the skin, loss of hair follicles and sebaceous glands, and cutaneous calcifications [Desbois and Cacoub, 2016, Krieg and Takehara, 2009].

The disease severeness can be rapidly progressive or mild and prolonged, progressing slowly over many years. Still today, patients with systemic sclerosis frequently remain undiagnosed for long periods of time, sometimes several years or even decades [Bellando-Randone et al., 2012]. Due to its heterogeneity, SSc is difficult to discover and to diagnose in an early stage.

Skin involvement. As systemic sclerosis progresses, the skin of diffuse cutaneous SSc patients typically undergoes 3 pathologic stages: in the early stage it worsens, then it reaches a plateau, and spontaneously improves in later stages [Clements et al., 2000, Rodnan et al., 1979].

Skin involvement has 3 phases, first skin is edematous (phenotypically visible as swollen fingers), then indurative (characterized by thickening and hardening of the skin) and lastly atrophic (skin softens). Typically, skin thickening begins in the fingers and is called sclerodactyly. In addition, skin involvement shows at the skin of the face, which shows hardening. Also, mimic folds disappear, but deep wrinkles appear on the upper and lower lip. [Ferreli et al., 2017]

Vascular abnormalities. Altered vascular changes occur before fibrosis [Varga and Abraham, 2007]. Vascular abnormalities usually begin with Raynaud's phenomenon of the fingers. Other typical vascular manifestations include digital ulcers, renal crisis, and pulmonary arterial hypertension. Raynaud's phenomenon exemplifies the generalised and progressive nature of the vascular dystrophy in the disease and is therefore a key component of SSc [Kahaleh, 2004]. It is generally known, that Raynaud's phenomenon is caused by cold-induced vascular spasms, resulting in episodes of reduced blood flow to the fingers, which characteristically turn white in coloration. It is present in over 90% of the patients with systemic sclerosis [Knobler et al., 2017b] and is defined by at least a 2-phase color change (red, white) in the fingers, usually due to response to cold exposure [van den Hoogen et al., 2013]. The fact that vascular abnormalities occur before other non-Raynaud's clinical feature of the disease [Walker et al., 2007] indicates that there is a pre-clincal stage of the disease, where the disease could be efficiently treated and later complications prevented [Becker et al., 2010].

1.2.2 Organ Involvement

Apart from skin manifestations, internal organ involvement is a common, but not a mandatory clinical feature for the definition of systemic sclerosis. It is a feature of systemic sclerosis that deserves particular attention, because severe organ involvement

is most often the cause of death in SSc patients [Steen and Medsger, 2007, Elhai et al., 2017].

The prevalence of pulmonary fibrosis is estimated at 53% of all SSc patients. Gastrointestinal manifestations such as in the oesophagus are extremely common (68% of patients have oesophageal involvement). Cardiovascular diseases such as diastolic dysfunction (17% of patients) vary according to the manifestation of myocardial fibrosis. Renal dysfunctions such as hypertensive renal crisis are less common (4% of patients). [Walker et al., 2007]

1.2.3 Clinical Skin Score: the Modified Rodnan Skin Score

To this date, the clinical characterisation ('gold standard') of skin thickness in systemic sclerosis patients is performed by measuring the modified Rodnan skin score (mRSS) [Khanna et al., 2017, Knobler et al., 2017a, Czirják et al., 2007]. Skin thickening/fibrosis is caused by accumulation of collagen in the dermis [Gabrielli et al., 2009]. In the indurative phase of the disease, pinching the skin of SSc patients into a normal skin fold is hindered through skin thickening [Rodnan et al., 1979]. This skin pinching is the basis on which the clinical examination and skin scoring (mRSS) is built [Khanna et al., 2017]. The Rodnan skin score was first described by Rodnan et al. [1979] and later simplified to the modified Rodnan skin score [Clements et al., 1995]. The modified Rodnan skin score is performed by palpating 17 different points of the skin. A score from 0-3 is given (0 being uninvolved skin, 1 mild thickening, 2 moderate thickening and 3 severe thickening). The maximum mRSS is a total score of 51 points. [Clements et al., 1995, 1993]

This method for evaluating skin thickness is challenging, not objective, and is dependent on the clinician and his or her abilities [Czirják et al., 2007]. It can have a high intraobserver variability [Czirják et al., 2007], but as stated above, it remains the gold standard of measuring skin involvement in systemic sclerosis.

The mRSS is often used as a score for skin sclerosis progression or as an outcome measure in clinical treatment trials, for example in [Clements et al., 2000]. Their data showed a high link between skin score and increased mortality. However, as they found, this is only true for the diffuse cutaneous subtype (dcSSc) of systemic sclerosis. In addition, it is generally accepted that for dcSSc the skin score worsens in the early phase of the disease (first 1-3 years), but regresses and improves in later phases (skin softens). Thus, using mRSS as a measurement tool for outcome in clinical trials has been argued to be ambigious [Shand et al., 2007], because this skin fibrosis can spontaneously regress. Moreover, the disease course is highly variable between different patients. Some researchers found that as the disease progresses, the mRSS improves [Amjadi et al., 2009]. This is, however, not in accordance with recent findings by Herrick et al. [2018]. They defined disease progression as mRSS worsening. In a previous study, Shand et al. [2007] also found an association between a high skin thickness score and increased mortality. However, they question the usefulness of the mRSS as a primary outcome variable. In

conclusion, these studies show that there is no definite consensus on skin score as a diagnostic endpoint measure.

1.2.4 Cutaneous Subtypes

As first described by LeRoy et al. [1988], patients can be classified into two subtypes of systemic sclerosis, based on the pattern of skin involvement: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc). In daily clinical practice this subtype division of SSc patients is the most commonly used.

Limited cutaneous SSc is characterised by sclerosis which is limited to the hands, face, feet and forearms or even absent skin sclerosis. Patients have usually had Raynaud's phenomenon for years, and have a late onset of pulmonary hypertension; see Table 1.1 [LeRoy et al., 1988]. Widely accepted as a form of limited cutaneous SSc are also the so called 'CREST-syndrome' (CREST: calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia) and 'systemic sclerosis sine scleroderma' (SSc without skin symptoms) [Knobler et al., 2017b].

The term diffuse cutaneous SSc is synonymous with progressive cutaneous systemic scleroderma. This subtype is characterised by skin fibrosis of the torso as well as the extremities. It is defined by early incidence of diffuse and rapidly progressive fibrosis of the skin, lungs, gastrointestinal system and other internal organs; see Table 1.1. [LeRoy et al., 1988]

At the onset of the disease, patients with dcSSc tend to have puffy fingers (swollen fingers). Later, they are followed by sclerotic changes, leading to skin fibrosis. The skin fibrosis usually begins in the fingertips (sclerodactyly) and as the disease progresses, the fibrosis spreads more proximal to the forearm. In later disease stages, internal organ involvement may be present, while fibrotic skin symptoms diminish. [Knobler et al., 2017b]

Limited cutaneous SSc (lcSSc)	**Diffuse cutaneous SSc (dcSSc)**
• Acral or absent skin involvement	• Truncal and acral skin involvement
• Raynaud's for years	• Early onset of Raynaud's, typically within 1 year of onset of skin changes
• Late incidence of pulmonary involvement	• Early and significant incidence of intestinal lung disease, oliguric renal failure, diffuse gastrintestinal disease or myocardial involvement
• Nailfold capillary dilation, usually without capillary dropout	• Nailfold capillary dilation and capillary destruction
• Anti-centromere-antibodies	• Anti-topisomerase-1-antibodies

Table 1.1 Subtype classification of systemic sclerosis (SSc). Adapted from [LeRoy et al., 1988, p. 202, Table 1].

1.2.5 Overlap Syndrome

Not all patients with systemic sclerosis can be easily classified into one of the above describe cutaneous subsets. There are courses of the disease, in which there is no clear distinction into lcSSc or dcSSc. So called 'overlap syndromes', for example, broaden the spectrum of subsets beyond the two cutaneous subtypes. Patients with systemic sclerosis may also exhibit features of various other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis, Sjörgen's syndrome and many more; see e.g. [Knobler et al., 2017a]. This presentation of multiple diseases at once is called 'overlap syndrome'. Moinzadeh et al. [2015] propose that overlap syndromes should be regarded as a distinct SSc subset. A detailed description of overlap syndromes is beyond the scope of this doctoral thesis, but can be found for example in the following paper: [Knobler et al., 2017a].

1.2.6 Classification and Diagnostic Criteria

In addition to skin manifestations, diagnosis is also based on organ involvement, laboratory parameters and nailfold capillaroscopy, see e.g. [Knobler et al., 2017a]. Due to the clinical heterogeneity of systemic sclerosis, the diagnosis of patients with this disease is difficult and often not conclusive. To this date, the global clinical SSc community debates on the best classification criteria for SSc and has not yet reached a consensus on the optimum approach to diagnosing systemic sclerosis [Johnson et al., 2018]. Diagnostics and therapeutic standards still have a wide variation across different countries and clinics [Matucci-Cerinic et al., 2009]. Bellando-Randone and Matucci-Cerinic [2019] summarize the debate about diagnosis of SSc very well with their statement that all SSc criteria proposed up to date are actually classification and not diagnostic criteria.

Actually, in the past four decades, researchers have tried to classify characteristic traits of systemic sclerosis into diagnostic criteria. In 1980, the American College of Rheumatology (ACR) developed a set of classification criteria [Masi et al., 1980]. The criteria consist of one major criterion, proximal scleroderma, and two or more minor criteria, i.e. sclerodactyly, digital pitting scars of fingertips, and bilateral basilar pulmonary fibrosis. Later, in 1988, LeRoy developed a classification system based on cutaneous manifestations (described in Subsection 1.2.4), in which he defined two subtypes: 1) diffuse cutaneous SSc and 2) limited cutaneous SSc.

EULAR/ACR criteria. Finally, in 2013, a joint committee, the European League against Rheumatism (EULAR) and the American College of Rheumatology (ACR) developed a nine point classification system, primarily for clinical research purposes [van den Hoogen et al., 2013]. These classification criteria are the basis of diagnosing SSc for clinical trials and research until today. The EULAR/ACR classification criteria are summarized in Table 1.2. Within these criteria, skin thickening of one or more fingers of both hands results in a score of 9, which is in turn sufficient for classifying a

Item	Subitem	Score*
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	–	9
Skin thickening of the fingers	Puffy fingers	2
	Sclerodactyly of the fingers (distal to MCP but proximal to PIP)	4
Fingertip lesions	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	–	2
Abnormal nailfold capillaries	–	2
Pulmonary arterial hypertension and/or interstitial lung disease	Pulmonary arterial hypertension	2
	Intestitial lung disease	2
Raynaud's phenomenon	–	3
SSc-related autoantibodies	Anti-centromere	3
	Anti-topoisomerase I	
	Anti-RNA-Polymerase III	

Table 1.2 The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for the classification of systemic sclerosis (SSc). Table adapted from [van den Hoogen et al., 2013, p. 2741, Table 1].
*The total score is determined by adding the score of each item/subitem. If the total score is equal to or higher than nine, patients are classified as having definite SSc.
MCP: Metacarpophalangeal joint **PIP**: Proximal interphalangeal joint

person as having systemic sclerosis (see Table 1.2). The sensitivity and specificity of this EULAR/ACR classification system is respectively 0.91% and 0.92% in detecting patients with SSc [van den Hoogen et al., 2013].

Early and very early SSc. However, these criteria have limitations in diagnosing early SSc and thus the approach to diagnosing SSc has shifted. Nowadays, more publications focus on early and very early diagnosis of SSc, for example [Valentini et al., 2011]. The ACR/EULAR classification criteria are not sensitive enough to identify SSc in an early and very early stage [Matucci-Cerinic et al., 2013]. Often the diagnosis of SSc is delayed for several years, even after first presentation of vascular changes such as Raynaud's phenomenon [Walker et al., 2007]. In a review article, Bellando-Randone et al. [2012] state the urgency of identifying systemic sclerosis in a very early stage of the disease, when skin or organ fibrosis are not yet visible. This way, therapy is not delayed until the disease has progressed to a state in which many symptoms are already irreversible.

Valentini et al. [2011] showed that while the clinical internal organ involvement is not visible, subclinical involvement was detected early in a number of cases. In a recent

Delphi exercise, Avouac et al. [2011] identified red flags for the Very Early Diagnosis of Systemic Sclerosis (VEDOSS). They classified patients as having high clinical relevance for VEDOSS when showing the following four features: Raynaud's phenomenon, puffy swollen digits turning into sclerodactily, abnormal capillarscopy with scleroderma pattern, and positive antibodies (anticentromere and antitopoisomerase-I antibodies). However, these criteria are not SSc-specific and can occur in other connective tissue diseases as well. Experts believe that the 'red flags' will however lead to an earlier diagnosis of SSc, resulting in earlier treatment [Avouac et al., 2011]. To this end, a Very Early Diagnosis of Systemic Sclerosis (VEDOSS) project has been launched by the EULAR Scleroderma Trial and Research group (VEDOSS project; http://www.eustar.org). Preliminary results from the VEDOSS study confirm that patients with the above described clinical features show a predisposition to develop early SSc. The only reliable early detector of SSc up to date is a close follow-up of VEDOSS patients, to detect early development of SSc-specific symptoms [Bellando-Randone and Matucci-Cerinic, 2019].

Future research should, as emphasized by Johnson et al. [2018], focus on developing new systemic sclerosis subset classification criteria that also include skin change over time. Johnson et al. [2018] state that in the future antibody profiling, genetic markers, and biomarkers will play a more prominent role in earlier identification of SSc.

Autoantibodies. As a diagnostic tool, autoantibodies targeting nuclear antigens have already become extremely important for characterising systemic sclerosis. In serum probes of virtually all patients with SSc, one can detect specific autoantibodies [Varga and Abraham, 2007]. A study completed by the German Network for Systemic Scleroderma [Mierau et al., 2011], shows that five antigens are sufficient for detecting 95% of the known SSc-associated autoantibody responses in ANA-positive SSc patients: antinuclear antibodies (ANA) such as antibodies against centromers (ACA: anticentromere-antibodies), topoisomerase I (also known as Scl70-antibodies), RNA-polymerase III, PM-Scl (polymyositis-sclerodermia), and U1-RNP. However, it has been argued that autoantibodies may also disappear in the course of the disease [Kuwana et al., 2000] and are therefore not a reliable source for identification and classification of SSc.

1.2.7 Differential Diagnosis: other Skin Diseases with Fibrosis

As dermatologists are often first contact persons for SSc patients, their task is to differentiate and properly depict SSc for other sclerotic skin disorders. However, cutaneous fibrosis leading to sclerosis of the skin does not only prevail in systemic sclerosis. According to Morgan and Hummers [2016] so called scleroderma mimikers can show close resemblance to dermal features of systemic sclerosis, thus increasing the difficulty of the task of accurately diagnosing SSc. Scleroderma mimikers include scleredema, skleromyxedema, morphea, diabetic chieroarthropathy, nephrogenic systemic fibrosis, and eosinophilic fasciitis [Morgan and Hummers, 2016]. In a paper by Jendrek et al. one can find an informative schematic illustration on common sites of skin involvement of the different

scleroderma mimikers [Jendrek et al., 2019, Fig. 1]. Knobler et al. [2017a,b] give a detailed outline on the European Dermatology Forum S1-guideline on the diagnosis and treatment of sclerosing diseases of the skin. In this paragraph, other sclerotic skin diseases are only briefly mentioned to create awareness of the difficulty in diagnosing systemic sclerosis. It is important to keep these differential diagnosis in mind and to differentiate these fibrotic disorders.

1.3 Prognosis and Current Treatment

The main focus of the thesis is the diagnosis of systemic sclerosis. As a background information we give a concise overview of the treatment.

The estimated survival rate of patients with systemic sclerosis is 62,5% following 10 years after diagnosis [Rubio-Rivas et al., 2014]. A study from the EULAR Scleroderma Trials and Research (EUSTAR) found that the majority of deaths are SSc-related [Tyndall et al., 2010]. They state that the most common cause of death is due to lung fibrosis, followed by pulmonary arterial hypertension (PAH) and cardiac causes. Of the non-SSc-related causes of death, the majority had an infectious disease, followed by neoplastic and cardiovascular causes [Tyndall et al., 2010].

Until today, SSc is considered incurable and still has a very high mortality rate [Rubio-Rivas et al., 2014]. Due to the complex pathology, the heterogeneity, and the non-linear disease progression, SSc is primarily treated according to clinical symptoms [Kowal-Bielecka et al., 2017a]. Treatment plans, disease-modifying treatment, and clear guidelines for systemic sclerosis have not yet been established. Thus, treatment mainly consists of the management of SSc-related complications.

The focus of many studies is finding appropriate targets for therapies. Treatment is closely linked to clinical features of SSc or targets different pathogenic processes. It is often tailored to the individual SSc subset (lcSSc/dcSSc), organ involvement, and disease severity. In consideration of this, a standardised homogenous treatment plan can not be developed. Instead, EULAR's recommendations of treatment are based on SSc-symptoms (Raynaud's phenomenon, digital ulcers, PAH, skin disease, SRC, and gastrointestinal disease). [Kowal-Bielecka et al., 2017a].

Patients are treated by "targeted therapy". The term targeted therapy includes several different aspects: (1) targeting organ-specific complications, (2) targeting pathological processes such as immune activation, skin/organ fibrosis and vascular complications and lastly (3) targeting specific cell types or interactions between cells. [Denton, 2015]

EULAR recommendations summarize treatments of SSc-related organ-specific complications: Raynaud's phenomenon (RP), digital ulcers, PAH, skin disease, SRC, and gastrointestinal disease [Kowal-Bielecka et al., 2017a]. These recommendations include, for example, PDE5-inhibitors and dihydropyridine-type calcium antagonists against SSc-RP attacks. ACE-inhibitors are recommended for patients with severe renal crisis.

Kowal-Bielecka et al. recommend Methotrexate (MTX, an antifolate drug) in early diffuse cutaneous SSc for skin manifestations. Moreover, haematopoietic stem cell transplantation has shown to improve skin manifestations; see reviews [Del Papa et al., 2018, Walker et al., 2018].

In the following we would like to shortly reflect the newest therapeutic drugs according to their targets in pathologic processes.

Treatments against inflammatory components. According to Sierra-Sepúlveda et al. [2019] literature review, treatments targeting immune mediators include immunosuppressive drugs such as Anifrolumab (antibody against type I interferon receptor), Tocilizumab (antibody against interleukin-6 receptor), Rituximab (antibody against the protein CD20), Efalizumab (antibody against CD11), Abatacept (antibody against CD80 and CD86), and many more.

A newly published review article states that molecular targeted therapy with monoclonal antibodies have shown promising clinical outcomes in the **treatment of skin involvement** [Asano, 2017] . The following three antibodies are particularly interesting: Tocilizumab, Rituximab, and Fresolimumab (anti-TGF-β-antibody). Further elaboration on these treatments can be found in the article by Asano. Another recent article identified the cytokines IL-13 and IL-4 as possible therapeutic targets of SSc [Gasparini et al., 2020].

Treatments against fibrosis. Typically, skin fibrosis is first treated through general measures such as skin protection from trauma, moisturizing skin care, physiotherapy, and lymphatic drainage. Systemically, fibrosis is treated with pharmacologic drugs such as Methotrexate, Mycophenolate mofetil, and Cyclophosphamide [Knobler et al., 2017b]. Methotrexate treatment has been analyzed though randomized controlled clinical studies and it was shown that this drug provokes a slight decrease in skin fibrosis in early diffuse SSc [Pope et al., 2001, van den Hoogen et al., 1996]. As discussed above, MTX is also included in the EULAR recommendations for skin disease [Kowal-Bielecka et al., 2017a].

Treatments against vascular complications. Vascular manifestations of SSc such as Raynaud's phenomenon (RP) are foremost treated by general measures such as avoidance to cold exposure, wearing heated gloves, prohibition of smoking and paraffin baths [Knobler et al., 2017a]. Pharmacologic treatment consists of first-line therapies with calcium antagonists such as Nifedipine and second-line therapies with PDE-5-inhibitors. The updated EULAR recommendations also include Fluoxetine (a selective serotonin reuptake inhibitor, SSRI) for RP. [Kowal-Bielecka et al., 2017a]

Another vascular complication, digital ulcers, is first treated with general measures such as wound care, vasodilatory physical therapy and then typically treated with intravenous Iloprost (a synthetic analog of prostacyclin PGI_2) [Kowal-Bielecka et al., 2017b].

Although a variety of medications have been used extensively in the treatment of the disease, no therapy has yet been discovered which reverses or slows the progression of tissue fibrosis or substantially modifies the natural progression of SSc.

1.4 Diagnostic Instruments of the Skin

As described in Section 1.1.2, pathogenic processes in SSc include abnormal skin fibrosis, caused by excessive collagen production and deposition. Van Praet et al. [2011] demonstrated in their study that mean epidermal thickness is associated with local skin involvement. Thus, functional measurements of the epidermal layer of the skin of SSc patients may help identify and differentiate skin manifestations.

The stratum corneum (the outermost layer of the epidermis) is the location of the functional skin barrier and transepidermal water loss (TEWL) is acclaimed as the main indicator of skin barrier function [Werner and Lindberg, 1985, Akdeniz et al., 2018, Alexander et al., 2018]. In previous studies, e.g. by Werner and Lindberg [1985] and Seidenari and Giusti [1995], TEWL has been used as a measure to evaluate skin barrier function, especially in patients with atopic dermatitis. TEWL is an established scientific method, which has been used to study the water loss through the skin since 1977 [Nilsson, 1977]. Several guidelines have been previously developed to ensure constancy and accuracy in research studies [Pinnagoda et al., 1990, Rogiers and EEMCO Group, 2001]. TEWL is used as a research tool to assess skin barrier function and it is known to correlate with the severity of atopic eczema [Alexander et al., 2018].

Similar to transepidermal water loss, two other skin parameters, pH and skin hydration, are commonly used in studies of healthy skin, atopic eczema and other skin disorders, e.g. to quantify skin function [Cannavò et al., 2017, Prakash et al., 2017, Jaeger et al., 2015, Constantin et al., 2014, Lambers et al., 2006, Eberlein-König et al., 2000, Seidenari and Giusti, 1995]. Since the pathology of SSc is not fully understood, we cannot yet understand the processes in the skin, which lead to changes that can be measured by diagnostic instruments of this study. However, we assume that epidermal thickness may result in skin changes which can be quantified through TEWL, skin hydration, and pH measurements.

A literature research shows that previous studies of systemic sclerosis have not yet performed in-depth analysis on these three skin parameters. The literature about skin TEWL measurements in SSc patients, obtained with a PubMed search using the string '(TEWL OR transepidermal water loss OR evaporimet*) AND (systemic sclerosis OR scleroderma)' yields only 5 results: [Sim et al., 2018, Ďurčanská et al., 2016, Muratore et al., 2013, Sogabe et al., 2002, Serup et al., 2006]. We will discuss these papers in detail in Chapter 4. The literature about skin pH measurements in SSc patients, obtained with a PubMed search using the string '(pHmet* OR pH-met* OR pH-value) AND (systemic sclerosis OR scleroderma)' yields only 18 results, none relevant for SSc skin manifestations. The literature about skin hydration measurements in SSc patients, obtained with a PubMed search using the string '(corneo* OR corneometry OR skin hydration OR capacitance) AND (systemic sclerosis OR scleroderma)' yields only 17 results, one being relevant for SSc skin manifestations: [Muratore et al., 2013]. In this prospective study, Muratore et al. [2013] perform measurements on the forearm of SSc patients, comparing TEWL and moisture of the skin after Iloprost and cleaning/moisturizing formulation.

In this study we analyzed the functional properties e.g. TEWL, skin hydration, and pH of SSc skin. The evaluation of skin surface parameters can be helpful in gaining a better understanding of the disease and its quantitative skin characteristics.

1.5 Rationale of the Study

To this date, systemic sclerosis (SSc) remains a rare disease, but it has gained increasing importance in the scientific world. In PubMed, one can find that publications about 'systemic sclerosis' or 'scleroderma' have increased substantially, especially in the last two decades.

Due to its rarity, few clinical studies have been performed which address objective measures of the skin of SSc patients. Cutaneous involvement is the major criterion in classification systems for systemic sclerosis [van den Hoogen et al., 2013]. In the most recent classification criteria, the 2013 ACR/EULAR classification criteria, highest scores are given to skin thickening (Table 1.2). As discussed in Section 1.2.3, the 'gold standard' for clinical scoring of skin involvement in systemic sclerosis is a semi-quantative skin scoring system: the modified Rodnan skin score (mRSS). The skin score is determined through manual palpation of 17 different skin sites by a clinical examiner and measures the skin thickness. The mRSS often used as an outcome measure in clinical trials of systemic sclerosis and to measure SSc disease activity, severity, and mortality [Khanna et al., 2017]. According to Furst et al. [2007] the mRSS has proven as not sensitive enough as an outcome measure in clinical studies. Consequently, there is need for a more objective scoring system both for clinical practice and in research settings.

The objective of this doctoral thesis was testing the prospective application of skin functional measurements for diagnostics of systemic sclerosis in the setting of a clinical study. We aimed to find objective parameters for the clinical examination and mapping of disease severity in systemic sclerosis.

The study of physical functions of the skin is essential for the assessment of skin-related diseases such as systemic sclerosis. In a clinicopathological study, Van Praet et al. [2011] found an increased epidermal thickness in biopsies with local clinical skin involvement. Dermal or skin thickening might impact the epidermal humidity (e.g. skin hydration), pH and transepidermal water loss by a disturbance of the normal skin architecture, microcirculation and sweat gland function. Thus, we examined the skin of patients with systemic sclerosis through three objective skin measurements: transepidermal water loss (TEWL), skin hydration and pH at nine pre-defined skin sites of the body. Using diagnostic instruments which are already standard instruments for other diseases (e.g. TEWL for atopic eczema [Alexander et al., 2018]), we wanted to test whether there is a simple-to-use, quick, but specific method to objectively analyse the skin of SSc patients.

Furthermore, we hoped to gain new insights on systemic sclerosis and to find prospective applications of these skin measurements.

To this end, we created the following study design. We chose a study population of 72 test subjects, consisting of four groups (1) systemic sclerosis, and as a comparison, test subjects with (2) atopic eczema, (3) acne vulgaris, and (4) healthy skin. As discussed above, we then conducted the following skin measurements at nine pre-defined skin sites: (1) transepidermal water loss (TEWL), (2) skin hydration, and (3) pH.

Chapter 2

Patients, Materials and Methods

2.1 Study Design

The study design of this doctor thesis is a prospective clinical study. It was conducted by a single observer on patients with systemic sclerosis in the framework of a specialised interdisciplinary consultation hour, in a collaboration of dermatologists and rheumatologists. As control groups, three cohorts of test persons were analysed: test subjects with (1) atopic eczema, (2) acne vulgaris and (3) healthy skin. The study was carried out at the clinic for dermatology and allergology at Biederstein, which is part of the Technical University of Munich. The recruitment of subjects and the completion of measurements was carried out over a period of nine months, from May 2017 until January 2018.

All patients gave oral and written consent and participated willingly in this clinical study. The recruited data was used exclusively for the purpose of this study and patient data was kept anonymous. There is no linkage between the data of the patients and the study results.

The clinical study was in accordance with the ethical standards of the Technical University of Munich and was approved by the local ethics committee (Registration number: 227 16S).

2.2 Study Population

The study population includes seventytwo test subjects. Ages ranged from 18 to 80 years and consisted of 49 females and 23 males. Figure 2.1 shows an overview of the analysed study population. As described above, the study comprised four cohorts of test persons. The demographics of test subjects are shown in Table 2.1. Our main study cohort, the 'Systemic Sclerosis Group', consisted of 18 test persons. As can be derived from Table 2.1, the female to male ratio with SSc is 15 to 3 (which is typical for this disease, see Chapter 1.1.1). We found a higher representation of patients with a limited cutaneous systemic sclerosis (lcSSc) subtype than with diffuse cutaneous systemic sclerosis (dcSSc), the ratio being 13 to 3. Two patients showed no cutaneous signs of systemic sclerosis and were therefore grouped under the subtype VEDOSS. Cohort 2 comprised 19 test persons with atopic eczema (9 male, 10 female), cohort 3 included 22 test persons with

Test Group	Sex Ratio	Age
Limited SSc	11:2	64 ± 20
Diffuse SSc	2:1	57 ± 23
VEDOSS	2:0	61 ± 16
Atopic eczema	10:9	44 ± 17
Acne vulgaris	14:8	22 ± 17
Healthy subjects	9:3	43 ± 19

Table 2.1 Demographics of test subjects. $n = 71$. The table shows the sex ratio (female:male) and mean age ($\mu \pm \sigma$) of test subjects at baseline of the study.

acne vulgaris (8 male, 14 female), and cohort 4 included 12 test subjects with healthy skin (3 male, 9 female).

Systemic sclerosis patients were recruited through the above mentioned interdisciplinary consultation hour. Study patients with acne vulgaris or atopic eczema were either recruited directly through the clinic of dermatology or through a flyer, which was distributed in the clinic and in a dermatology practice in Munich.

For a comparison, we chose patients with atopic eczema (AE) as dermatologic disorder with dry skin and often times defect skin barrier. We also chose patients with acne vulgaris (AV), because patients with this disorder are a clinically well defined group and we assumed they have an intact skin barrier.

Exclusion criteria were: (1) being under the age of 18, (2) pregnancy, (3) systemic corticosteriod therapy within the past 7 days; (4) any application of topical drugs at

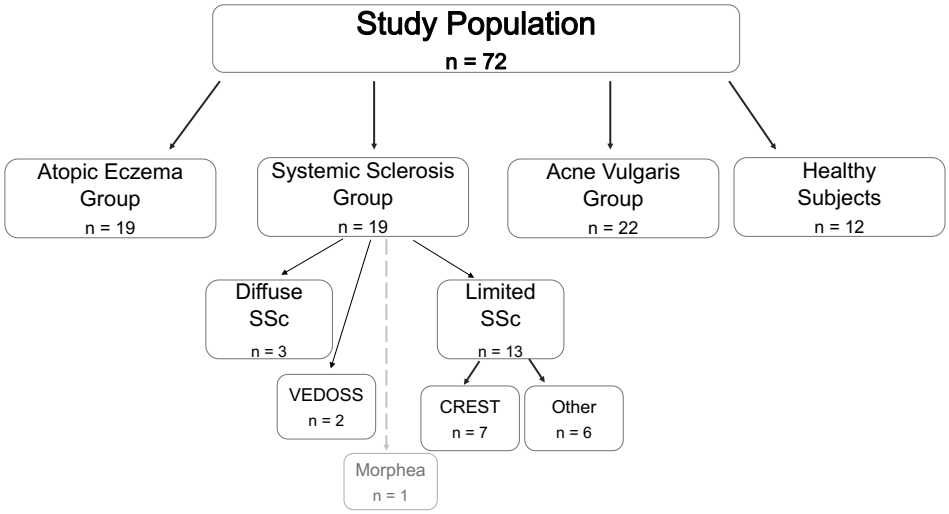

Figure 2.1 Study population. Illustration showing an overview of the different cohorts of the study population of this clinical study.

least 24h before measurements with the exception of applying regular moisturisers. Also, the patient with morphea was excluded from further analyses, because it is not a form of systemic sclerosis.

2.3 Clinical Characteristics

In order to confirm the progression of systemic sclerosis and the individual severity of skin and organ fibrosis, each patient with systemic sclerosis was undertaken clinical examination and careful anamnesis. During the interdisciplinary consultation hours, study patients with systemic sclerosis were analysed for the following clinical characteristics during their first visit and further evaluated during follow-up visits: modified Rodnan skin score, subtype of systemic sclerosis, underlying diseases, first appearance of sclerosis specific symptoms, skin changes such as the presence of digital ulcers, telangiectasias, contracted tongue frenulum, swelling of the hands and fingers (for a full list see Table 2.2). Organ-oriented diagnostics also were part of the general examination of patients. Patients were investigated for symptoms of abnormalities of inner organs such as the esophagus, lung, heart or the gastrointestinal tract. Other evaluated parameters were the date of the first onset of Raynaud's phenomenon, the current treatment of the disease and laboratory parameters such as blood count, CRP, clinical chemistry (including liver function and renal function), and antinuclear-antibodies (ANA). Data for laboratory parameters used in this study and clinical characteristics were taken from previous medical records.

2.4 Objective Parameters

The following devices were used to conduct skin measurements: the condenser-chamber TEWL instrument AquaFlux AF200 (Biox Systems Ltd., London, UK), the Corneometer CM 820 and the Skin-pH-Meter PH 900 (Courage and Khazaka Electronic GmbH, Cologne, Germany). As mentioned in the introduction, the aim of this study was testing the diagnostic potential of skin measurements in systemic sclerosis and to learn more about the skin attributes of the disease.

2.4.1 AquaFlux: a TEWL Measurement Instrument

The AquaFlux model AF200 is a measurement device, which determines the transepidermal water loss (TEWL). In this study, it was used in combination with the BioX AquaFlux Software Version 9.1. As described by Imhof et al. [2009], the AquaFlux uses a condenser-chamber-measurement method. In the following we give a short description of the AquaFlux and its measurement method as described by Imhof et al. [2009] and in the instruction manual of the AquaFlux [AquaFlux200, 2016]. This device, illustrated in Fig.

Category	**Diagnostic Procedure**
General	Weightloss in the last 6 months, profession, alcohol-abuse, nicotine-abusue, underlying diseases, family history, relatives with SSc, allergies, contraception
SSc-specific skin symptoms	First appearance of sclerosis specific symptoms, first appearance of Raynaud's phenomenon, hardened skin areas, previous biopsies, SSc subtype
Previous illnesses	High blood pressure, low blood pressure, diabetes, polyneuropathy, rheumatological disorders, autoimmune disorders, cancer, thrombosis, heart attacks, stroke, asthma, chronic obstructive pulmonary disease (COPD), infectious gastrointestinal disorders, depression
Previous treatment	Cortisone cream, cortisone tablets, aspirin, pain-medicine, azathioprine, methotrexate, mycophenolate mofetil, cyclophosphamide, nifedipine, iloprost, sildenafil, proton pump inhibitors (PPI), bosentan, lymphatic drainage, paraffin baths, physiotherapy
Organ involvement	*Skin:* Digital ulcerations, skin abnormalities. *Musculoskeletal:* joint pain. *Gastrointestinal:* Oesophageal complaints, gastral complaints, intestinal abnormalities. *Heart:* dyspnea, NYHA stadium. *Lung:* pulmonary arterial hypertension (PAH)
Clinical examination	Height, weight, blood pressure, pulse, swelling of hands/fingers, clinical signs for synovitis, tendon rubbing, contractures, muscle strength, mRSS, telangiectansias, microstomia, shortened tongue frenulum
Laboratory parameters	Blood count, erythrocyte sedimentation rate, serum chemistry, antinuclear antibodies
Systemic diagnostics	Pulmonary function test, 6min-walking-test, electrocardiography, echocardiography, abdominal sonography, oesophageal manometry, chest X-ray, chest HR-CT

Table 2.2 Clinical parameters of systemic sclerosis patients. Each patient with suspected or diagnosed systemic sclerosis was characterized for most of these elements during the interdisciplinary consultation hour.

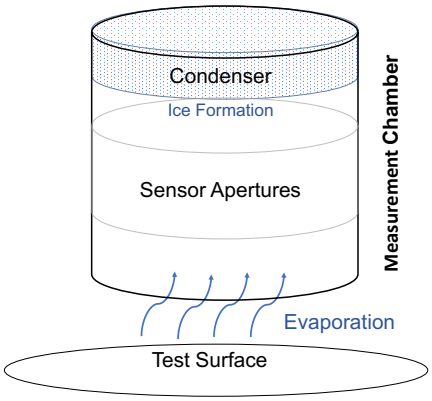

Figure 2.2 The condenser-chamber of the AquaFlux. The illustration shows a test surface, e.g. the skin, from which water evaporates into the closed measurement chamber (illustrated in the form of a hollow cylinder) and then forms ice on the condenser. The chamber consists of sensor apertures (in the middle of the chamber) and is closed by a condenser (on the top). Adapted from [Imhof et al., 2009, p. 101, Figure 3].

2.2, consists of a measurement chamber of a defined volume, in which sensor apertures for humidity and temperature are located. During the measurement period of about 90 seconds, water evaporates from the skin and is captured in the measurement chamber of the instrument. This water vapour accumulates as ice on the condenser. Consequently, the humidity of the air in the measurement chamber is high near the skin and low near the condenser. Sensor apertures in the condenser-chamber probe measure the flux density $[g/(m^2h)]$, which is the flow of water through the measurement chamber. Indirectly, this correlates to the steady-state flux density of water diffusing through the skin barrier.

It is important that the contact between the measurement chamber and the skin is tightly sealed by adding enough pressure to the instrument while pressing it perpendicularly to the skin surface. This way the water evaporating from the skin is solely captured in the measurement chamber. A microprocessor then calculates the transepidermal water loss (TEWL), which is the steady state flux density or flow of water escaping from the skin surface. The value it calculates is the evaporation rate and is expressed in grams per hour per square meter $[g/(m^2h)]$.

2.4.2 Corneometer

The skin surface hydration or water content of the skin in the stratum corneum is measured with the corneometer (model: Corneometer CM 820). As described in the instruction manual by Courage and Khazaka [Corneometer CM820, 2017], the instrument measures the skin hydration of the stratum corneum using a capacitive measurement method. This measurement principle is based on the dielectric qualities of the epidermal

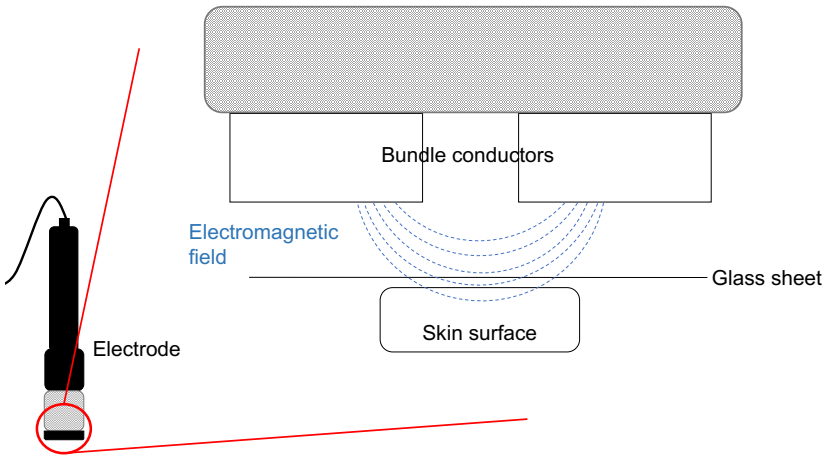

Figure 2.3 The measurement principle of the corneometer. The illustration on the left is the electrode probe. The diameter of the contact area of the device with the skin is about 1.5 cm. The tip of the electrode is enlarged in the middle, showing an illustration of the inside of the corneometer electrode. The probe, indicated in grey, is connected to the metal bundle conductors, shown as the two white squares. These generate an electromagnetic field (indicated in blue). The bundle conductors are separated from the skin surface by a glass sheet, indicated as the black line. The skin hydration (corneometry) is measured by pressing the tip (black box on the tip of the electrode) against the skin surface and holding it there for about 3 seconds. The illustration is adapted from the corneometer instruction manual [Corneometer CM820, 2017].

stratum corneum. Water has the highest dielectric constant in the skin, therefore an increase in water content raises capacitance values [Seidenari and Giusti, 1995]. Dry skin has a different conductivity than hydrated skin and therefore has a different dielectric quality.

Figure 2.3 illustrates the measurement principle of the corneometer. The electrode probe consists of gold bundle conductors. These are separated from the skin probe through a thin glass sheet. Through an electric field, which is created by the different charges of the bundle conductors, a sensor measures the capacity and calculates the water content of the investigated skin surface. A spring in the probe ensures a constant pressure during measurements and the device has a maximum penetration depth of 10-20 micrometers.

The instrument calculates arbitrary Corneometer® units, ranging from 0 to 120. Very dry skin typically shows values lower than 60 for skin areas at the arms, hands, legs, and elbows. Skin of the forehead, cheek, chest, back, and neck under 75 corneometer units. Dry skin displays values between 60-75 for the first mentioned skin areas and 75-85 for the later mentioned skin areas. Normal or sufficiently moist skin displays values above 75 corneometer units for arms, hands, legs, and elbows areas and above 85 for the forehead, cheek, chest, back, and neck areas.

Corneometry is a well established measuring method and has been used in many studies (the link to multiple papers on corneometry can be found on the courage and khazaka website: *https://www.courage-khazaka.de/en/downloads-en/category/study-lists*; for example papers from Constantin et al. [2014] and Korponyai et al. [2017]).

2.4.3 Skin-pH-Meter

The measurement of the skin surface pH was performed using the Skin-pH-Meter PH 900 (Courage and Khazaka Electronic GmbH, Cologne, Germany). A normal pH of the skin ranges from 5.4 to 5.9 [Braun-Falco and Korting, 1986]. The concept of the pH was developed by Schade and Marchionini [1928], who stated that the acid mantle of the skin is built when sweat and sebum are combined and together create a barrier against viruses, bacteria and other potential skin toxins.

Figure 2.4 The pH-meter probe. The glass probe is held in 90 degree angle to the area of the skin one would like to measure, shown in the image is a measurement of the lower part of the arm. Figure retrieved from the Courage and Khazaka website (https://www.courage-khazaka.de/de/downloads/item/foto-probes-ph-arm; 04.01.2019; downloaded 04.11.2019).

As described in the instruction manual of by Courage and Khazaka [Skin-pH-Meter PH905, 2017], the instrument measures the pH of the skin surface. There are two electrodes in one hand-held the probe; see Fig. 2.4 : (1) the reference electrode and (2) the measurement electrode. Through these electrodes, the device measures the electric potential of the skin surface by sensing the hydrogen ion concentration of the skin. It then compares that value to the voltage of a known solution located in the reference electrode of the glass pH probe. To beginn the measurement of the skin pH, the glass pH probe is rinsed with distilled water and after the excess water is shaken off. The glass pH probe is then placed on the respective skin area and held in this position until a value appears on the screen of the Skin-pH-Meter [Prakash et al., 2017].

2.5 Measurement Sites

The measurements were carried out at nine pre-defined skin sites on the left side of the body. As discussed in the introduction, skin fibrosis in SSc patients can be acral, truncal or it can be absent, depending on the individual expression of the disease. Typically, skin involvement is seen at the fingers, hands, and forearms of SSc patients; see ACR/EULAR classification in Table 1.2. The sites measured for the clinical study of this thesis included typical fibrosis sites (e.g. extremities: finger, hand, forearm, foot) as well as sites generally not as affected by skin fibrosis (e.g. face and trunk). Figure 2.5 shows an illustration of these measurement sites. Skin parameters (TEWL, skin hydration, and pH) were measured starting at the (1) left middle finger (between the MCP and PIP), (2) dorsal site of the left hand (in the middle), (3) left forearm (5 cm distal of the antecubal flexure, on the extensor side), (4) median-left side of the forehead, (5) left cheek, (6) left upper chest (2 cm distal to the collar bone), (7) left upper back, proximal of the scapula, (8) lower left leg, lateral of the tibial bone, and lastly on the (9) dorsal part of the left foot. Due to personal preferences, one of the subjects with systemic sclerosis declined to have the cheek and forehead measured. Otherwise, the same nine sites were measured on test subjects and always measured in the same order, starting with the middle finger of the left hand side.

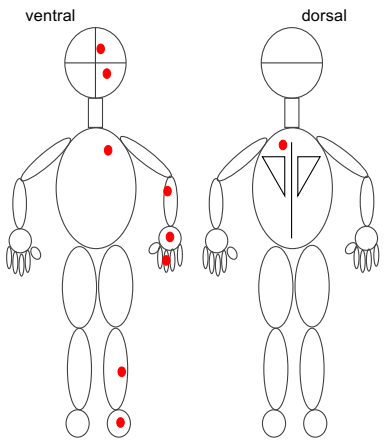

Figure 2.5 Illustration of the nine measurement sites. The red dots show points on the body where TEWL, pH, and skin hydration were measured. **Ventral**, from top to bottom: (1) median-left side of the forehead, (2) left cheek, (3) left upper chest, (4) left forearm, (5) dorsal site of the left hand, (6) left middle finger, (7) lower left leg, lateral of the tibial bone, and lastly on the (8) left foot. **Dorsal**: left upper back, proximal of the scapula.

2.6 Experimental Conditions

The above described skin measurements were performed in a closed room at the clinic for dermatology of the Technical University of Munich. Transepidermal water loss, skin hydration and pH were measured using a standardised procedure on nine predefined points of the body; see Figure 2.5 in Section 2.5. To minimize discrepancies, especially due to perspiration, skin measurements were performed under standardised conditions for temperature and humidity. Measurements were performed in a closed room at an ambient room temperature of about 21°C. To ensure this constant room temperature, air conditioning of the room was set at 21°C at least 30 min before conducting measurements. Every test person was acclimated in the air conditioned room for 10-15 min in a resting position (lying on a stretcher). This acclimatization was important to diminish sweating and thus evaporation, which could confound the replicability of measurements. The amount of time spent on acclimatization varied on the state of humidity and the temperature on the particular measurement day. Figure 2.6 shows the humidity and temperature of all 72 measurements. While the temperature was set at a constant level, the relative humidity in the room varied between a minimum of 25% and a maximum of 55%. Measurements of skin parameters were made in the following order: first transepidermal water loss, starting at the finger and then moving proximal to the hand, forearm, forehead, cheek, then to the chest, back and lastly to the leg and dorsal foot. Secondly, after TEWL measurements, corneometer measurements and lastly pH measurements were carried out.

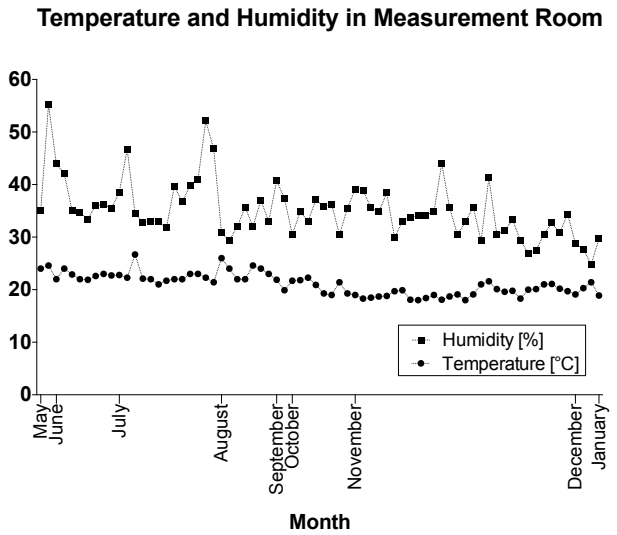

Figure 2.6 Temperature and humidity. Line graph showing the temperature [°C] and humidity [%] in the measurement room in the time period of May 2017 till January 2018. From the analysis of the statistical data one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$). Total mean temperature is 21 ± 2 °C. Total mean humidity is 35 ± 5 %.

2.7 Statistical Analyses

In this section we give a short summary of the main statistical tools used in this study, and list the methods and programs used for the statistical analysis.

A linear regression analysis determines the line of best fit, representing the trend of the data. The coefficient of determination, r^2, is defined as the part of the variance in the dependent variable, so the variable on the y-axis, that is predictable from the independent variable, so the variable on the x-axis. In other words, the value r^2 quantifies the goodness-of-fit of a linear regression models. R^2 is defined as variance explained by the model divided by the total variance. Values of r^2 range between 0.0 and 1.0. The value of r^2 is a measure of how well the logistic regression model explains the outcome. The larger the value is, the better it explains the outcome. Like the coefficient of determination, r^2, the Pearson correlation coefficient, r, describes the strength of the association between two variables. It is defined as the square root of r^2. [McDonald, 2014]

The coefficient of variation (CV) expresses the variance of a distribution. It measures the extent of variability in relation to the mean and is defined as the ratio $CV = \sigma/\mu$ of the standard deviation σ to the mean μ and expressed in percentage. [McDonald, 2014]

The program GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) as well as Microsoft Excel were used for the statistical analysis of our results. All data are displayed as mean ± standard deviation ($\mu \pm \sigma$), unless otherwise specified. Mean TEWL values and skin hydration values were compared using Student's unpaired t-test (two-tailed, with Welch's correlation: unequal SD). Differences in pH values between groups (SSc, AE, AV and HS) were calculated using a Student's unpaired t-test (two-tailed, with equal SD). A p-value of $p < 0.05$ was considered as statistically significant.

The coefficient of variation (CV) was used in this study to show the dispersion of TEWL measurements in each respective group. Differences in CV between groups (SSc, AE, AV and HS) were calculated using a Student's unpaired t-test (two-tailed, with equal SD). A p-value of $p < 0.05$ was considered as statistically significant.

Linear regression was used to investigate the correlation between TEWL values and local modified Rodnan skin score (mRSS) and to investigate the correlation between TEWL and laboratory parameters, respectively GFR values. Linear regression lines, Pearson coefficients (r), and coefficients of determination (r^2), and p-values were calculated using GraphPad Prism 6.0. A p-value of $p < 0.05$ was considered as statistically significant.

Non-linear regression was used to examine the correlation between TEWL and ANA values. Non-linear regression analyses, Pearson coefficients (r), and coefficients of determination (r^2), and p-values were calculated using GraphPad Prism 6.0. A p-value of $p < 0.05$ was considered as statistically significant.

Chapter 3

Results

This chapter comprises the results of the clinical study, which was performed from May 2017 to January 2018 at the clinic for dermatology and allergology of the Technical University of Munich. It is structured as follows: first, clinical features of SSc patients are listed and analysed. Second, an illustration of results of skin parameters, respectively TEWL, skin hydration, and pH, is given. Lastly, we correlate results from skin measurements to clinical features, specifically to mRSS, ANA and GFR.

3.1 Clinical Features of Systemic Sclerosis Patients

As part of the systemic sclerosis consultation hour, several clinical parameters, as listed in Table 2.2 in Chapter 2, were recorded for all patients with systemic sclerosis. Table 3.1 lists three clinical features, which were of interest for a deeper understanding of baseline characteristics of our SSc patients: age at disease diagnosis, duration of systemic sclerosis and the total mRSS. As can be inferred from Table 3.1, the mean age at disease diagnosis of systemic sclerosis was about 54 years (age range: 33-75 years). Hence, SSc duration of our SSc patients was about 12 years for limited SSc patients, and 5 years for diffuse SSc patients. Of the 18 SSc patients, 2 patients (11%) had no clinical skin involvement (VEDOSS), and 16 patients (89%) had skin involvement (15 lcSSc, and 3 dcSSc patients). The skin involvement was evaluated using the modified Rodnan skin score (mRSS), which was on average higher in diffuse SSc subsets (12 ± 7) than in limited SSc subsets (5.8 ± 4.4). All SSc patients had skin scores that were lower than 20, and 5 patients had scores higher than 10.

Test Group	Age at Disease Diagnosis	SSc Duration	Total mRSS
Limited SSc ($n = 15$)	52 ± 12	12 ± 8	5.8 ± 4.4
Diffuse SSc ($n = 3$)	53 ± 19	4.7 ± 4.6	12 ± 7
VEDOSS ($n = 2$)	56 ± 16	5 ± 0	0

Table 3.1 Clinical features of systemic sclerosis patients. The table summarizes the statistical analysis of age at disease diagnosis, SSc duration, and total mRSS for $n = 18$ SSc patients. Shown are the mean value μ and the respective standard deviation σ ($\mu \pm \sigma$).

3.2 Laboratory Parameters of Systemic Sclerosis Patients

We chose to study two laboratory parameters (ANA and GFR), which we assumed might have a correlation to the skin parameters of our study. Literature has shown that autoantibodies (e.g. antinuclear antibodies (ANA)) are diagnostic markers and correlate with disease severity [Gabrielli et al., 2009]. Therefore, in order to understand variables that are associated with the disease progression, it was important to include values for antinuclear antibodies (ANA) in our study. Kidney function is often impaired in SSc patients [Steen and Medsger, 2000]. Hence, we chose to include a renal parameter, the glomerular filtration rate (GFR) for correlation analyses, which will be discussed in Section 3.4.

In this study, a variety of laboratory parameters were recorded for each systemic sclerosis patient (Table 2.2, Chapter 2). Regular blood examinations took place during patient visits at the systemic sclerosis consultation hour (which varied from every 3 months over every 6 months to once a year). For the data acquisition of this study, we

Test Subject	GFR	ANA
dcSSc patient 1	96	1 : 160
dcSSc patient 2	ND	ND
dcSSc patient 3	43	1 : 3840
lcSSc patient 1	49	1 : 160
lcSSc patient 2	61	1 : 3840
lcSSc patient 3	95	1 : 640
lcSSc patient 4	ND	1 : 1280
lcSSc patient 5	89	1 : 12800
lcSSc patient 6	53	1 : 1280
lcSSc patient 7	76	1 : 7680
lcSSc patient 8	71	1 : 640
lcSSc patient 9	69	1 : 2560
lcSSc patient 10	45	1 : 3840
lcSSc patient 11	92	1 : 320
lcSSc patient 12	88	1 : 640
lcSSc patient 13	100	1 : 240
VEDOSS patient 1	83	1 : 1280
VEDOSS patient 2	115	1 : 640

Table 3.2 Glomerular filtration rate and antinuclear antibodies of systemic sclerosis patients. Listed are ANA and GFR values of test subjects with systemic sclerosis ($n = 18$). lcSSc = limited cutaneous systemic sclerosis; dcSSC = diffuse cutaneous systemic sclerosis; ND = not determined.

took the latest laboratory parameters into account. These laboratory parameters included blood count, electrolytes, C-reactive protein (CRP), liver enzymes, renal parameters such as glomerular filtration rate (GFR), and antinuclear antibodies (ANA). Table 3.2 lists ANA and GFR values obtained from our systemic sclerosis patients.

As can be seen Table 3.2, ANA values found in patients of this study showed extreme scattering, ranging from a $1:160$ to a $1:12800$ serum dilution. We assume the scattering is most likely attributed to the heterogeneity of systemic sclerosis. We also calculated mean GFR values (and the respective standard deviation) for patients with limited cutaneous systemic sclerosis (68 ± 19), for diffused cutaneous systemic sclerosis (70 ± 38) and for patients with VEDOSS subtype (99 ± 23). However, since the number of patients was only $n = 2$ for dcSSc and VEDOSS, the relevance of the mean for these values is questionable.

3.3 Skin Parameters

The objective of this clinical study was testing the prospective application of skin functional measurements or skin parameters for the diagnostics of systemic sclerosis. We determined values of three skin parameters: TEWL, skin hydration, and pH for four different skin conditions: systemic sclerosis, atopic eczema, acne vulgaris and healthy skin. The results obtained for the three skin parameters will be discussed in the following sections. The data shown in Figures 3.1, 3.3, and 3.4 has been presented as an E-poster at the EAACI (European Academy of Allergy and Clinical Immunology) in Munich in May 2018 (Access to the E-poster can be found in the EAACI Media Library: *http://webcast.eaaci.cyim.com/*) and as a scientific poster at ADF (Arbeitsgemeinschaft Dermatologischer Forschung) Conference in Zurich, Switzerland in March 2018.

3.3.1 Transepidermal Water Loss Values

As stated in Chapter 1, the aim of this doctoral thesis was testing the diagnostic potential of skin functional measurements in systemic sclerosis in the setting of a clinical study.

The statistical analysis of transepidermal water loss (TEWL) values lead to three important general insights. First, unpaired Student's t-tests show no significant difference between TEWL values of subjects with systemic sclerosis and subjects with healthy skin. Second, there is little significant difference between TEWL values of subjects with systemic sclerosis and those with acne vulgaris. Figure 3.1A, for example, shows that TEWL values of the finger of systemic sclerosis patients are not significantly different to those of acne vulgaris test subjects ($[p] = 0.3$, see Table 3.4). Third, when comparing TEWL values of subjects with systemic sclerosis and those with atopic eczema, statistical analyses show significant differences between TEWL values of these two subject groups. Figure 3.1A clearly shows a significant difference ($[p] = 0.04$, see Table 3.4) between TEWL values of the finger for patients with systemic sclerosis and those with atopic

eczema. TEWL values of the hand are also significantly different in subjects with systemic sclerosis compared to subjects with atopic eczema ($[p] = 0.002$, see Table 3.4). Differences in TEWL values were most prominent in measurements made at the forearm; see Fig. 3.1C. Here, significant differences are observed in systemic sclerosis subjects, when comparing TEWL values to atopic eczema ($[p] < 0.0001$) and to acne vulgaris ($[p] = 0.006$). However, no significant difference are found between SSc and subjects with healthy skin ($[p] = 0.7$); see Table 3.4.

In addition, mean transepidermal water loss values are typically lower in SSc patients than in subjects with acne vulgaris, atopic eczema or healthy skin. For example, the mean TEWL value in the hand is 17.1 g/hm^2 for patients with systemic sclerosis; see Table 3.3. In contrast, it is 35.1 g/hm^2 in subjects with atopic eczema, 18.9 g/hm^2 in subjects with acne vulgaris, and 19.8 g/hm^2 in subjects with healthy skin. Since the TEWL value describes the water loss over the skin surface, this suggests that the low TEWL value in systemic sclerosis means that these patients have a more intact skin barrier or less evaporation of water across this barrier. Lower values for transepidermal water loss could also result from the pathological atrophy of sweat glands [Katsumoto et al., 2011]. Less sweating could in turn lead to less water loss across the skin barrier. As a comparison, typical mean TEWL values for the back of the left hand are 21.2 g/hm^2 for healthy adults [Akdeniz et al., 2018, Kottner et al., 2013]. Kottner et al. [2013] performed a systematic review and meta-analysis of TEWL values is healthy adults, in which he focused on identifying generalized TEWL values for different skin areas.

Measurements of the TEWL of the skin surface of the cheek (Fig. 3.1D) and the forehead (Fig. 3.1E) show no significant differences between systemic sclerosis and acne vulgaris as well as healthy skin; see p-values in Table 3.4. However, there is a

TEWL	Atopic eczema	Systemic sclerosis	Acne vulgaris	Healthy subjects
Finger	35.8 ± 15.4	27 ± 7.9	30.1 ± 12.1	29.3 ± 13.2
Hand	35.1 ± 21.0	17.1 ± 5.2	18.9 ± 5.4	19.8 ± 9.0
Forearm	26.0 ± 12.4	10.7 ± 1.8	12.8 ± 2.8	10.4 ± 2.0
Forehead	37.9 ± 19.8	20.7 ± 4.8 (n=17)	26.0 ± 13.1	26.1 ± 13.4
Cheek	28.4 ± 10.2	20.1 ± 5.2 (n=17)	26.6 ± 14.0	18.7 ± 5.9
Chest	21.8 ± 15.9	9.4 ± 1.7	14.1 ± 8.2	9.7 ± 2.0
Back	37.7 ± 28.6	11.6 ± 5.3	13.7 ± 4.5	13.0 ± 7.1
Leg	24.6 ± 13.3	11.8 ± 3.8	13.9 ± 5.5	15.4 ± 15.5
Foot	35.6 ± 23.4	19.4 ± 7.5	23.9 ± 14.7	23.2 ± 18.0

Table 3.3 Mean values of transepidermal water loss measurements of four test subject groups measured at nine skin sites. Listed are TEWL values of four different test subject groups: atopic eczema (n = 19), systemic sclerosis (n = 18), acne vulgaris (n = 22), and healthy subjects (n = 12). From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$).

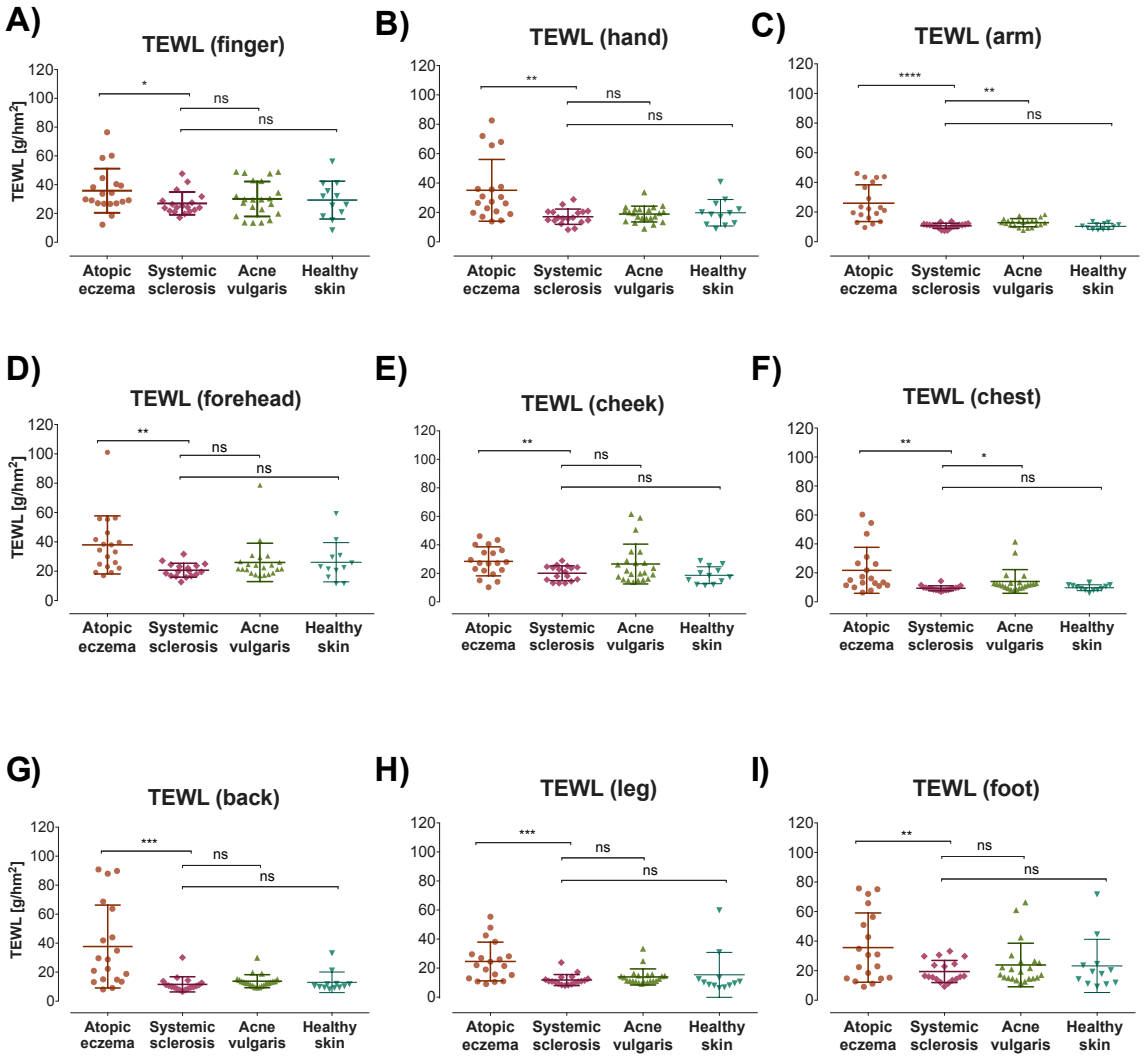

Figure 3.1 Transepidermal water loss (TEWL) of nine measurement sites. Scatter dot plots for TEWL values of test subjects with different skin conditions, as indicated in the graph. Shown are mean values and standard deviations indicated as error bars. The different panels show the results obtained from TEWL measurements performed on the **A)** finger, **B)** hand, **C)** forearm, **D)** forehead, **E)** cheek, **F)** chest, **G)** back, **H)** leg, and **I)** foot. From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$). Mean TEWL values are listed in Table 3.3. P-values are indicated as such: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant. P-values are listed in Table 3.4.

significant difference between TEWL values of subjects with systemic sclerosis compared to subjects with atopic eczema (forehead: $[p] = 0.0015$, cheek: $[p] = 0.004$; see Table 3.4). As discussed in Chapter 1, transepidermal water loss is an important trait for characterising skin barrier function. In patients with atopic eczema the skin barrier is impaired. Therefore, we generally expected higher TEWL results for atopic eczema. As a reference, Seidenari and Giusti [1995] found TEWL values of around 30 g/hm^2 in involved skin of atopic eczema patients. Hence, a significant difference between atopic eczema and systemic sclerosis is due to the fact that atopic eczema has such increased TEWL values.

Significant differences between TEWL values can be seen in measurements made at the skin surface of the chest (Fig. 3.1F). Results of TEWL measurements show significant differences in patients with systemic sclerosis compared to atopic eczema ($[p] = 0.003$) and to acne vulgaris ($[p] = 0.015$). TEWL values of healthy subjects are not significantly different; see p-value in Table 3.4.

Figure 3.1G shows TEWL values made of the dorsal side (back) of test subjects. These measurements show a set of outliers in data points (TEWL-value > 80 g/hm^2) in test subjects with atopic eczema. This could be caused by a variety of reasons, for example due to humidity, temperature, or stress-level. In the discussion, possible causes will be analysed in further detail.

In Fig. 3.1H one can see a significant difference in the transepidermal water loss of the leg between systemic sclerosis and atopic eczema subjects ($[p] = 0.0006$). In comparison, there is no significant difference when comparing values derived from systemic sclerosis patients to those with acne vulgaris ($[p] = 0.15$) and healthy subjects ($[p] = 0.4$).

In accordance with the results obtained for the leg, TEWL values of the foot (Fig. 3.1I) show similar results. Again, there is no significant difference between patients with

TEWL	SSc and AE	SSc and AV	SSc and HS
Finger	$[p] = 0.04$	$[p] = 0.3$	$[p] = 0.6$
Hand	$[p] = 0.002$	$[p] = 0.3$	$[p] = 0.4$
Forearm	$[p] < 0.0001$	$[p] = 0.006$	$[p] = 0.6$
Forehead	$[p] = 0.0015$	$[p] = 0.09$	$[p] = 0.2$
Cheek	$[p] = 0.004$	$[p] = 0.06$	$[p] = 0.5$
Chest	$[p] = 0.003$	$[p] = 0.015$	$[p] = 0.6$
Back	$[p] = 0.0009$	$[p] = 0.2$	$[p] = 0.6$
Leg	$[p] = 0.0006$	$[p] = 0.2$	$[p] = 0.4$
Foot	$[p] = 0.008$	$[p] = 0.2$	$[p] = 0.5$

Table 3.4 Pairwise comparison of TEWL results obtained from systemic sclerosis patients to three skin conditions: atopic eczema, acne vulgaris, and healthy skin. From the statistical analysis one obtains the respective p-values as indicated in the table.
SSc: systemic sclerosis, AE: atopic eczema, AV: acne vulgaris, HS: healthy subjects.

systemic sclerosis and those with acne vulgaris ($[p] = 0.2$) and in healthy skin subjects ($[p] = 0.5$).

There are three main conclusions from statistical analyses of TEWL measurements performed in this study. (1) In the comparison of systemic sclerosis and acne vulgaris, the most significant differences are found in measurements made at the forearm. Hence, (2) when comparing patients with systemic sclerosis and with atopic eczema, TEWL values are significantly different at all nine measurement sites. This suggests that (3) TEWL values of systemic sclerosis patients are not significantly different to TEWL values of test subjects with healthy skin. Implications of these results are discussed in more depth in Chapter 4.

3.3.2 TEWL Values are Narrowly Distributed in SSc Patients

Figure 3.1 has shown us that transepidermal water loss (TEWL) values of test subjects with systemic sclerosis are narrowly distributed. In contrast, in atopic eczema, acne vulgaris, and healthy test subjects, TEWL results are more broadly distributed.

This distribution can be quantified through of the coefficient of variation (CV). As described in the Section 2.7, the coefficient of variation (CV) measures the dispersion or variance of a distribution. It is defined as the ratio of the standard deviation to the mean ($CV = \sigma/\mu$). The TEWL values of the four cohorts are shown in Figure 3.2 as a bar plot of the coefficient of variation with the standard deviation indicated by the error bars. The mean CV for the systemic sclerosis group is 29%, which is half of that of

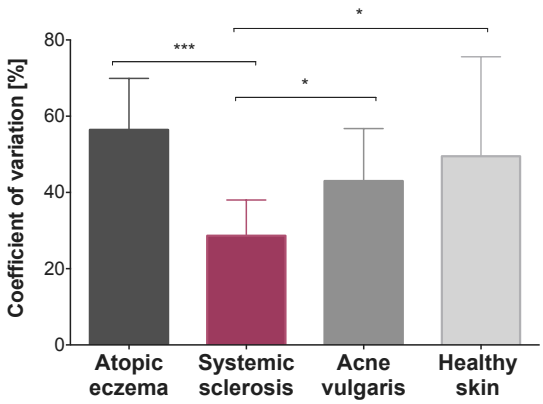

Figure 3.2 Coefficient of variation (CV) for TEWL values. Column bar graph for the coefficient of variation for TEWL values of test subjects with different skin conditions, as indicated in the graph. Shown are columns, indicating the mean value and the standard deviation indicated by the error bar. From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$) : atopic eczema: 56% ± 13.5%, systemic sclerosis: 29% ± 9%, acne vulgaris: 43% ± 14%, healthy skin: 49.5% ± 26%. P-values are indicated as such: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

the atopic eczema group (CV = 56%). Compared to TEWL values of test subjects with healthy skin (CV = 49,5%) or subjects with acne vulgaris (CV = 43%), test subjects with systemic sclerosis have less dispersion (CV = 29%). These findings suggest that under comparable conditions, TEWL values are more narrowly distributed in SSc patients. One concludes that even though systemic sclerosis is clinically a very heterogenous disease, TEWL values show homogenous results. Homogenous TEWL results suggest that the skin barrier of all systemic sclerosis patients in this study is similarly altered, even though patients had different clinical features; see Section 3.1.

3.3.3 Skin Hydration Values

Previous studies state that typical values for capacitance measurements in subjects with healthy skin range from 53 to 90 arbitary units (a.u.) of the corneometer and values in subjects with atopic eczema range from 21 to 77 a.u. of the corneometer [Lodén et al., 1992, Seidenari and Giusti, 1995, Sator et al., 2003]. In our study, mean skin hydration values of the 71 test subjects ranged from 21.1 to 64.8 a.u. of the corneometer; see Figure 3.3 and Table 3.5. We observed no significant difference ($p > 0.05$) between the skin hydration of test subjects with systemic sclerosis and those with acne vulgaris as well as to those with healthy skin; see Fig. 3.3 and Table 3.6.

Similar to results obtained from TEWL measurements, there are significant differences between test subjects with systemic sclerosis and subjects with atopic eczema (Fig. 3.3). Seven measurement sites showed significant differences between skin hydration values of test subjects with systemic sclerosis and those with atopic eczema; see p-values in Table 3.6. In our interpretation, these significant differences are due to decreased values

Skin hydration	Atopic eczema	Systemic sclerosis	Acne vulgaris	Healthy subj.
Finger	25.2 ± 8.0	32.2 ± 10.9	32.9 ± 9.2	38.6 ± 8.6
Hand	21.1 ± 9.8	35.9 ± 10.3	39.8 ± 14.4	35.3 ± 7.4
Forearm	26.4 ± 11.4	34.6 ± 11.3	35.8 ± 6.0	41.2 ± 6.3
Forehead	41.4 ± 16.3	51.9 ± 10.8 (n=17)	55.1 ± 11.6	56.4 ± 12.1
Cheek	47.3 ± 13.7	57.1 ± 9.5 (n=17)	56.8 ± 11.0	54.5 ± 10.1
Chest	41.3 ± 17.3	56.2 ± 14.0	57.1 ± 10.8	61.8 ± 15.7
Back	38.8 ± 16.9	54.6 ± 12.2	55.1 ± 9.8	64.8 ± 14.6
Leg	26.7 ± 11.0	32.1 ± 9.2	38.6 ± 12.4	39.7 ± 12.4
Foot	27.7 ± 11.7	34.8 ± 10.4	38.1 ± 10.0	40.8 ± 9.0

Table 3.5 Mean values of corneometer measurements of four test subject groups measured at nine skin sites. Listed are skin hydration values of four different test subject groups: atopic eczema (n = 19), systemic sclerosis (n = 18), acne vulgaris (n = 22), and healthy subjects (n = 12). All measurements were carried out on the left side of the body. From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$).

of skin hydration in atopic eczema. As discussed, for example, by Lodén et al. [1992], skin hydration is significantly lower in atopic eczema patients than in healthy control subjects. Hence, a significant difference between atopic eczema and systemic sclerosis is due to the fact that atopic eczema has decreased skin hydration values.

Skin hydration	SSc and AE	SSc and AV	SSc and HS
Finger	$[p] = 0.03$	$[p] = 0.8$	$[p] = 0.08$
Hand	$[p] < 0.0001$	$[p] = 0.3$	$[p] = 0.8$
Forearm	$[p] = 0.03$	$[p] = 0.7$	$[p] = 0.05$
Forehead	$[p] = 0.03$	$[p] = 0.4$	$[p] = 0.3$
Cheek	$[p] = 0.017$	$[p] = 0.9$	$[p] = 0.5$
Chest	$[p] = 0.007$	$[p] = 0.8$	$[p] = 0.3$
Back	$[p] = 0.002$	$[p] = 0.9$	$[p] = 0.06$
Leg	$[p] = 0.1$	$[p] = 0.06$	$[p] = 0.09$
Foot	$[p] = 0.06$	$[p] = 0.3$	$[p] = 0.1$

Table 3.6 Pairwise comparison of skin hydration results obtained from systemic sclerosis patients to three skin conditions: atopic eczema, acne vulgaris, and healthy skin. From the statistical analysis one obtains the repective p-values, as indicated in the table.
SSc: systemic sclerosis, AE: atopic eczema, AV: acne vulgaris, HS: healthy subjects.

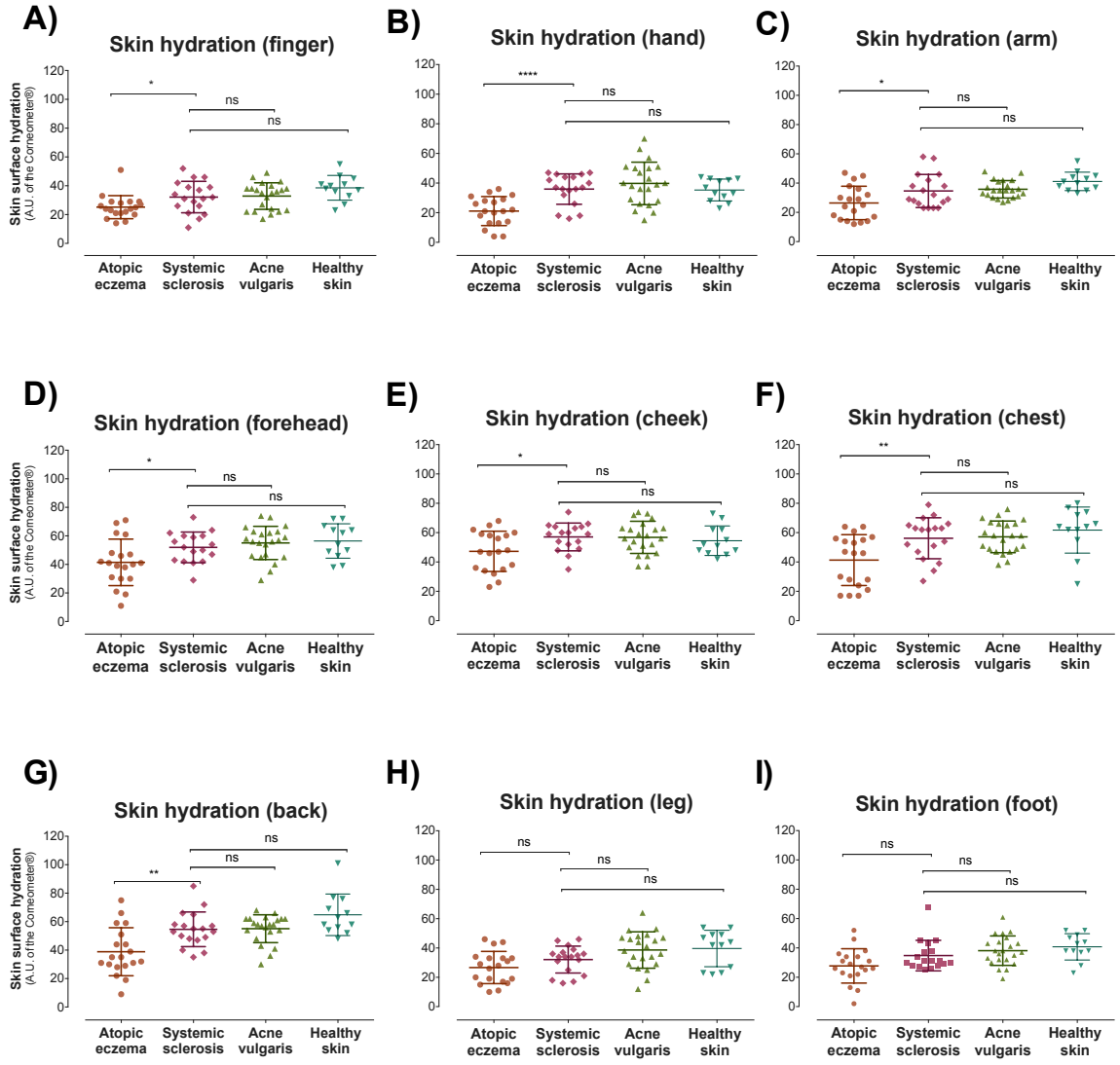

Figure 3.3 Skin hydration of nine measurement sites. Scatter dot plots for corneometer values of test subjects with different skin conditions, as indicated in the graph. Shown are mean values and standard deviations indicated as error bars. The different panels show the results obtained from corneometer measurements performed on the **A)** finger, **B)** hand, **C)** forearm, **D)** forehead, **E)** cheek, **F)** chest, **G)** back, **H)** leg, and **I)** foot. From the statistical analysis, one obtains the mean values μ and respective standard deviation σ ($\mu \pm \sigma$), as listed in Table 3.5. P-values are indicated as such: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant. P-values are listed in Table 3.6.

3.3.4 Skin pH Values

Lastly, we measured pH values of systemic sclerosis patients and compared these to test subjects with atopic eczema, acne vulgaris and healthy skin. Previous studies have found that pH values are significantly higher in involved skin of atopic eczema patients [Eberlein-König et al., 2000] than in the healthy skin of control subjects. In other skin diseases such as psoriasis, pH values have also been studied [Cannavò et al., 2017] and showed lower values in involved skin than in healthy skin. PH values in the skin of systemic sclerosis patients have up to today not been determined.
Figure 3.4 shows results our pH measurements. Mean pH values range from 5.2 to 5.8 (Table 3.7). For most of the nine measurement sites, scatter dot plots, as illustrated in Fig. 3.4, show no significant differences between pH values of all four cohorts.
However, pH values of measurements of the back (Fig. 3.4G) are significantly different in patients with SSc. At this measurement site, patients with systemic sclerosis showed mean pH values of $\mu = 5.8$. Subjects with atopic eczema, acne vulgaris and healthy skin showed lower mean pH-values (Table 3.7: atopic eczema: $\mu = 5.4$, acne vulgaris: $\mu = 5.3$, healthy skin: $\mu = 5.4$). Significant differences can be seen when comparing SSc values to atopic eczema ($[p] = 0.03$) and to acne vulgaris ($[p] = 0.0016$), as well as to healthy subjects ($[p] = 0.03$).

Taken together, we calculate that the observed variation of pH values of systemic sclerosis patients is within the bounds typically observed for the pH of healthy adults. On the one hand, Schmid-Wendtner and Korting [2006] state in their review article that the pH of a healthy adult is typically between the bounds of 5.4-5.9 obtained from an overall evaluation of publications. On the other hand, Lambers et al. [2006] disagree and state that the pH is on average 4.7 in healthy adults. These two papers show how complex evaluating data from physical measurements such as pH-measurements is. Hence, the

pH	Atopic eczema	Systemic sclerosis	Acne vulgaris	Healthy subjects
Finger	5.8 ± 0.6	5.7 ± 0.5	5.5 ± 0.6	5.45 ± 0.5
Hand	5.6 ± 0.6	5.4 ± 0.5	5.4 ± 0.5	5.3 ± 0.65
Forearm	5.6 ± 0.6	5.5 ± 0.4	5.5 ± 0.5	5.35 ± 0.6
Forehead	5.45 ± 0.6	5.3 ± 0.4 (n=17)	5.3 ± 0.4	5.2 ± 0.6
Cheek	5.45 ± 0.5	5.45 ± 0.4 (n=17)	5.4 ± 0.4	5.3 ± 0.6
Chest	5.45 ± 0.5	5.6 ± 0.4	5.4 ± 0.5	5.3 ± 0.6
Back	5.4 ± 0.7	5.8 ± 0.4	5.3 ± 0.4	5.4 ± 0.5
Leg	5.45 ± 0.7	5.8 ± 0.6	5.4 ± 0.5	5.5 ± 0.5
Foot	5.45 ± 0.75	5.5 ± 0.5	5.2 ± 0.4	5.3 ± 0.55

Table 3.7 Mean values of pH measurements of four test subject groups measured at nine skin sites. Listed are pH-values of four different test subject groups: atopic eczema (n = 19), systemic sclerosis (n = 18), acne vulgaris (n = 22), and healthy subjects (n = 12). From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$).

pH	SSc and AE	SSc and AV	SSc and HS
Finger	$[p] = 0.6$	$[p] = 0.5$	$[p] = 0.3$
Hand	$[p] = 0.4$	$[p] = 0.96$	$[p] = 0.5$
Forearm	$[p] = 0.8$	$[p] = 0.8$	$[p] = 0.4$
Forehead	$[p] = 0.4$	$[p] = 0.8$	$[p] = 0.6$
Cheek	$[p] = 0.97$	$[p] = 0.9$	$[p] = 0.6$
Chest	$[p] = 0.4$	$[p] = 0.1$	$[p] = 0.2$
Back	$[p] = 0.03$	$[p] = 0.002$	$[p] = 0.03$
Leg	$[p] = 0.2$	$[p] = 0.04$	$[p] = 0.2$
Foot	$[p] = 0.95$	$[p] = 0.06$	$[p] = 0.3$

Table 3.8 Pairwise comparison of pH results obtained from systemic sclerosis patients to three skin conditions: atopic eczema, acne vulgaris, and healthy skin. From the statistical analysis one obtains the repective p-values, as indicated in the table.
SSc: systemic sclerosis, AE: atopic eczema, AV: acne vulgaris, HS: healthy subjects.

skin pH may not be a distinct measure for showing differences in the four skin conditions systemic sclerosis, atopic eczema, acne vulgaris, and healthy skin.

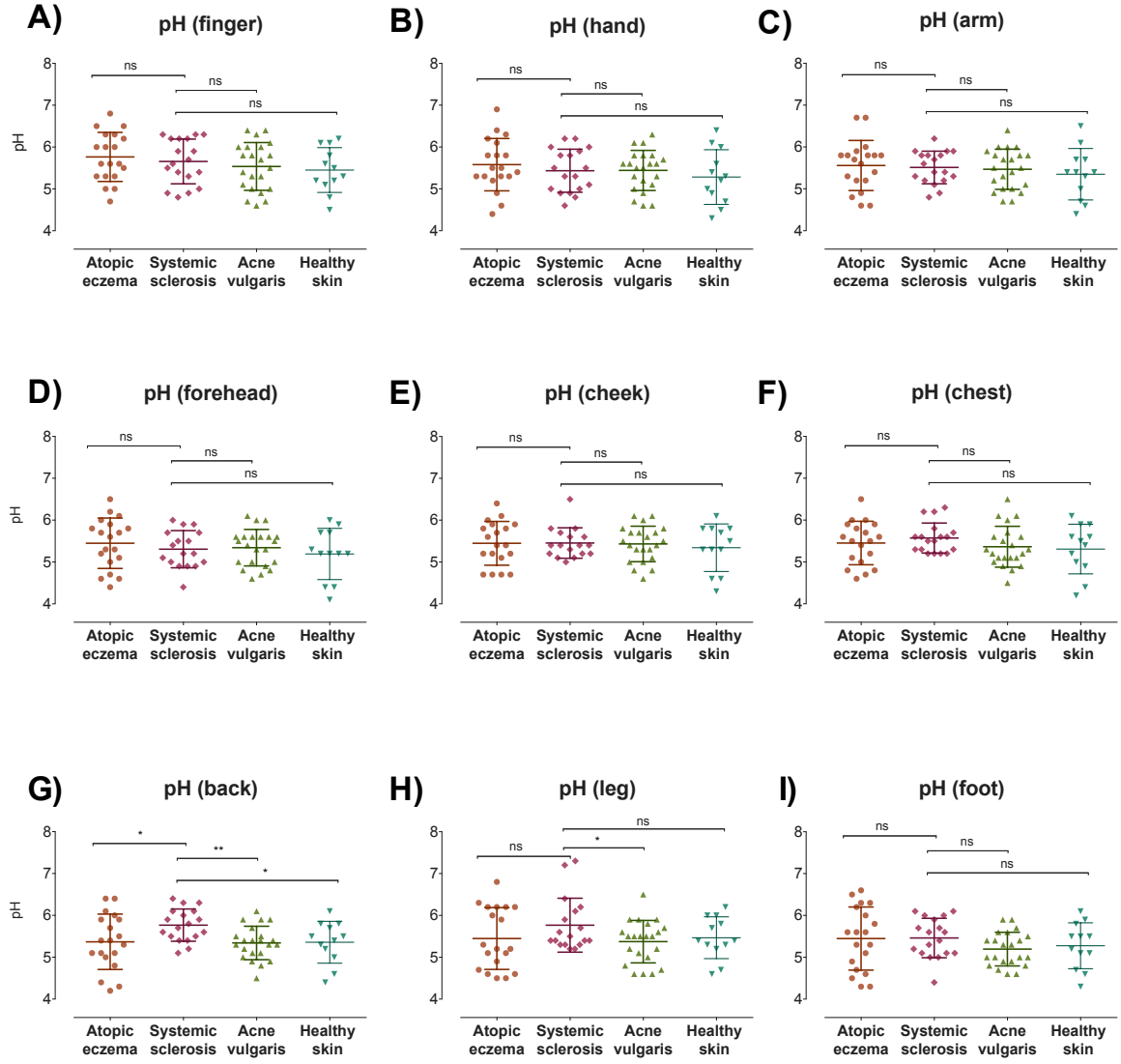

Figure 3.4 Skin pH of nine measurement sites. Scatter dot plots for pH values of test subjects with different skin conditions, as indicated in the graph. Shown are mean values and standard deviations indicated as error bars. The different panels show the results obtained from pH measurements performed on the **A)** finger, **B)** hand, **C)** forearm, **D)** forehead, **E)** cheek, **F)** chest, **G)** back, **H)** leg, and **I)** foot. From the statistical analysis one obtains the mean value μ and respective standard deviation σ ($\mu \pm \sigma$), as listed in Table 3.7. P-values are indicated as such: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant. The p-values are listed in Table 3.8.

3.4 Correlation of Clinical Features with Skin Measurements

After performing skin measurements, we looked for a correlation to various known disease parameters such as the skin score, and laboratory parameters. In the following, we want to investigate the following questions: Are skin parameters of our study (TEWL, skin hydration, and pH) correlated with the modified Rodnan skin score (mRSS)? Do we see a correlation between our results and laboratory measurements such as SSc-related autoantibodies or renal function (e.g. GFR)?

As we have learned in Section 3.3, transepidermal water loss, skin hydration and pH values in systemic sclerosis are not significantly different from test subjects with acne vulgaris and healthy skin. Hence, as such they are not useful as a diagnostic tool for systemic sclerosis. However, when paired with clinical features, they could become informative about the state of disease. Therefore, we explored the correlation of skin measurements with values obtained from the clinical data of systemic sclerosis patients.

Moreover, because we observed the most significant differences between our test subject groups in TEWL values, we focused on these values in the following and performed an in-depth statistical analysis. In particular, we determined Pearson correlations between TEWL values and for various clinical features such as local modified Rodnan skin score (mRSS), ANA values, and glomerular filtration rate (GFR).

3.4.1 TEWL and the Modified Rodnan Skin Score (mRSS)

As discussed in Section 1.2.3, the modified Rodnan skin score (mRSS) is the 'gold standard' for measuring skin thickness. Hence, we analysed whether SSc patients with a higher modified Rodnan skin score (mRSS) and conclusively higher clinical skin thickness show a correlation to their skin hydration, pH, and transepidermal water loss (TEWL) value. We found no correlation of the skin score (mRSS) to the skin hydration or the pH, therefore in the following we discuss only the findings between the mRSS and TEWL values. Since most of our SSc patients (15 of 18 SSc patients) can be grouped in the subtype limited cutaneous systemic sclerosis, their skin involvement is typically limited to the extremities distal to the elbows and knees [LeRoy et al., 1988]. Thus, we focussed on correlation analyses of following three skin sites: finger, hand, and forearm. We correlated TEWL values of the finger, hand, and forearm to the respective local modified Rodnan skin score. To this end, we performed linear regression analyses, which are shown, together with the coefficient of determination r^2 in Fig. 3.5. The value for the coefficient of determination r^2 is very small for all three linear regression lines (Fig. 3.5 A) $[r^2] = 0.002$, B) $[r^2] = 0.002$, C) $[r^2] = 0.1$). Therefore the goodness-of-fit, meaning the extent to which the the observed data matches the theoretically expected values, is not very meaningful. We also determined the Pearson coefficient, r, (Figure 3.5 A) $[r] = -0.04$, B) $[r] = 0.04$, C) $[r] = 0.3$). The Pearson coefficient, as well as the p-value

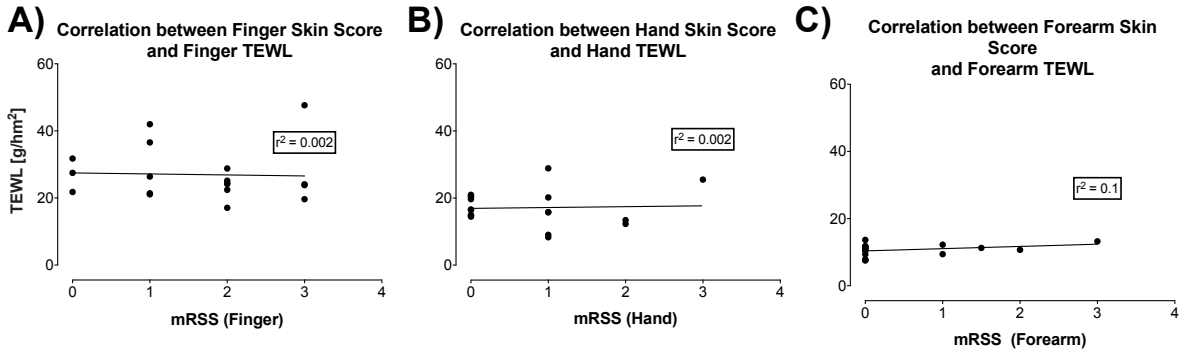

Figure 3.5 Correlation graphs of transepidermal water loss (TEWL) and site specific modified Rodnan skin score (mRSS). Scatter dot plots for the TEWL values compared to the site specific mRSS, as indicated in the graphs. The solid line shows the linear regression line. From the analysis of the statistical data one obtains the respective coefficient of determination r^2: **A)** TEWL of the finger correlated with mRSS of the finger: $[r^2] = 0.002$, **B)** TEWL of the hand correlated with mRSS of the hand: $[r^2] = 0.002$, **C)** TEWL of the forearm correlated with mRSS of the forearm: $[r^2] = 0.1$.

(Fig. 3.5 A) $[p] = 0.9$, B) $[p] = 0.9$, C) $[p] = 0.2$) demonstrate that there is no significant correlation between the TEWL value and the local Rodnan skin score. In conclusion, our data suggests that there is no correlation between TEWL values and the local mRSS.

3.4.2 TEWL and Antinuclear Antibodies (ANA)

Autoantibody formation is one of many characteristics of SSc [Salazar et al., 2015]. Antibodies against nuclear antigens also known als antinuclear antibodies (ANA) are present almost universally in SSc patients [Mierau et al., 2011], are associated with skin involvement and function as a diagnostic tool in the ACR/EULAR classification criteria [van den Hoogen et al., 2013]. We compared this important marker of systemic sclerosis to TEWL values obtained in this study, a value not yet used for diagnostics of systemic sclerosis. For the statistical analysis of our data we used nonlinear regression lines, comparing TEWL values to values obtained for antinuclear antibodies (ANA) (Fig. 3.6). The aim of this analysis was to test whether there are significant correlations between ANA and TEWL values. Figure 3.6 shows the regression analyses for all nine measurement sites. We find that the value for the coefficient of determination, r^2, is very small for all nine regression lines (minimum value for $[r^2] = 0.0002$, maximum value for $[r^2] = 0.2$), indicating that the goodness-of-fit is not informative. We also determined the Pearson coefficient r and the respective p-value (Fig. 3.6 A) finger: $[r] = 0.25$, $[p] = n.s$ B) hand: $[r] = -0.03$, $[p] = n.s$, C) forearm: $[r] = -0.4$, $[p] = n.s$, D) forehead: $[r] = 0.0015$, $[p] = n.s$, E) cheek: $[r] = 0.3$, $[p] = n.s$, F) chest: $[r] = -0.09$, $[p] = n.s$, G) back: $[r] = -0.1$,

$[p] = n.s$, H) leg: $[r] = -0.03$, $[p] = n.s$, I) foot: $[r] = -0.15$ $[p] = n.s$). The Pearson coefficients as well as the calculated p-values demonstrate that there is no significant correlation between the TEWL value and the ANA value. In summary, our data suggest that there is no correlation between TEWL and values obtained for antinuclear antibodies.

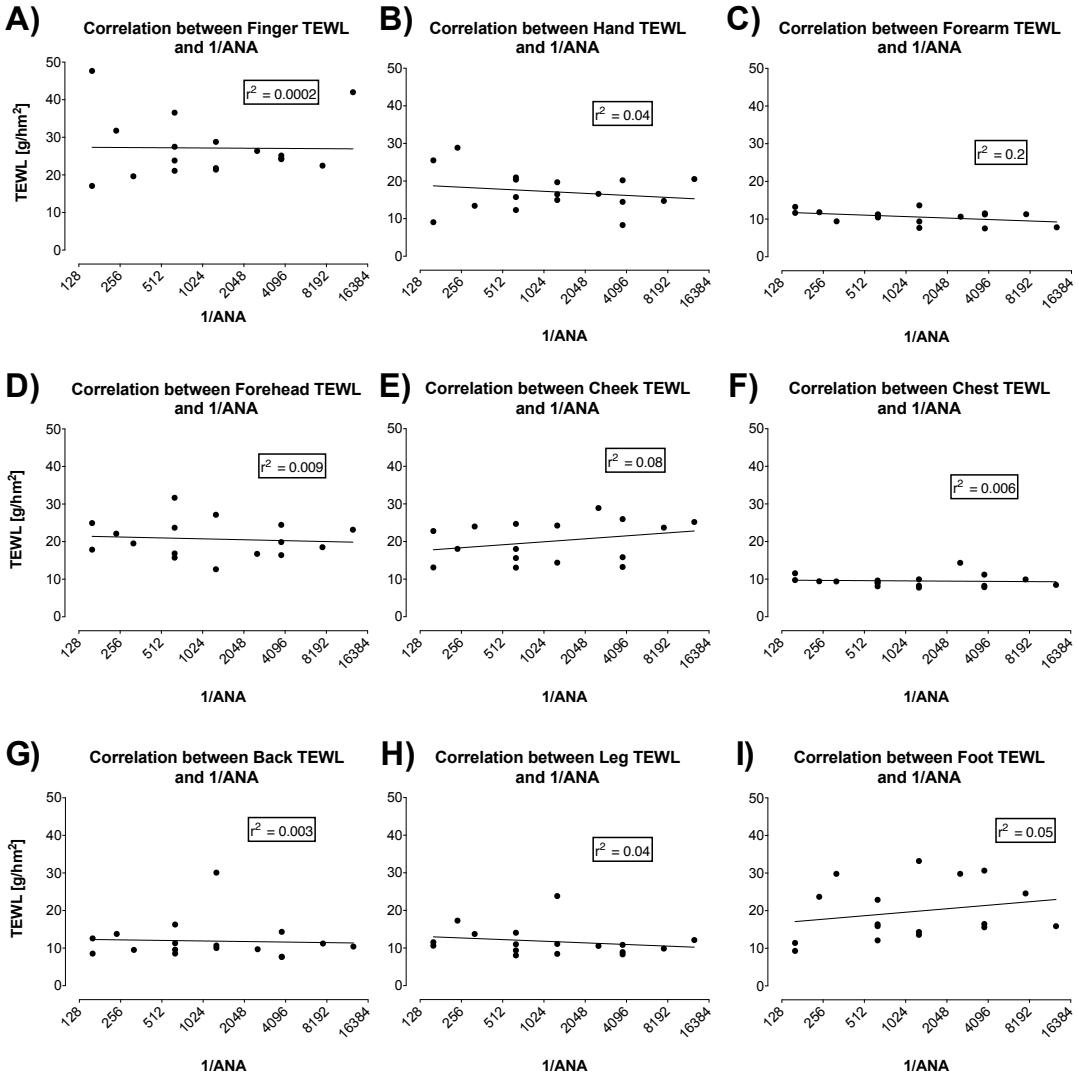

Figure 3.6 Correlation graphs of TEWL and ANA values. Scatter dot plots for site-specific TEWL values as a function of the inverse ANA value on a semi-log scale, where the inverse ANA values are given on a base-2 log scale. The regression analysis was performed for the data on that semi-log scale. From the statistical analysis one obtains the coefficient of determination r^2, as indicated in the respective graphs. The different panels show the results obtained from measurements performed on the **A)** finger, **B)** hand, **C)** forearm, **D)** forehead, **E)** cheek, **F)** chest, **G)** back, **H)** leg, and **I)** foot.

3.4.3 TEWL and the Glomerular Filtration Rate (GFR)

A glomerular filtration rate (GFR) of 90 ml/min or higher is typical for normal kidney function [Herold, 2020]. Hence, a lower GFR value indicates irregular kidney function or even kidney failure, and the GFR is thus an important tool for diagnosing renal disease. In a recent review article, Woodworth et al. [2016] describe renal involvement and dysfunction in systemic sclerosis. They discuss studies, which investigated the effect of renal dysfunction on the glomerular filtration rate and found mostly comorbid causes for the decrease of GFR (such as hypertension, cardiac involvement and other nephropathies not associated with SSc). Overall, Woodworth et al. [2016] conclude that the glomerular filtration rate is not significantly effected by SSc-related renal dysfunction and can not be differentiated from comorbid causes. To this date, researchers have not yet analyzed correlations between renal laboratory parameters such as GFR and physical skin measurements such as transepidermal water loss (TEWL). To test these correlations, we investigated whether skin measurements (specifically transepidermal water loss) of our study showed a correlation to the glomerular filtration rate (GFR).

To this end, we investigated renal laboratory parameters of our 18 SSc patients. First, we evaluated creatinine levels. With the exception of 2 patients, which had a creatinine level of 1.2 mg/dl, all other patients showed normal creatinine levels ($<$ 1.1 mg/dl [Herold, 2020]). Second, we looked at the GFR values (which were calculated with the CKD-EPI formula) of our SSc patients. As can be inferred from Table 3.2, 11 out of 16 patients (2 patients showed no record of GFR values) showed an irregular kidney function, e.g. decreased GFR values at the time of measurement. Five SSc patients showed GFR values $>$ 90 ml/min, seven SSc patients had GFR values between 60 and 89 ml/min, and 4 had GFR values $<$ 60 ml/min. Of the 2 patients with the elevated creatinine level, both had a measured GFR $<$ 50 ml/min, one with a limited cutaneous subtype of SSc (GFR $=$ 45 ml/min), and the other diffuse cutaneous SSc (GFR $=$ 43 ml/min).

In a next step, we correlated GFR values to TEWL values. Figure 3.7 shows linear regression analyses of TEWL and GFR values. We found that the TEWL of the hand and forehead showed a correlation with GFR values (Fig. 3.7B hand: $[r] = 0.59$, $[r^2] = 0.34$, $[p] = 0.02$ and Fig. 3.7D forehead: $[r] = 0.54$, $[r^2] = 0.29$, $[p] = 0.04$). As indicated in Fig. 3.7B and D, there is a positive correlation between GFR and TEWL values of the hand and forehead. For example, for a TEWL value of 9 g/hm^2, the GFR was 49 ml/min. In contrast, the correlation was low between TEWL and GFR values of the finger, forearm, cheek, chest, back, leg, and foot (See Fig. 3.7A) finger: $[r] = 0.297$, $[r^2] = 0.09$, $[p] = n.s$, C) forearm: $[r] = -0.05$, $[r^2] = 0.003$, $[p] = n.s$, E) cheek: $[r] = 0.28$, $[r^2] = 0.08$, $[p] = n.s$, F) chest: $[r] = -0.13$, $[r^2] = 0.02$, $[p] = n.s$, G) back: $[r] = 0.2$, $[r^2] = 0.04$, $[p] = n.s$, H) leg: $[r] = 0.12$, $[r^2] = 0.01$, $[p] = n.s$, I) foot: $[r] = 0.11$, $[r^2] = 0.01$, $[p] = n.s$).

In summary, our data suggests that a low value for transepidermal water loss of a patient with systemic sclerosis correlates with a low GFR value. This correlation is only

evident in the TEWL value at the hands, as indicated by the red-framed graph in Fig. 3.7. Hence, a low TEWL value could predict a low GFR value.

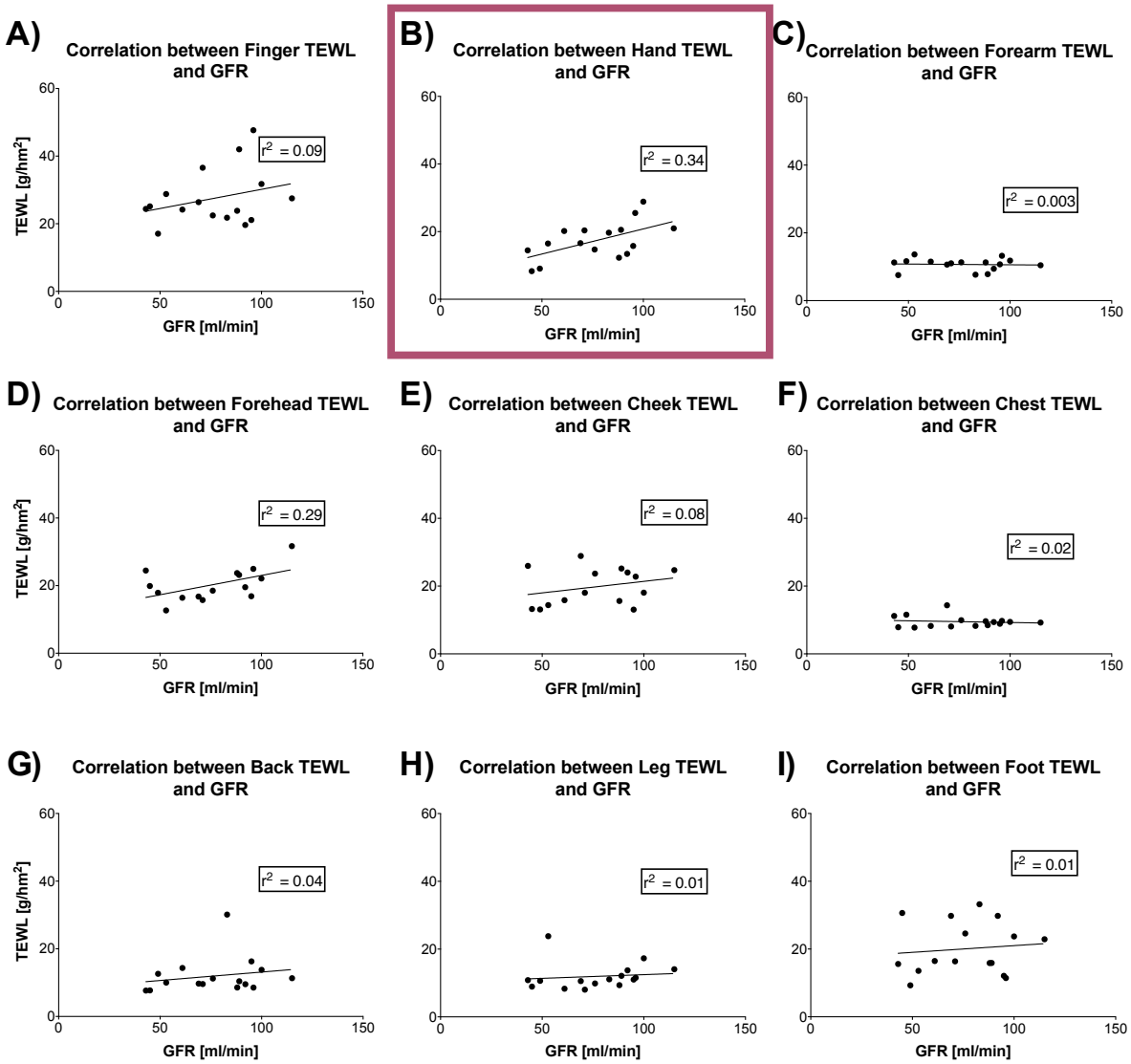

Figure 3.7 Correlation graphs of TEWL and GFR values. Scatter dot plots for site-specific TEWL values as a function of GFR values. From the statistical analysis one obtains the coefficient of determination r^2, as indicated in the respective graphs. The different panels show results obtained from measurements performed on the **A)** finger, **B)** hand, **C)** forearm, **D)** forehead, **E)** cheek, **F)** chest, **G)** back, **H)** leg, and **I)** foot. To highlight the most significant graph, a red frame was placed around it.

Chapter 4

Discussion

4.1 Summary

Systemic sclerosis is a heterogenous disease with a high case specific mortality rate [Rubio-Rivas et al., 2014, Tyndall et al., 2010], a complex pathobiology [Katsumoto et al., 2011, Gabrielli et al., 2009], and a diverse clinical appearance [Wollheim, 2005]. The hallmark of systemic sclerosis is skin fibrosis, which clinically shows as increased hardening of the skin. This is often times the initial visual manifestation of the disease and therefore dermatologists are the first contact persons a SSc patient encounters. While previous studies have not found objective diagnostics for skin features of systemic sclerosis, this study now investigates several different skin physical measurements of the epidermis in patients with systemic sclerosis. Furthermore, in the means of this study, we were able to collect data on several clinical features through the systemic sclerosis consultation hour, which we used for correlation analyses.

In this study, the skin of systemic sclerosis (SSc) patients was investigated by means of three quantitative skin measurements: transepidermal water loss (TEWL), skin hydration, and pH. To the best of our knowledge, previous studies have not analyzed transepidermal water loss combined with skin hydration and pH in subjects with systemic sclerosis. Therefore, we found it interesting for an in-depth analysis. Using the above described tools, we measured the skin of 71 test subjects and investigated 18 test subjects with systemic sclerosis, which we compared to 19 test subjects with atopic eczema, 22 with acne vulgaris, and 12 with healthy skin.

We performed statistical analyses, comparing the three skin parameters: TEWL, skin hydration, and pH between the four different skin conditions: systemic sclerosis, atopic eczema, acne vulgaris and healthy skin. In summary, these statistical analyses showed no significant differences in skin measurements (TEWL, skin hydration, and pH) between skin of patients with systemic sclerosis and test subjects with healthy skin or those with acne vulgaris. We did, however, find significant differences to TEWL and skin hydration values of atopic eczema patients. Second, we performed correlation analysis of skin values (TEWL) and clinical features (skin score and laboratory parameters). We did not find a correlation of our skin parameters, namely TEWL values, to the local skin score (mRSS) or to ANA values.

Finally, one interesting finding of our study was that in SSc patients, transepidermal water loss values measured at the hand and forehead showed a significant positive correlation to the glomerular filtration rate (GFR).

4.2 Discussion

When reviewing the literature, a common theme was that the complexity of systemic sclerosis (SSc) pathology [Sierra-Sepúlveda et al., 2019, Katsumoto et al., 2011] continues to challenge researchers to develop good clinical measures of treatment effect (outcome measures) [Denton, 2019, Furst et al., 2007, Matucci-Cerinic et al., 2007]. Good measures for the assessment of systemic sclerosis would be helpful to gain a deeper understanding of the disease. Therefore, new objective outcome measures are still needed to help better differentiate subtypes, to aid in the determination of prognostic factors, and to recognise SSc in an early stage. We will discuss the challenges in clinical studies of systemic sclerosis in the first part of this chapter.

The focus of our clinical study was on the dermatological aspects of systemic sclerosis. Our intent was to propose tools for measuring disease activity and thereby to help distinguish between severe and less severe skin involvement. In the second part of the discussion, we will critically review the current assessment methods of skin in systemic sclerosis. Then, we will analyze the suitability of our methods for the assessment of SSc skin. Third, we will evaluate the correlation between transepidermal water loss and the glomerular filtration rate. And lastly, we give an outlook on the future in SSc diagnostics.

4.2.1 Challenges in Clinical Studies of Systemic Sclerosis

Systemic sclerosis is, as the word says, a *systemic* disease. Systemic diseases are defined as diseases affecting multiple organs and tissues of the body. Therefore, as previously discussed in this doctoral thesis, SSc patients show a variety of different clinical features ranging from skin manifestations over vascular dysfunctions to internal organ failure. Thus, the implementation of practical applications in grasping various aspects of the disease is challenging. In this way, the design of a study concept which captures disease severity (disease severity: quantifying the degree of manifestation the disease has on the patient) in patients with systemic sclerosis is difficult, due to the multifaceted nature of the disease.

Clinical studies are designed to answer questions in a well-defined relatively *homogenous* patient group. In a heterogenous disease as systemic scleorsis there is not even a consent on the existing and well-established subset groups. Recently, Johnson et al. [2018] reiterated this issue. He conducted semi-structured interviews with randomly sampled international SSc experts and found that the idea of SSc subsets merely exists as a complex latent construct and should be revised [Johnson et al., 2018, p. 5].

Furthermore, there is no guideline for treatment of SSc, merely recommendations from the European League Against Rheumatism (EULAR) Scleroderma Trial and Research group [Kowal-Bielecka et al., 2017b,a]. In this paragraph, we would like to explore the fact that finding an appropriate end-point for measuring treatment success (outcome measure) is a complex endeavour. Many clinical trials on the treatment of SSc have been performed; see e.g. references [van den Hoogen et al., 1996, van Laar et al., 2014, Walker et al., 2018]. Until recently, the lack of a definitive clinical trial template made interpretation and comparison of findings difficult [Matucci-Cerinic et al., 2007]. In 2007, Matucci-Cerinic et al. reviewed data from previous clinical trials and concluded that advances that have been made in the treatment of SSc, however these advances are hindered by the complex nature of SSc and disease heterogeneity. In 2015, 8 years later, Khanna et al. [2015] interviewed 13 international SSc experts on their experiences in SSc clinical trial design. Together, they developed 22 points to consider for future clinical trials in systemic sclerosis. They concluded that a more uniform trial design is needed, because previously the complexity has hindered comparisons between trials. So, yet again the complexity stands in the way of adequate clinical studies. More than 10 years later, Denton [2019], as one of the experts in the field of systemic sclerosis, again addresses the same difficulties in trial design in his review on the challenges of systemic sclerosis trial design. In this respect, existing markers for SSc are still insufficient in predicting clinical outcomes or in assessing response to therapy.

To optimize individual patient outcomes, accurate and reliable tools are important for predicting disease progression and analyzing disease severity. For these reasons, we would like to discuss the role of the skin as an adequate tool as an outcome measure in clinical trials and its role in the assessment of systemic sclerosis.

4.2.2 Assessment Tools of Systemic Sclerosis

To put our study in a broader context, in this section we discuss recent advances in the design of assessment tools and their application for skin changes in SSc. There is still much need for improving the assessment of systemic sclerosis skin manifestations, disease severity, prognosis and treatment response. Concerning the assessment of systemic sclerosis, one main question has crossed my mind: Is the modified Rodnan skin score (mRSS) a good measure of skin involvement or is there a better assessment tool for skin manifestations in SSc? As we have elaborated in the introduction, skin changes are not a mandatory trait for the diagnosis of systemic sclerosis. The standard for diagnosing SSc is through the 2013 ACR/EULAR classification criteria, which include skin thickening as a main criteria [van den Hoogen et al., 2013]. To be clear, the diagnosis of systemic sclerosis can also be made without the presence of skin changes. However, despite the various manifestations of the disease, it is widely accepted that skin sclerosis is virtually universal in SSc. Skin involvement is present in over 90% of SSc patients [Knobler et al., 2017b].

One of the most important tools in measuring the outcome of, for example, drug trials is the modified Rodnan skin score. The mRSS is a skin score which measures skin thickness by palpation; see Section 1.2.3. Previous studies have shown that the mRSS accurately assesses the severity of skin sclerosis and thus making it a good outcome measure for the success or non-success of clinical trials [Clements et al., 2000]. Although the skin score is a good and validated measure for the prognosis in systemic sclerosis [Clements et al., 1990, Steen and Medsger, 2001], it has limitations. These limitations include the potential for interobserver variability [Clements et al., 1993, 1995], the need for extensive training of investigators to reduce variability [Khanna et al., 2017, Low et al., 2019], and that mRSS is only a validated outcome measure for diffuse cutaneous SSc [Shand et al., 2007]. Moreover, despite the ominous fact that the skin score is a viable measure for skin thickness, the mRSS does not differentiate skin thickness from skin hardness or tethering [Khanna et al., 2017]. In addition, changes in the skin are variable and the skin score can improve as the disease progresses [Clements and Furst, 2004]. Therefore, the mRSS can be misleading for disease activity. To sum up, the modified Rodnan skin score has various limitations and for this reason there is need for a more objective, accurate, and specific measures of SSc skin, which would allow a clearer detection of skin changes in drug trials.

In the last two decades, there have been other attempts to use new tools for skin assessment. Balbir-Gurman et al. [2002] assessed biomechanical properties of SSc skin with a suction device called BTC-2000. Another group of researchers, Kissin et al. [2006], tested the validity of the durometer as an objective measure of skin hardness. Bendeck and Jacobe [2007] found that skin measurements with ultrasound had the potential of assessing diseases with skin thickening. For a more detailed comparison of new skin assessment tools, the reviews from Czirjak et al. [2008] and Kumánovics et al. [2017] are good references.

In summary, there have been advances in the development of assessment tools for SSc skin manifestations, but these are still unsatisfactory for the concrete representation of skin changes.

4.2.3 Are our Methods Suitable for the Assessment of Skin in SSc Patients?

In our study, we sought to find alternatives to the mRSS for the assessment of the skin. The results of this study show that independent of the measurement site (finger, hand, forearm, cheek, forehead, chest, back, leg, and foot) the skin parameters (TEWL, skin hydration, and pH) may not be adaquate measures for the skin assessment of systemic sclerosis. Some limiting factors to our study were the heterogenous pool of SSc patients, the diversity of cutaneous SSc subsets and the differences in SSc duration time (e.g. early or late SSc) of each SSc patient. In future studies, one should therefore consider these factors and selects patients accordingly.

The analysis of our data was difficult due to the **heterogenous pool of SSc patients**. This was mainly due to the rarity of the disease, which did not allow us to find a more homogenous/uniform group of SSc patients. We suggest that for a future analysis one should filter SSc patients according to their current stage of skin sclerosis and also to their individual disease duration time. The skin sclerotic sites change over time from early-stage oedemous to indurative to late-stage atrophic skin [Gabrielli et al., 2009]. Future studies should take this aspect of skin change into consideration when choosing the study cohort. In a previous study about localized scleroderma, Ďurčanská et al. [2016] analyzed the change of one of the skin parameters, TEWL, in different stages of local scleroderma skin lesions. He found that mean TEWL values were highest in inflammatory stages of the disease and decreased in the sclerotic stage and lowest in the atrophic stage. In comparison, our study population had a heterogenous SSc duration and SSc patients probably showed different stages of skin changes. Three of our patients (17%) had a SSc duration of over 20 years, 5 SSc patients (28%) had a SSc duration of 10 years or more, and 10 (55%) had a SSc duration between 2 and 9 years. Therefore, SSc patients of this study had both early and late forms of systemic sclerosis. Interestingly however, results from TEWL measurements were narrowly distributed, see Section 3.3.2. These results suggest two possible interpretations. First, that physical function of the epidermis of SSc patients are not representative for showing skin changes. Second, that SSc patients, despite their heterogeneity in clinical features, subsets etc., have similar epidermal manifestations of the disease. Since the pathology is elusive, the epidermal manifestations of SSc are not clear. Therefore we cannot draw any concrete conclusions from our observations.

In a majority of SSc studies, the main focus was on the dermis of the skin. Increasing evidence has recently been presented, which supports a key role of the **epidermis** in skin changes. Van Praet et al. [2011] studied the histopathology of SSc through biopsies (forearm and upper inner arm) and found epidermal thickening in involved skin, independent of the biopsy site. They also found that epidermal thickness correlated with the local clinical skin score. In another study, Nikitorowicz-Buniak et al. [2014] discovered a significant increase in epidermal thickness between the basal layer and stratum corneum.

The measurements of our study assessed physical properties of the skin epidermis. TEWL, for example, measures the transepidermal water loss in the epidermis and thus represents the skin barrier function. To the best of our knowledge, skin barrier function in SSc patients may have been influenced by pathological changes of the skin such as the synthesis of collagen an extracellular-matrix components which causes fibrosis in the skin [Gabrielli et al., 2009]. Moreover, depending on the stage of sclerosis (early or late SSc), our patients may have shown different results for TEWL, skin hydration, and pH measurements.

Our results support previous observations. In a past study, Sogabe et al. [2002] tested functional properties of the stratum corneum of the skin, namely TEWL and skin surface hydration, in patients with systemic sclerosis and hypertrophic scars/keloid. In their

examination, they studied the forearm skin of 39 SSc patients and 7 with hypertrophic scars. They found that TEWL and skin hydration in SSc skin was not significantly different from age-matched healthy controls ($n = 10$). Furthermore, they found that in keloid and hypertrophic scars TEWL was higher than in the control group, however without any statistical significance. Consistent with Sogabe et al. [2002], our TEWL and skin hydration results also show no significant difference to subjects with healthy skin. However, we performed a more elaborate study, measuring 9 skin sites and compared these not only with healthy controls but also to atopic skin and the skin of acne patients. This concludes that even when mapping several different skin sites, transepidermal water loss is still not significant in differentiating SSc to other skin conditions, namely acne vulgaris, atopic eczema or healthy skin.

In our literature search we found another recent publication, in which researchers compared TEWL and sclerotic skin. Ďurčanská et al. [2016] performed a study of patients with localized scleroderma (LS), measuring TEWL at skin sites which had a local skin hardening. They found that involved skin with localized scleroderma in the sclerotic stage showed a significant difference to uninvolved skin in the contralateral side. Here, TEWL values observed on LS skin sites were higher than in the non-involved skin sites. In contrast, in our study, average TEWL values of SSc patients were typically lower than in healthy controls. These findings suggest that sclerotic skin of localized scleroderma can be differentiated from systemic sclerosis skin through TEWL measurements. To examine these results in more detail one could, in a future analysis, perform a comparison of TEWL values between skin of patients with localized scleroderma and those with systemic sclerosis. Therby, one would find out if the observed conflicting differences were coincidental or whether a difference actually exists between these two types of sclerotic skin disorders.

This study is based on instruments (evaporimeter, corneometer, and pH-meter) used in several previous studies, especially in studies of atopic eczema, to mention a few selected examples: [Eberlein-König et al., 2000, Jungersted et al., 2010, Seidenari and Giusti, 1995, Lodén et al., 1992, Tupker et al., 1990, Werner and Lindberg, 1985, Werner, 1986]. As mentioned in these publications, skin functional measurements such as transepidermal water loss and skin hydration have been explored in atopic eczema and show decreased hydration and increased TEWL.

Similar to atopic eczema, our aim was to use these instruments (evaporimeter, corneometer, and pH-meter) to show skin changes in SSc and we hoped to find a new assessment tool to map disease severity. Our aim was creating new parameters for diagnostics of systemic sclerosis (SSc) and finding a measure with which to differentiate SSc from other skin diseases and healthy skin. But are our methods suitable for the task of better understanding and hence diagnosing SSc or even mapping disease severity? Our approach, trying to measure disease severity through physical measurements such as transepidermal water loss, skin hydration and pH, may have been too simple for this complex endeavour.

What we have learned from our study, is that the inclusion of a higher number of test subjects would increase the significance of our statistical analysis. A limiting factor was the number of test subjects within each cohort (three of our cohorts consisted of < 20 patients). However, the rarity of systemic sclerosis limits the number of patients available for trials. Hence making it difficult to conduct a study on a larger scale. In the future, one could increase the number of subjects to improve the significance of the study. In order to design studies with a higher statistical significance it will also be necessary to consider using age-matched controls for healthy subjects.

Other aspects which need to be discussed are limitations due to the chosen location of skin measurements, disturbances through faulty devices and disturbances due to environmental factors. As discussed above, SSc is heterogenous in its clinical appearance. Hence, we chose skin sites for our skin measurements, which were known to typically show skin hardening and skin sites where hardening was not typically visible. The anatomical location of measurements showed that the sites where we assumed a skin change (– typical locations of skin hardening in patients with limited cutaneous SSc are for example the forearm, hands and fingers [van den Hoogen et al., 2013] –) were also the ones where we saw the most interesting differences in skin functional measurements between cohorts. However, statistical analyses showed no significant difference in our skin measurements.

In the following we discuss limitations due to disturbances in the execution of measurements. Variability in TEWL, skin hydration, and pH measurements may be caused by differences in the placement of the devices on the skin (they should all be placed perpendicularly on the skin plane). This problem could be assessed in a future study, where we measure triplicates of all 3 skin parameters.

Another factor causing disturbances or variance in measurements could have been physical activity, which influences sweating and in turn affects TEWL [Pinnagoda et al., 1990]. However, none of our test subjects were involved in physical activity before measurement.

An important issue to consider when performing skin physical measurements is that the environmental factors are clearly defined and that their influence in skin condition is reduced to a minimum amount. Environmental factors such as temperature and humidity could also have an influence on skin measurements, especially transepidermal water loss. More humid or hot days in the summer could most likely cause test subjects to have a higher transpiration. Although we tried to keep avoid influences of temperature and humidity by acclimatization, the summer season may still have a role in higher transpiration and skin moisture. Sweating is caused by increased body temperature and according to Pinnagoda et al. [1990] if the ambient room temperature is below 20°C, the thermal sweat gland activity improbable and thereby cannot interfere with measurements. In the future, to avoid seasonal influence, one could to conduct all measurements in the winter. In the winter there is less humidity and hence the hydrolipidfilm is lower.

Possibly solutions to remedy these study deficiencies would be, for example, performing a standardized test on the investigator of the study. Here, we would also perform the

same three measurements on a pre-defined skin site on the exact date of the study measurements. These measurements would then later be used as a baseline/reference in our statistical analysis.

4.2.4 The Correlation Between Transepidermal Water Loss (TEWL) and the Glomerular Filtration Rate (GFR)

The prognosis of systemic sclerosis is frequently determined by internal organ manifestations and SSc-associated deaths are often caused by renal complications. The most severe of all renal complications is scleroderma renal crisis (SRC), one of the most studied renal complications in SSc. It is a complication observed in 5% of scleroderma cases, more often in dcSSc (and here within the first 2 years). [Penn et al., 2007]

Other renal dysfunctions are more common in systemic sclerosis than the SRC. These include for example isolated reduction in glomerular filtration rate (GFR), microalbuminuria, and renal function impairment without any other manifestations of renal disease; for a more comprehensive review see [Shanmugam and Steen, 2012]. Asymptomatic renal dysfunctions have been found in about 10-55% of SSc patients [Steen et al., 1984, Shanmugam and Steen, 2012]. Recently, researchers found a decreased GFR in SSc patients that do not have SRC, but merely abnormal renal function [Kingdon et al., 2003]. This leads to the conclusion, that GFR could be a marker for renal dysfunction.

In our study, we found several SSc patients with decreased glomerular filtration rates (GFR) recorded in their medical file. Eleven out of 16 patients (69%) showed an irregular renal function, e.g. decreased GFR values at the time of measurement. Seven SSc patients (44%) had GFR values between 60 and 89 ml/min, and four SSc patients (25%) had GFR values < 60 ml/min. The GFR can be used to identify SSc patients with an abnormal renal function [Kingdon et al., 2003]. Hence, we correlated skin parameters (TEWL, skin hydration, and pH) to GFR values of our SSc patients to see if these could maybe predict a renal dysfunction (without having to perform invasive blood investigations).

Interestingly, these correlation analyses showed that in systemic sclerosis patients, transepidermal water loss (TEWL) values measured at the hand and the forehead had a significant positive correlation to the glomerular filtration rate (GFR). There seems to be a connection between TEWL values and the glomerular filtration rate and hence renal impairment. To our knowledge, previous studies have not yet analyzed the correlation between GFR values and TEWL. This finding leads to the assumption that systemic sclerosis patients with an intact skin barrier function (normal TEWL value) at the hand and forehead are more likely to show abnormal renal function. TEWL is a measure of skin barrier function and is known to be increased in patients with dry skin a.e. atopic eczema [Lodén et al., 1992]. Patients with renal diseases, especially with chronic kidney disease frequently show pruritus and dry skin [Wojtowicz-Prus et al., 2016, Schmid-Simbeck et al., 2016]. Yosipovitch et al. [2007] found an abnormal skin barrier integrity and increased TEWL in patients with end-stage renal disease. In our study, we detected normal TEWL

values for decreased GFR ($< 50\ ml/min$). In contrast to previous findings, our data suggest that in systemic sclerosis renal abnormalities correlate to normal skin barrier integrity.

However, limitations to this correlation analysis must be noted. Although SSc patients were studied prospectively in our study, data for GFR values was collected retrospectively using medical files. Most recorded GFR values were measured close to, or 1-3 months before or after we performed skin measurements. However, some GFR values were 1 year old. This might influence the statistical analysis. In future analyses, more meaningful results on kidney function could be obtained by extending our analyses to include more specific clinical examinations such as proteinuria, hypertension, etc. In addition, comorbid diseases such as diabetes mellitus or medications could have had an influence on renal function and could cause a distortion in results. Therefore in the future, these should be documented or even excluded. For a better analysis of the correlation between GFR and SSc patients, the setting of the clinical study would have to be modified to create conditions that are favourable for an analysis of renal dysfunction.

In the future, a study focusing solely on the correlation between TEWL and GFR should meet inclusion criteria suggested by Galluccio et al. [2017]. The correct identification of renal dysfunction may be important in the design of future drug trails and clinical studies. And the correlation of skin parameters with laboratory markers such as GFR may allow further insights into the pathogenesis of systemic sclerosis and even give us information about the severity of the disease.

4.3 Outlook

There is a need for improved methods which determine the progression and severity in systemic sclerosis. Regarding the mapping of disease severity in systemic sclerosis, research tools such as transepidermal water loss, skin hydration, and pH are probably not the most resourceful. In this study, we learned that the complexity that is systemic sclerosis cannot be quantified in a straight-forward way. Therefore, in this outlook we would like to give a short overview of improvements in our study, which could lead to better outcomes.

Following our rationale, one should in the future conduct a study in which all skin measurements are made in a single device. This would hinder fluctuations between different measurements and could ensure for more accurate results of the measurements. In a recent study, scientists from Korea designed a truncated hollow cone for analyzing skin thickness and TEWL simultaneously [Sim et al., 2018]. They suggest that this new device may contribute to the diagnoses of various dermatological diseases including systemic sclerosis. In addition, Sim et al. state that their dual-measurement device could help decrease the measurement uncertainty of the measuring sites and reduce the time and effort of researchers. With these two methods, a more accurate quantification

would be possible. In addition, one would as a next step have to test the intra- and interobserver viability of these methods.

In recent years, there has been substantial progress in the assessment of systemic sclerosis. Several tools to assess the skin biomechanics, physical properties and the functional impairment have been developed [Allen et al., 2018, Merkel et al., 2008, Kissin et al., 2006, Balbir-Gurman et al., 2002, Bendeck and Jacobe, 2007].

However, disease classification based formally on the extent of skin involvement does not express the true heterogeneity of systemic sclerosis. In order to include the entire spectrum of cutaneous and extracutaneous manifestation of systemic sclerosis, it has recently been proposed by Milano et al. [2008] that gene expression profiling is useful for mapping disease severity in SSc. They demonstrated that through DNA microarrays the heterogeneity in scleroderma can be measured qualitatively. This finding is reinforced by Sargent and Whitfield [2011]. Mechanic measurements which measure the skin, such as the ones used in this study, can be prone to disruptions and external influences such as individual clinical characteristics can lead to fluctuations. Therefore, when looking at the study with a critical eye, one would have to state that the medical assumption that measuring skin quantitative measures for mapping disease severity is not the best way of studying SSc. A better way of characterizing a systemic disease would be though genetic expression profiles. The complexity of the disease cannot be captured merely through skin characteristics. We have gained merely an understanding of one of the aspects of the disease - the dermatologic aspect.

When viewing systemic sclerosis from a broad perspective, I would like to conclude with the following opinion: I think for characterizing the severity of the disease, the focus should be on the pathology instead of merely clinical symptoms. The main problem in understanding systemic sclerosis is that the causes and pathology are yet not fully understood. In my opinion, finding the causes of chronic systemic autoimmune diseases such as systemic sclerosis should be the main focus of research studies. However, steps which help guide the way towards a deeper understanding of such pathologically complex diseases are useful and important. The study of this doctoral thesis highlights the complex nature of systemic sclerosis and investigates one of the clinical manifestations of the disease – the skin.

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