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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Paper I investigated whether skin colonization with *S. aureus* had an effect on the skin barrier function in patients with AD, and also aimed to determine the effect of sensitization to skin-associated microorganisms on the skin barrier function. Paper II studied *MBL* gene polymorphism in patients with AD and the effect of this on sensitization to skin-associated microorganisms. Paper III was a study protocol for a prospective translational study that aimed to find predictive and prognostic biomarkers in skin and blood in patients with MF and SS. Paper IV studied the skin microbiome concerning the absolute quantities of *Staphylococci* and *Cutibacterium acnes (C. acnes)* in unaffected and affected skin in patients with MF and in the skin of healthy controls.

In paper I, *S. aureus*-culture positive patients with AD had a significantly higher SCORAD compared to patients not colonized with *S. aureus* ($P = 0.002$). *S. aureus*-culture positive patients with AD had a significantly higher TEWL compared to patients not colonized with *S. aureus* ($P < 0.05$). Patients with AD sensitized to three of the investigated skin-associated microorganisms had an increased TEWL, SCORAD and total IgE compared to patients sensitized to none, one or two of the investigated skin-associated microorganisms ($P = 0.026$, $P = 0.008$, $P < 0.001$).

In paper II, the prevalence of the *MBL* gene polymorphism associated with serum MBL deficiency was 13% in patients with AD. Patients with a *MBL* gene polymorphism associated with serum MBL deficiency were 6 times more likely to be sensitized to *Candida albicans* compared to patients with *MBL* gene polymorphism associated with no serum MBL deficiency (OR = 6.00, $P = 0.047$).

Paper IV showed that the gene copy ratio of *Staphylococci/C. acnes* was significantly higher in unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.01$ and $P < 0.001$). TARC/CCL17 was significantly higher in advanced stages of MF compared to early stages of MF ($P < 0.05$).

Key words: Atopic dermatitis, Mycosis fungoides, Sézary syndrome, biomarkers, skin barrier function

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This thesis is dedicated to memory of my beloved grandmother
Maria Elovsson, 1928-2021

"What we know is a drop, what we don´t know is an ocean."
Isaac Newton

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Jinnestål CL, Belfrage E, Bäck O, Schmidtchen A, Sonesson A. Skin barrier impairment correlates with cutaneous Staphylococcus aureus colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. Int J Dermatol. 2014 Jan;53(1):27-33.

Paper II

Belfrage E, Jinnestål CL, Jönsen A, Bengtsson A, Åkesson A, Schmidtchen A, Sonesson A. Role of mannose-binding lectin and association with microbial sensitization in a cohort of patients with atopic dermatitis. Acta Derm Venereol. 2023 Mar 30;103:adv2405.

Paper III

Belfrage E, Ek S, Johansson Å, Brauner H, Sonesson A, Drott K. Predictive and prognostic biomarkers in patients with Mycosis fungoides and Sézary syndrome (BIO-MUSE): Study protocol for a translational study. JMIR Res Protoc (forthcoming). doi:10.2196/55723.

Paper IV

Belfrage E, Feidenhans'l C, Brauner H, Ek S, Lood R, Drott K, Sonesson A. Low levels of Cutibacterium acnes correlates inversely with Staphylococci in patients with Mycosis fungoides. In manuscript.

Related papers not included in the thesis

Kalliara E, Belfrage E, Gullberg U, Drott K, Ek S. Spatially guided and single cell tools to map the microenvironment in cutaneous T-cell lymphoma. Cancers (Basel). 2023 Apr 18;15(8):2362.

Nenonen J, Winther AH, Leijonhufvud E, Belfrage E, Smedby KE, Brauner H. Overall survival and registration of cutaneous T-cell lymphoma patients in Sweden: a multi-center cohort and validation study. Acta Oncol. 2022 May;61(5):597-601.

Belfrage E, Relander T, Brauner H. New national treatment guidelines for primary cutaneous lymphoma. Läkartidningen. 2021 Aug 10;118:20238.

Preface

The road towards this thesis

When this project was initiated in 2013 with Andreas Sonesson and Artur Schmidtchen, the project had the title “Biomarkers and skin barrier function in atopic dermatitis”. The first two papers are focused on atopic dermatitis.

In 2012, I started working at the outpatient-clinic for primary cutaneous lymphomas alongside the oncologists at the Department of Oncology at Skåne University Hospital. This triggered my interest in primary cutaneous lymphomas, and since 2018 I have been the chairperson for the national Swedish guidelines for primary cutaneous lymphomas.

While I was doing research on the skin barrier function and *Staphylococcus aureus* in atopic dermatitis, I also became interested in the work of other research groups writing about *Staphylococcus aureus* and its effects on the primary cutaneous lymphomas, called mycosis fungoides and Sézary syndrome.

I contacted Kristina Drott in 2019, and the thesis took on a new orientation, also focusing on the skin barrier function and the microbiological aspects in mycosis fungoides and Sézary syndrome. Hanna Brauner also joined the project. Unfortunately, the start of the clinical study BIO-MUSE was initially delayed due to the pandemic. Andreas Sonesson has had an active role as supervisor throughout the project.

This thesis ended up becoming broader than just being about atopic dermatitis, and the title was changed to “Biomarkers and skin barrier function in atopic dermatitis, mycosis fungoides and Sézary syndrome.”

Abbreviations

AD	atopic dermatitis
AMPs	antimicrobial peptides
BSA	body surface area
CADM	contact agar disc method
CBCL	cutaneous B-cell lymphoma
cfu	colony-forming units
CTCL	cutaneous T-cell lymphoma
CT	computed tomography
C. acnes	*Cutibacterium acnes*
DLQI	Dermatology Life Quality Index
EASI	Eczema Area and Severity Index
ECP	extracorporeal photopheresis
EMA	European Medicines Agency
FDA	U. S. Food and Drug Administration
FLG	filaggrin gene
ICH-GCP	International Committee on Harmonisation of Good Clinical Practice
IgE	immunoglobulin E
IL	interleukin
ILC2s	group 2 innate lymphoid cells
MBL	mannose-binding lectin
MBL	mannose-binding lectin gene
MF	mycosis fungoides
MHC	major histocompatibility complex
MRSA	methicillin-resistent *Staphylococcus aureus*
mSWAT	Modified Severity-Weighted Assessment Tool
NB-UVB	narrow-band ultraviolet B
PCL	primary cutaneous lymphomas
PET-CT	positron emission tomography-computed tomography

PRRs	pattern recognition receptors
PUVA	psoralen and UVA
S. aureus	*Staphylococcus aureus*
SDT	skin-directed therapy
SEA	*staphylocoocal* enterotoxin A
SEB	*staphylocoocal* enterotoxin B
SS	Sézary syndrome
STAT3	signal transducers and activators of transcription 3
TBSA	total body-surface area
TCI	topical calcineurin inhibitor
TCR	T-cell receptor
TCS	topical corticosteroids
TEWL	transepidermal water loss
TLRs	toll-like receptors
TNMB	tumor node visceral blood
TSEBT	total skin electron beam therapy
TSLP	thymic stromal lymphopoietin
TSST-1	toxic shock syndrome toxin-1
TNF	tumor necrosis factor
UVA	ultraviolet A
UV-light	ultraviolet light
VAS	visual analogue scale
WHO	World Health Organization

Abstract

Atopic dermatitis (AD) is a common relapsing inflammatory skin disease. Mycosis fungoides (MF) and Sézary syndrome (SS) belong to the rare group of primary cutaneous T-cell lymphomas. Although AD is a benign disease and MF and SS are malignant diseases, they do have some similarities, including a Th2-dominated inflammation, a tendency to be colonized with *Staphylococcus aureus (S. aureus)* and an impaired skin barrier function.

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Paper IV showed that the gene copy ratio of *Staphylococci/C. acnes* was significantly higher in unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.01$ and $P < 0.001$). TARC/CCL17 was significantly higher in advanced stages of MF compared to early stages of MF ($P < 0.05$).

Populärvetenskaplig sammanfattning

Atopisk dermatit (AD) är en kliande inflammatorisk hudsjukdom som ofta har ett kroniskt förlopp med återkommande hudutslag. AD förekommer hos ca 15-30 % av barn och ca 2-10 % av vuxna. Sjukdomen debuterar ofta innan 2 års ålder, men kan debutera i alla åldrar. Hudutslagen har ett ofta typiskt utseende med en infiltrerad rodnad med eksem, som ofta sitter i böjveck, såsom armveck och knäveck. Diagnosen ställs baserat på hudutslagens utseende och förekomsten av andra atopiska manifestationer såsom rinit och astma hos patienten eller förstagradssläktingar. Orsaken till AD är komplex och beror på flera faktorer som inte är helt kartlagda. Vid sjukdomen ses en påverkad hudbarriär och en ökad inflammation i huden. Patienter med AD har en ökad benägenhet att bilda en sorts antikroppar som heter IgE mot olika ämnen och detta kallas för sensibilisering. Förekomst av bakterien *Staphylococcous aureus (S. aureus)* är vanligare i huden hos patienter med AD än hos friska personer.

Mycosis fungoides (MF) och Sézarys syndrom (SS) är hudlymfom, som är en grupp av ovanliga sjukdomar där sjukdomen debuterar i huden, och som vid diagnos oftast finns endast i huden. MF är det vanligaste hudlymfomet och brukar debutera kring 50-74 års ålder och är något vanligare hos män. Hudutslagen vid MF kallas för patch, plack eller tumör beroende på utseende och tidigare stadier kan likna andra vanliga hudsjukdomar såsom AD. De flesta patienter med tidigt stadium av MF har en god prognos, men sjukdomen kan utvecklas till mer avancerat stadium med en försämrad prognos. Vid SS ses en generell rodnad av hela huden, förstorade lymfkörtlar och sjuka T-celler i blod. SS har en betydligt sämre prognos än MF, men sjukdomarna liknar varandra på flera sätt. Förekomst av bakterien *S. aureus* är vanligare i huden hos patienter med MF/SS än hos friska personer.

AD och MF/SS är helt olika sjukdomar, men det finns vissa likheter såsom en ökad inflammation i huden, en försämrad hudbarriär och ett ofta kroniskt förlopp. Vid både AD och MF/SS är förekomsten av bakterien *S. aureus* i huden vanligare, vilket kallas för kolonisering, och bakterien tros påverka sjukdomarnas förlopp.

Genom patientnära forskning syftade detta doktorandprojekt till att undersöka flera faktorer som skulle kunna påverka sjukdomsutvecklingen vid både AD och MF/SS.

Delarbete I-II undersökte patienter med AD och delarbete III-IV studerade patienter med MF/SS.

Delarbete I undersökte om kolonisering av *S. aureus* i huden påverkade hudbarriärens funktion hos patienter med AD. Delarbete I undersökte också om sensibilisering mot bakterien *S. aureus* eller jästsvamparna *Candida albicans* och *Malassezia* påverkade funktionen hos hudbarriären hos patienter med AD. Delarbete I fann att patienter som var koloniserade med *S. aureus* hade en försämrad hudbarriär, och även en svårare sjukdom. Patienter som var sensibiliserade mot alla

tre av de undersökta hudmikroberna *(S. aureus, Candida albicans* och *Malassezia)* hade mer försämrad hudbarriär, svårare sjukdom och även högre totalt IgE i blodet jämfört med de patienter som var sensibiliserade mot inga, eller endast en eller två av de undersökta hudmikroberna.

Delarbete II studerade förekomsten av ett förändrat arvsanlag hos mannose-bindande lektin (MBL), som är en del av immunförsvaret, hos patienter med AD. Ett förändrat arvsanlag kan ge en brist på MBL i blodet, vilket är en vanlig immunbrist. Delarbete II undersökte om förekomst av förändrat arvsanlag för MBL påverkade sensibilisering mot bakterien *S. aureus* eller jästsvamparna *Candida albicans* och *Malassezia* hos patienter med AD. Delarbete II fann att förekomsten av förändrat arvsanlag för MBL som ger brist på MBL i blod var 13 % i undersökta gruppen med patienter med AD. De patienter med AD som hade ett förändrat arvsanslag för MBL som ger en brist på MBL i blod, hade en 6 gånger ökad risk att vara sensibiliserade mot jästsvampen *Candida albicans*.

Delarbete III var ett studieprotokoll för BIO-MUSE studien i syfte att undersöka prediktiva och prognostiska faktorer i hud och blod hos patienter med MF och SS.

Delarbete IV är en del av BIO-MUSE studien och här undersöktes förekomst av bakterierna *Staphylococci* (flera olika sorters stafylokocker, inklusive *S. aureus*) och bakterien *Cutibacterium acnes (C. acnes)* i sjuk hud och i intilliggande frisk hud hos patienter med tidigt och avancerat stadium av MF samt hos friska kontroller. Blodprover togs också för att se om man kunde hitta skillnader mellan patienter med tidigt och avancerat stadium av MF. Delarbete IV fann att förekomsten av *Staphylococci* var högre i sjuk hud och i intilliggande frisk hud hos patienter med MF jämfört med friska kontroller. Förekomsten av bakterien *C. acnes* var lägre i sjuk hud och i intilliggande frisk hud hos patienter med MF jämfört med friska kontroller. Hudbarriären var försämrad i sjuk hud och i intilliggande frisk hud hos patienter med MF jämfört med hos friska kontroller. Blodprovet TARC/CCL17 var högre hos patienter med avancerat stadium jämfört med hos patienter med tidigt stadium av MF.

Vid både AD och MF sågs en försämrad hudbarriär, som i sin tur troligen bidrar till kolonisering av bakterien *S. aureus* och därmed en obalans i hudens sammansättning av hudmikrober, vilket leder till att *S. aureus* triggar en ökad inflammation som kan bidra till försämrad sjukdom, vilket ytterligare försämrar hudbarriären.

Introduction

Normal skin and the skin barrier function

The skin is an effective barrier to the surrounding environment and gives protection against mechanical trauma and foreign substances such as allergens, irritants, UV radiation and microorganisms (1, 2). The skin is the largest organ of the human body with many functions, such as sensory receptiveness, regulation of body temperature and water loss, and synthesis of several essential molecules, such as vitamin D3 (3).

The epidermis is the outermost layer and is further subdivided into several layers of squamous epithelium (the stratum corneum, stratum lucidum, stratum granulosum, stratum spinousum and stratum basale), primarily composed of keratinocytes and a minor number of melanocytes, Merkel cells and immune cells, such as Langerhans cells and intradermal T-cells. The keratinocytes in the stratum basale divide and migrate over the course of 3-4 weeks to the outermost layer, the stratum corneum, where they are shed (4). During epidermal differentiation, the keratinocytes produce lipids that are extruded into the extracellular space. The lipid matrix consists of cholesterol, free fatty acids, and ceramides (2). The keratinocytes contribute to controlling inflammation, regulating the skin barrier, and produce antimicrobial peptides (AMPs), which kill microorganisms in the skin (5). One of the most important functions of the stratum corneum is to withstand mechanical trauma and microorganisms; this is maintained by synthesis of the cornified envelope and by a protective acidic film composed of sebum, sweat, proteases, antimicrobial peptides, and products of the commensal microbiome. Filaggrin is a protein that contributes to the aggregation of the keratin cytoskeleton and the skin barrier function. Tight junctions and desmosomes are paracellular proteins that form a permeability barrier between adjacent cells and contribute to cell adhesion and skin barrier function (3).

The dermis is composed of connective tissue and consists of the upper papillary dermis and the deeper reticular dermis. Most cells in the dermis are fibroblasts, and the other cells present are mast cells, macrophages, dendritic cells, melanocytes, and B- and T-cells. The extracellular matrix is made up of proteoglycans, glycoproteins, collagen, and elastin. The dermis also contains a network of nerves, free nerve endings, blood vessels, hair follicles and sweat glands and sebaceous glands (3).

The subcutis is a layer of fat tissue beneath the dermis (Figure 1).

The skin barrier function can be measured as transepidermal water loss (TEWL) (6, 7).

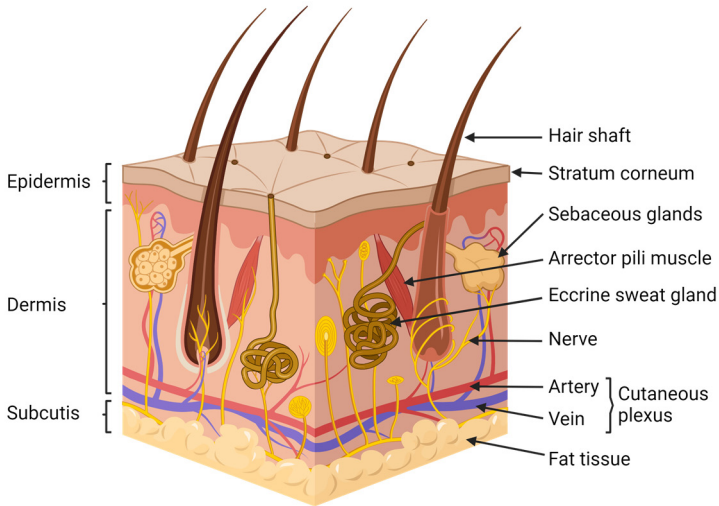

Figure 1.
Skin anatomy. The skin is composed of three layers. The outermost layer, the epidermis, provides a protective barrier to the environment. The dermis contains hair follicles, sweat and sebaceous glands, nerves and blood vessels. The innermost layer, the subcutis, contains fat tissue and cutaneous plexuses. Created with BioRender.com

The skin microbiome

The skin is one of the largest areas of the body that interacts with microorganisms. Its outer layers have several types of naturally occurring microorganisms, such as bacteria, yeasts, and viruses (8). Microorganisms that normally occur in the skin are called commensals and they play an important role in the defense against pathogens and contribute to the innate and adaptive immune defenses (8-11). They can affect the production of AMPs and various cytokines in the skin, such as IL-1, IL-17, IL-22, and TNF (9). Microorganisms that can cause infections and contribute to diseases are called pathogens. The combined genetic material of the microorganisms found on the skin is called the skin microbiome (8, 12, 13).

Some bacteria, such as *Cutibacterium*, *Corynebacterium and Staphylococci,* are common in the healthy skin microbiome, while other bacteria, such as *Staphylococcus aureus (S. aureus),* are seen less often (14). About 5-30% of the healthy population is colonized with *S. aureus* (15, 16). If the skin barrier is impaired, or if the microbial balance between commensals and pathogens is affected, skin diseases and even systemic diseases can be triggered (8). It is also

established that an environment with chronic inflammation can cause malignant cells, including malignant T-cells, to proliferate (14).

The microenvironment in the skin, and thus also the skin microbiome, are affected by differences in anatomical location, pH, temperature, humidity, sebaceous secretion, and UV light exposure (8). Based on this, the skin is divided into different areas: sebaceous (face, chest, back), moist (arm creases, groin, knee creases) and dry (upper arms, palms). Depending on the location of the skin, the microbiome can vary, but in each area, the microbiome tends to be relatively constant over time (9, 17). All areas are colonized by *Cutibacterium, Corynebacterium* and *Staphylococci* species. The sebaceous sites are dominated by *Cutibacterium* and *Staphylococci* species, while the moist sites mostly have *Corynebacterium* and *Staphylococci* species. The dry areas have the lowest range of bacteria, but a high diversity of species of bacteria, including *Proteobacteria, Corynebacterium, Cutibacterium and Staphylococci* (18). The anterior part of the nostrils (nares) can be a reservoir for *S. aureus* colonization with an increased risk of sepsis after surgery and dialysis (15).

There is a delicate balance between the commensal bacteria in the skin, the skin barrier, and the innate and adaptive immunity to maintain a healthy skin microbiome and prevent pathogens in the skin, cutaneous infections, and inflammation (19).

The innate and the adaptive immune system

The immune system is a protective system divided into the innate and the adaptive immune systems (20). The innate immune system is mainly unspecific, rapid and relies on conserved sets of receptors that provide the first line of defense, while the adaptive immune system can be more specific, coordinated and have an immunological memory. However, the activation and amplification of specific immune cells in the adaptive immune system after initial recognition takes hours to several days and is therefore regarded as the second line of defense. The adaptive immune system is also referred to as the acquired immune system. The immune system is complex and the innate and the adaptive immune systems interact in multiple ways (21, 22).

T- and B-lymphocytes, also called T- and B-cells, constitute most of the cells in the adaptive immune system. The lymphocytes have specific receptors that can recognize a specific antigen. B-cell receptors can recognize an intact antigen and T-cell receptors can recognize a processed antigen presented on an antigen-presenting cell. Antigen-presenting cells, such as Langerhans cells, macrophages, and dendritic cells, are central for the activation of the adaptive immune system as they can internalize antigens and migrate to the draining lymph nodes and present the antigen to the T-cells (21).

The antigen receptor of the T-cells, called the T-cell receptor (TCR), is usually composed of an alpha (α) chain and a beta (β) chain, or more rarely, of a gamma (γ) and a delta (δ) chain, and these are combined in a blend of variants that results in a unique specificity. The antigen is presented to the T-cell by the major histocompatibility complex (MHC) molecule on an antigen-presenting cell. The T-cells depend on the MHC molecule for recognition of the antigen and subsequent activation. A certain T-cell is only activated when a specific combination of MHC molecules with bound antigens are available. There are two types of MHC molecules. MHC class II are found on antigen-presenting cells, such as dendritic cells and macrophages, which bind to T-cells (21).

The number of T cells present in the skin is almost twice as many as in the peripheral blood and most of the T-cells in the skin are found in the dermis (21, 23). Skin resident memory T (T_{RM}) cells is a subset of long-lived memory T cells that occupies the skin, without recirculating. The main function of skin T_{RM} cells is to protect against infections, but they can also be involved in pathological conditions, such as psoriasis, vitiligo, and cutaneous T-cell lymphomas (CTCL). In CTCL, there is a clonal expansion of transformed T_{RM} cells taking place primarily in the skin. T_{RM} in the skin express the skin homing antigens cutaneous lymphocyte antigen (CLA) and CC chemokine receptor 8 (CCR8) (23).

There are two subsets of T-cells expressing TCR αβ, called the T helper (Th-cells), which are CD4+ based on expression of surface proteins, and the cytotoxic T-cells (Tc-cells or killer cells), which are CD8+. Resting CD4+ Th-cells become activated when they are introduced to an antigen by an antigen-presenting cells and start to differentiate and proliferate. The naïve CD4+ Th-cell can differentiate to Th1, Th2, Th9, Th17, Th22, regulatory Treg and follicular helper T cell, depending on the local cytokine milieu during T-cell priming. Th1-cells secrete the cytokines IL-2 and interferon-γ. Th2-cells secrete the cytokines IL-4, IL-5, IL-9, IL-10, and IL-13 and are involved in the immunity to kill pathogens such as bacteria and viruses. IL-4 is a potent activator of B-cells to produce immunoglobulin E (IgE).

The main function of B-cells is to produce antibodies and take up antigens and present them to T-cells. There are five classes of antibodies, called immunoglobulins A-E. In response to contact with a previously unknown antigen, the B-cells produce IgM antibodies, and if re-exposure to the antigen occurs, a secondary immune response with primarily IgG will occur. Immunoglobulins are proteins expressed on the surface of B-cells that bind to pathogens and serve as B-cells' pathogen recognition receptors specific for an antigen. The immunoglobulins can also be secreted in a soluble form in extracellular fluids, such as plasma. Immunoglobulin E is important in the protection against worms and protozoa. IgE has also been shown to be of interest in atopic dermatitis (AD), asthma and allergy where an IgE response to allergens has been seen (20).

The complement system and mannose-binding lectin

The complement system is part of the innate immunity and is an interface between the innate and adaptive immune systems (21, 22). The complement system consists of a group of plasma proteins, of which some exist as soluble inactive precursors. Each precursor can be cleaved into active fragments, which then can cleave molecules in the next component in a cascade-like manner. Complement activation can be initiated via three specific pathways to target pathogens: the classical pathway, the alternative pathway, and the lectin pathway. All three share the same late steps and achieve the same results with destruction of pathogens. The lectin pathway is initiated by the binding of mannose-binding lectin (MBL) to certain polysaccharides in the membrane of the pathogen, activating the mannose-binding lectin-associated serine proteases (MASP-1, MASP-2, and MASP-3) (24, 25).

MBL is produced by the liver and is an acute phase protein. MBL belongs to a family of circulating pattern recognition receptors (PRRs) called collectins, which bind to the surface of many pathogens, including bacteria, yeasts, and viruses. MBL functions as an opsonin; it can induce phagocytosis of the pathogen and induce activation of the complement system (26-28).

The *MBL* gene is located on chromosome 10 and there are several possible gene polymorphisms. Polymorphism of the associated promotor region and the structural *MBL* gene can result in a dysfunctional protein and a MBL deficiency in serum (29, 30). MBL deficiency is a common immunodeficiency and found in approximately 5-10% of the population in almost all parts of the word (28-30).

Several reports have shown an association between *MBL* gene polymorphism associated with MBL deficiency in serum and an increased risk of infections (30-38), but on the other hand, some studies have shown no association between *MBL* gene polymorphism associated with MBL deficiency in serum and risk of infections (28, 39). MBL has also been investigated in autoimmune diseases, with the same contradictory results (38, 40, 41).

Several factors can contribute to complicating the interpretations of *MBL* gene polymorphism and the corresponding MBL levels in serum (29, 42-44). The MBL serum concentrations can overlap between different *MBL* gene polymorphism, and a variability in MBL serum concentrations can also be seen between individuals with the same *MBL* gene polymorphism. Different definitions of MBL deficiency in serum have also been used.

MBL has been reported to be a part of the defense against *Candida* species (45, 46). Blocking MBL in mice resulted in an increased *C. albicans* colonization in the gut (47). The role of *MBL* gene polymorphism in dermatological diseases is relatively unexplored.

Staphylococcus aureus and bacterial superantigens

Staphylococci are gram-positive cocci that are divided into coagulase producing strains, such as *S. aureus*, and non-producing strains, collectively called coagulase-negative *staphylococci*, such as *Staphylococcus epidermidis (S. epidermidis)*, a non-pathogenic member of the skin microbiome.

S. aureus can cause a range of infections from skin infections, such as impetigo, cellulitis, abscesses and ecthyma, to life-threatening diseases, including staphylococcal scalded skin syndrome, endocarditis, osteomyelitis, toxic shock syndrome and sepsis. *S. aureus* has also been described to colonizing the skin in various skin diseases without resulting in acute infections, but instead modulating the inflammatory response and leading to a possible disease progression.

S. aureus produces several virulence factors, including enzymes (proteases, lipases), surface proteins (fibronectin-binding proteins), cytotoxins (hemolysins) and bacterial superantigens (enterotoxins, toxic shock syndrome toxin), and all these are crucial for adhesion to the epithelium, colonization and sometimes infections (48, 49) (Figure 2).

Bacterial superantigens are a family of secreted protein toxins produced by *S. aureus* and other bacteria (51-53). *S. aureus* can produce toxins called enterotoxins, such as staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB) and toxic shock syndrome toxin (TSST-1), which are extremely potent activators of T-cells (51). While common antigen peptides must first be processed by antigen-presenting cells before the MHC molecule can present them to antigen-specific T-cells, the bacterial superantigens can bind directly as whole proteins to MHC class II molecules outside the antigen-peptide binding portion and induce activation. Bacterial superantigens interact with the TCR molecules on T-cells by binding primarily to the variable region of the β chain called the Vβ domain, resulting in an oligoclonal stimulation of defined T-cell repertoire, potentially activating > 20% of all T-cells. In some families of T-cell receptors (TCRs), the Vβ chains that cross-link TCR complexes can induce T-cell activation at extremely low concentrations (51).

Systemic intoxication by bacterial superantigens can induce life-threatening conditions, such as toxic shock syndrome, caused by a sudden cytokine storm when large numbers of T-cells are stimulated by the bacterial superantigen. The bacterial superantigens produced by *S. aureus* have also been reported to trigger T-cells and contribute to worsening of atopic dermatitis and disease progression in cutaneous T-cell lymphomas (CTCL) (52, 54).

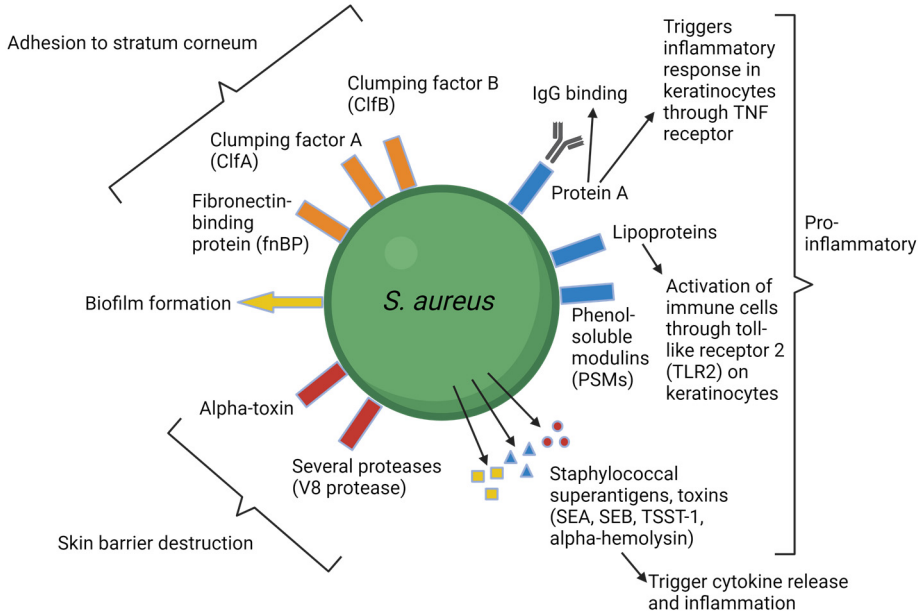

Figure 2.
Virulence factors of *S. aureus*. *S. aureus* has several cell-wall proteins and secreted factors that enable it to adhere to the stratum corneum, imapir the skin barrier and trigger inflammation. Modified from Paller et al. 2019 (50). Created with BioRender.com

Definition of biomarker

The term "biomarker" was defined in 1998 as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" by the National Institutes of Health Biomarkers Definitions Working Group (55). It was later defined by the WHO in 2001 as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" (56). The FDA and the National Institutes of Health established their joint criteria called "Biomarkers, EndpointS, and other Tools (BEST)" and defined a biomarker as "a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" (57). Several subtypes of biomarkers have been defined, including diagnostic biomarker, monitoring biomarker, pharmacodynamic/response biomarker, predictive biomarker, prognostic biomarker, safety biomarker or susceptibility/risk biomarker. A prognostic biomarker measures the outcome of a disease, and a predictive biomarker discriminates those who will respond or not

respond to therapy. An ideal biomarker should be safe, easy to measure, cost efficient and consistent across gender and ethnic groups.

Atopic dermatitis

Background

Atopic dermatitis (AD) is an inflammatory and often chronic relapsing skin disease with an intense itch. The first description of a dermatitis resembling AD dates to the Roman Era, but it was not until 1923 that Arthur Coca and Robert Cooke defined the concept of "atopy" based on an association between allergic rhinitis and asthma, and in 1933 the term "atopic dermatitis" was proposed by Fred Wise and Marion Sulzberger (58).

The prevalence of AD is about 15-30% in children and 2-10% in adults (59-62). The onset of disease is often prior to 2 years of age, but the disease can begin at any time in life. In 50% of the patients the debut is before 1 year of age and in 85% the onset is before 5 years of age (63).

The pathogenesis of AD is complex and multifactorial, where the heredity and environmental factors induce inflammation and an impaired skin barrier function (1, 64).

Due to the inflammation and a deteriorated skin barrier, patients with AD have an increased risk of being colonized with bacteria, yeasts, and viruses (65). *S. aureus* frequently colonizes the skin of patients with AD, and *S. aureus* has been reported to exacerbate AD and cause skin infections (66, 67). There is also an increased risk of herpes simplex infection in patients with AD, where the patients with AD may develop a severe generalized infection with herpes simplex, called eczema herpeticum (68).

AD is strongly associated with the development of food allergy, allergic asthma, and rhinitis, which is commonly referred to as the atopic march (69-73). Food allergies are reported in up to 33% of children with severe AD, but in mild AD food allergies are only present in around 6% of the patients, and food allergies are rarely a cause of exacerbation of AD (74).

Clinical presentation of atopic dermatitis

The skin lesions in AD have an infiltrated erythema and erosions caused by scratching and sometimes lichenified areas. In skin of color the skin lesions in AD have less erythema and an increased tendency to present as prurigo-like lesions. AD

can typically be limited to the flexural areas or be more widespread (Figure 3). The typical anatomical locations of the skin lesions vary depending on age. About 70-80% have mild disease, 15-20% have moderate and 2-5% have severe AD (75).

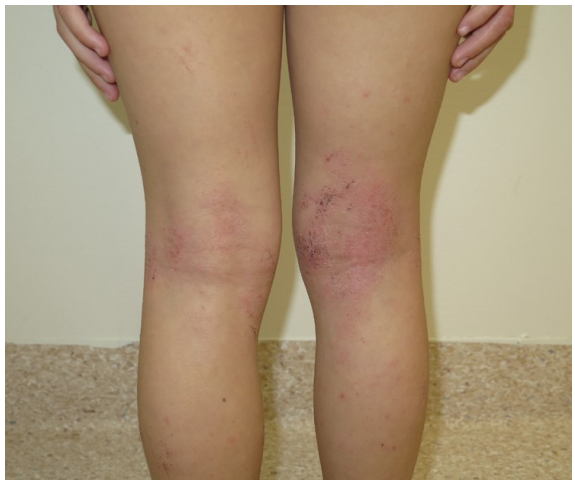

Figure 3.
Clinical manifestations of atopic dermatitis. Published with permission of the patient. Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund.

Diagnostic criteria of atopic dermatitis

The first diagnostic criteria for AD were defined in 1980 by Hanifin and Rajka and evolved over time to the UK Working Party's diagnostic criteria for AD from 1994, which are based on personal history and the clinical picture (76, 77) (Figure 4).

1. Presence of itchy skin condition (required) and
2. At least three of the following:
   - Visual flexural dermatitis (or cheeks, forehead or extension surfaces < age 10 years)
   - Personal history of flexural disease as above
   - Personal history of asthma and allergic rhinitis (or atopic dermatitis in a first-degree relative in children < age 4 years)
   - Personal history of dry skin in the last 12 months
   - Onset < age 2 years (in children > age 4 years)

Figure 4.
The UK Working Party's diagnostic criteria for atopic dermatitis.

Treatment of atopic dermatitis

The treatment of AD aims to reduce inflammation, improve the skin barrier, reduce pruritus, and avoid trigger factors. The goal of treatment is for the patient to be free of symptoms from their eczema for the longest periods possible (78, 79).

Basic emollient treatment is recommended. The first line of treatment for AD is topical corticosteroids (TCS), which are anti-inflammatory and antipruritic. TCS are available in different strengths, and as absorption varies depending on the thickness of the skin, a weaker topical corticosteroid is recommended for areas with thin skin, such as the face and skin folds. TCS should be applied once daily and tapered off when there is a response to treatment. Treatment with TCS needs to be repeated when the disease flares up again. Topical calcineurin inhibitors (TCI) are the second line of treatment for AD; they are also a good alternative for thin skin, as they do not have the side-effect of causing atrophy of the skin. Other treatment options include UV-light therapy with narrowband UVB (NB-UVB) or UVA1. If there is need for optimized compliance, eczema school might be relevant (80). Trigger factors should be avoided if possible. Moderate to severe AD may require systemic treatment. The systemic treatment options are methotrexate, cyclosporine, mycophenolate mofetil, and in recent years there are new systemic treatments for AD such as monoclonal antibodies, including dupilumab and tralokinumab, and oral JAK-inhibitors, including abrocitinib, baricitinib and upadacitinib (78, 79, 81-83). The choice of systemic treatment needs to follow clinical guidelines and be individualized according to several factors such as age, pregnancy, and comorbidities (84).

Differential diagnosis and comorbidities of atopic dermatitis

In patients with AD unresponsive to basic topical treatment, patch testing is essential to exclude allergic contact dermatitis. There are many differential diagnoses of AD including scabies, infections, allergic contact dermatitis, irritative-toxic eczema, seborrheic dermatitis, immunodeficiency syndromes and cutaneous T-cell lymphoma (CTCL), and sometimes other investigations are necessary.

Attention deficit hyperactivity disorder (ADHD), depression and anxiety disorders are some comorbidities that are more common in patients with severe AD (85, 86).

Quality of life with atopic dermatitis

Having children with mild AD has the same impact on a family's quality of life as having children with insulin-treated diabetes, and the impact of moderate to severe AD is even greater (87, 88). The severe itching and accompanying stigmatization of AD can lead to marked psychosocial comorbidity and distress that significantly impairs the quality of life (85, 89).

Skin barrier function in atopic dermatitis

Patients with AD have an impaired skin barrier and an increased transepidermal water loss (TEWL) that correlates with increased severity of AD (90-92). Multiple factors, including the Th2 cytokines, contribute to the impaired skin barrier in AD with decreased proteins (filaggrin, loricrin), deficiency of AMPs, altered lipid compositions in the stratum corneum and a changed skin microbiome (1).

The epidermal protein filaggrin is needed for the keratin fibers to bind to epidermal cells and a mutation in the *FLG* gene results in an impaired skin barrier (93, 94). An *FLG* mutation is an important genetic factor in AD and is reported to be a predisposing factor for AD (95, 96). Approximately 25-50% of patients with AD have an *FLG* mutation. However, about 10% of the European population have an *FLG* mutation, and only about 40% of those with such a mutation develop AD (95, 97-99).

The dysfunctional skin barrier allows microorganisms and allergens to penetrate the skin leading to an increased risk of cutaneous IgE sensitization (100-102). The Th2 cytokines and the deficiency of AMPs facilitate colonization with *S. aureus*.

Immunological changes in atopic dermatitis

In the acute phase of AD there is primarily a Th2-dominated inflammation, and the predominating cytokines are IL-4, IL-5, IL-9, IL-10, IL-13, and IL-31. During progression of AD, it has been reported that activation of Th1, Th17 and Th22-cells may contribute to the chronic inflammation (103, 104).

Total IgE and sensitization to skin-associated microorganisms in patients with atopic dermatitis

High serum levels of total IgE are reported to be more common in patients with AD compared to healthy controls and have also been associated with a poor long-term outcome of AD (105-107). However, the role of total IgE in pathogenesis and worsening of AD remains incompletely understood (105, 106, 108). The Th2 inflammation in AD and the cytokines IL-4, IL-13 and thymic stromal lymphopoietin (TSLP) promote the production of allergen-specific IgE by plasma cells.

Several studies of patients with AD have also demonstrated an increased rate of IgE to specific antigens or allergens, such as food, aeroallergens, and skin-associated microorganisms. This is called sensitization (109-112). Sensitization can be demonstrated with a positive skin prick test or elevated levels of IgE antibodies to specific antigens in serum. However, evidence of a sensitization is not proof of a clinically relevant allergy. An allergy requires both an allergen-specific IgE

measured in blood or skin testing and symptoms of allergy when exposed to the allergen. Patients with AD often have elevated serum IgE without any relevant allergy to most of the allergens (108).

Sensitization to the airborne allergen house dust mites has been reported to worsen AD (113). There appears to be an association between sensitization to skin-associated microorganisms, including *S. aureus, Malassezia* and *C. albicans*, and high levels of total IgE, impairment of the skin barrier function and severe AD (67, 114-117).

The role of *Staphylococcus aureus* in atopic dermatitis

S. aureus colonizes the skin in 50-90% of patients with AD (118-122). In flare-ups of AD, an increased dominance of *S. aureus* in the skin microbiome has been reported, correlating with disease severity (119).

S. aureus produces virulence factors, such as *S. aureus* proteases, to increase its survival, and these can cause increased inflammation in the skin and a worsening of AD (123). *S. aureus* can also produce biofilms that enhance the bacteria's ability to bind to the skin surface and evade treatment with oral antibiotics. In addition, *S. aureus* also produces several toxins such as staphylococcus enterotoxin A (SEA), staphylococcus enterotoxin B (SEB) and toxic shock syndrome toxin-1 (TSST-1), which affect the host's immune system, facilitate tissue invasion, and secrete proinflammatory cytokines.

The keratinocytes in the epidermis constitute the barrier to the environment and can recognize pathogens through pattern recognition receptors (PRRs) and toll-like receptors (TLRs), which then start an immunological response (124, 125). *S. aureus* can stimulate the production of the keratinocyte-produced cytokines TSLP and IL-33 to recruit immune cells, and this leads to the production of Th2 cytokines IL-4, IL-5 and IL-13 from basophils, eosinophils, mast cells, Th2-cells, and group 2 innate lymphoid cells (ILC2s) (16, 104).

Mouse models with transgenic mice with increased expression of IL-4 and IL-13 have induced an itchy dermatitis. Mice with increased expression of epithelial cell-producing cytokines such as TSLP and IL-33 have also tended to develop an atopic dermatitis-like phenotype (104) (Figure 5).

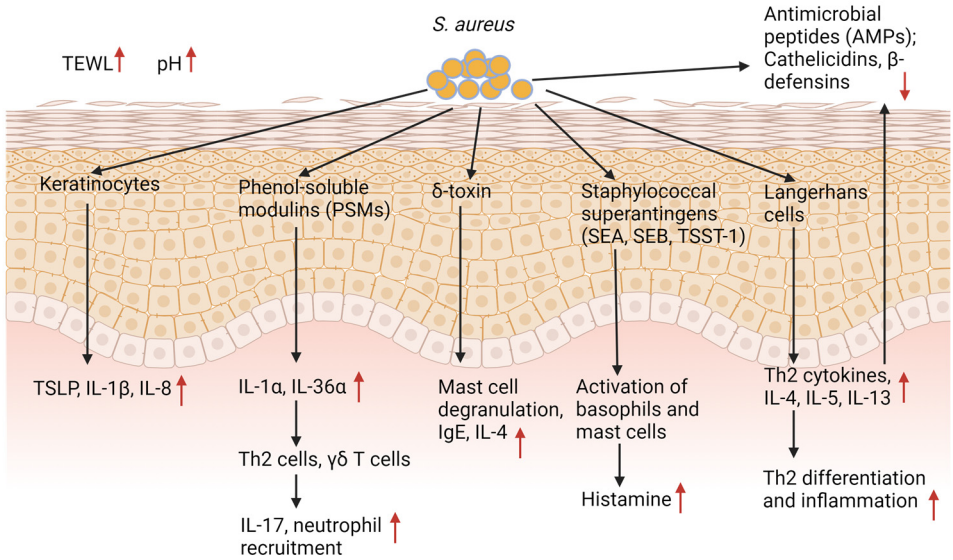

Figure 5.
The proposed role of *S. aureus* in atopic dermatitis. Modified from Alam et al. 2022 (126), Yang et al. 2020 (127). Created with BioRender.com

The role of yeasts in atopic dermatitis

Patients with AD have an increased susceptibility to microbial colonization with yeasts, which has been reported to trigger a worsening of the disease (128-131). The impaired skin barrier allows microorganisms to penetrate the skin and affect the immune system with a consequent inflammation and sensitization (132, 133). Sensitization to antigens from the yeasts *Malassezia* and *Candida albicans* tends to be more common in adults with severe AD (134).

Antifungal treatments in patients with AD sensitized to *Malassezia* and *Candida albicans* have shown to significantly decrease the levels of IgE to *Malassezia* and *Candida albicans* and to improvement of their AD (114, 128, 134-137). Especially in patients with AD with head- and neck dermatitis, oral itraconazole or ketoconazole treatments have been shown to be effective (114, 137, 138).

Candida albicans can be found in the normal microbiome of the mucous membranes and may also colonize the skin. Compared to patients with psoriasis and healthy controls, *Candida* species are more common in the skin microbiome of patients with AD (139). In a cohort of patients with AD, a significant correlation was seen between sensitization to *Candida albicans* and AD symptoms in a subgroup of

patients with gastrointestinal growth of *Candida albicans* (140). Another study reported that specific IgE to *Candida albicans* was significantly higher compared to healthy controls, and treatment with ketoconazole or fluconazole for three months significantly improved AD and reduced specific IgE to *Candida albicans* (117). It was speculated that *Candida albicans* can colonize the skin, oral mucosa, and intestine, and then stimulate the immune system to a sensitization to *Candida albicans*, which can then further exacerbate AD through a hypersensitivity reaction (117).

The clinical implications of sensitization to yeasts in patients with AD is debated and the role yeast in the pathogenesis and progression of AD is not yet fully understood (141).

The role of mannose-binding lectin in atopic dermatitis

The role MBL might play in AD is not fully understood and the studies are few and contradictory. Some studies have reported an association between the *MBL* gene polymorphism causing MBL deficiency and AD (142-144).

Carrera et al. examined the *MBL* gene in 131 children with AD and 165 healthy controls and found that the prevalence of an *MBL* gene polymorphism causing MBL deficiency was significantly more frequent in the group with AD, and may possibly predispose to AD, but no association to disease activity was found (142).

Hashimoto et al. examined 68 patients with AD and 32 healthy controls regarding the *MBL* gene polymorphism and found no difference between the two groups regarding the occurrence of *MBL* gene polymorphisms causing serum MBL deficiency (143). Hashimoto et al found no associations between an *MBL* gene polymorphism causing serum MBL deficiency and the levels of total IgE, severity of eczema or presence of asthma (143).

Brandão et al. examined the *MBL* gene polymorphism in 165 children with AD and in a control group of 165 healthy adults and found that the *MBL* gene polymorphism causing MBL deficiency was significantly more common in patients with AD compared to the control group (144).

Primary cutaneous lymphomas

Primary cutaneous lymphomas (PCLs) are a rare group of non-Hodgkin lymphomas that primarily develop in the skin and in most cases have no extracutaneous manifestations at the time of diagnosis (145, 146). PCLs usually remain restricted to the skin over time. The incidence of PCLs is about 1:100,000 (147). The subtypes of PCLs demonstrate a broad spectrum of clinical manifestations, histological

features, and prognoses. Most of the PCL subtypes are indolent diseases, while others have a more aggressive course. Cutaneous T-cell lymphomas (CTCL) constitute about 70% of PCLs and cutaneous B-cell lymphomas constitutes about 30% (147).

Over the last two decades there have been several classifications of PCLs. The WHO classification from 2005 (148) was updated in 2018 to the 4th edition (149-151). In 2022 two new classifications for hematolymphoid tumors were published, but they are partly contradictory (146, 152). In Sweden a decision was made to still use the 4th edition of the WHO classification from 2018, but that the pathologist will also refer to both classifications from 2022 when relevant.

Mycosis fungoides and Sézary syndrome

Background

Mycosis fungoides (MF) is the most common form of CTCL, with an incidence of about 0.5:100,000 (147, 153). Since it is an indolent disease, the prevalence is higher. MF has a higher incidence in men than women (1.6:1.0) and a peak age incidence between 50 and 74 years, although it can also occur in younger adults and children (147).

The term "mycosis fungoides" was first used in 1806 by Jean Louis Alibert who suggested the name because the skin lesions in a patient had a mushroom-like appearance (154).

In 1870, Ernest Bazin described the natural evolution of the disease and defined the current skin staging with patches, plaques, and tumors. The term "cutaneous T-cell lymphoma" was introduced in 1975 by Edelson and in the 1980s and 1990s the classification evolved further to distinguish the subtypes CTCL and CBCL (155, 156).

Clinical presentation of mycosis fungoides and Sézary syndrome

The clinical manifestations of MF in the skin are called patches, plaques, tumors, and erythroderma (157). Patches are flat, but often scaly or have textural changes (Figure 6). Plaques ae defined as slightly raised lesions that can be smooth, scaly, crusted or ulcerated (Figure 7). A tumor is a lesion at least 1 cm in diameter that has vertical growth or depth (Figure 8). Most of the lesions are erythematous, but they can also be hyper- or hypopigmented. When the skin lesions or an erythema covers 80% or more of the body surface area, this meets the criteria for erythroderma (157). The skin lesions usually present on the trunk and buttocks, but they can appear

anywhere on the skin. In advanced stages, MF can also involve lymph nodes, blood and other extracutaneous organs.

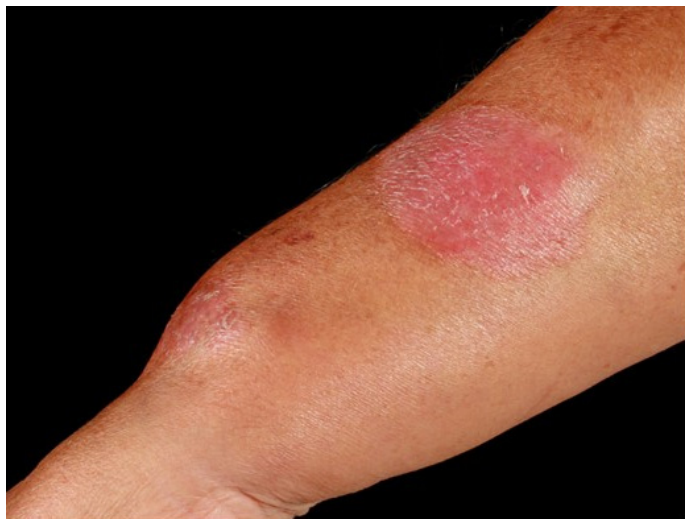

Figure 6.
Clinical manifestations of mycosis fungoides. Patches. Published with permission of the patient. Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund.

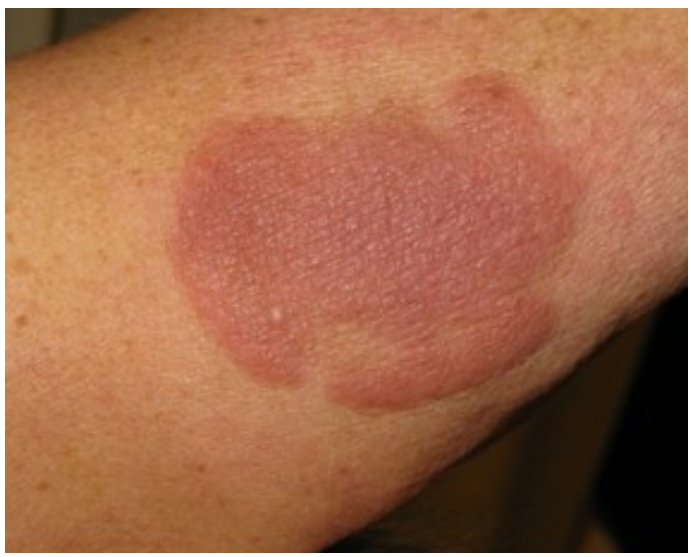

Figure 7.
Clincal manifestations of mycosis fungoides. Plaques. Published with permission of the patient. Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund.

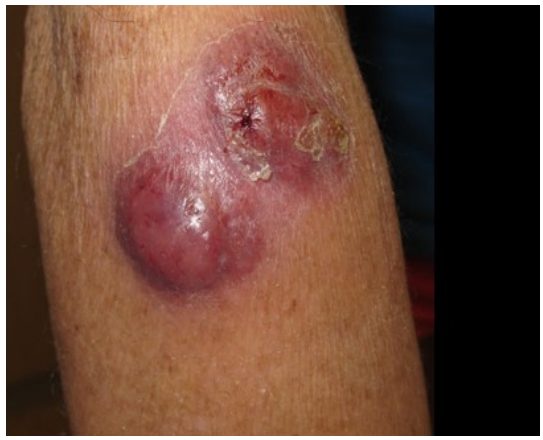

Figure 8.
Clincal manifestations of mycosis fungoides. Tumor. Published with permission of the patient. Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund.

Sézary syndrome (SS) was first described in 1938 by Albert Sézary. SS consists of a triad of erythroderma, generalized lymph node enlargement and blood involvement (Figure 9). Sézary syndrome is an aggressive leukemic variant of MF and constitutes only 5% of CTCL (145). SS most often affects middle-aged or older individuals, and more often men than women. Pruritus is a common symptom in MF and SS. Hyperkeratosis in the palms and soles, alopecia and nail manifestations are also seen in both MF and SS.

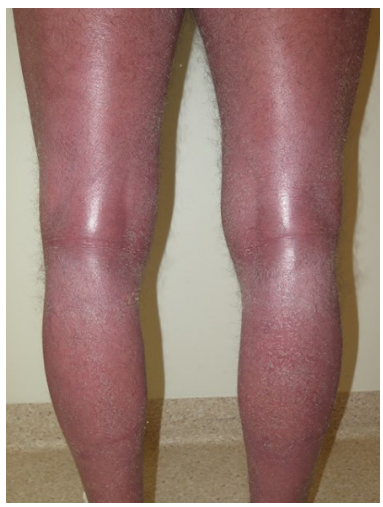

Figure 9.
Clincal manifestations of Sézary syndrome. Published with permission of the patient. Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund.

Diagnosis of mycosis fungoides and Sézary syndrome

The diagnosis of MF or SS requires clinicopathologic correlation and is based on a representative punch biopsy suggestive of MF or SS in combination with consistent clinical manifestations. In early stages of MF, the histological changes can be subtle, and the histologic diagnosis is challenging, and therefore several repeated biopsies over time can be necessary to establish the diagnosis. The most indurated lesion should be biopsied and two biopsies at least 4 mm in diameter from different lesions is recommended. The lymphocytic infiltrate can be affected by TCS, so it is important for the patient to be off these for at least 2 weeks before punch biopsy if the histological diagnosis is challenging. The punch biopsy will be examined with hematoxylin and eosin to investigate histological changes, and immunophenotyping with various T-cell surface markers, such as CD2, CD3, CD4, CD5, CD8, CD7, CD30 and CD20 (B-cell marker) and PCR to find a potential clone of T-cell receptor (TCR) gene rearrangement.

With MF, histological changes are seen with infiltrating atypical lymphocytes in the dermis and epidermis. Atypical lymphocytes in the epidermis are referred to as epidermotropism. Pautrier's microabscesses are a presentation of atypical lymphocytes within intraepidermal vesicles, which is seen in around 25% of the patients (Figure 10). The malignant T-cells in MF and SS typically exhibit the phenotype of skin-homing CD4+ T-cells expressing receptors such as cutaneous lymphocyte antigen (CLA) and CC chemokine receptor 4 (CCR4) (158, 159). The immunophenotype is often CD3+, CD4+, CD7-, CD8- (but can be CD8+) and CD30-/+. Clonal T-cell receptor (TCR) gene rearrangements may be present, especially in more advanced stages (160, 161).

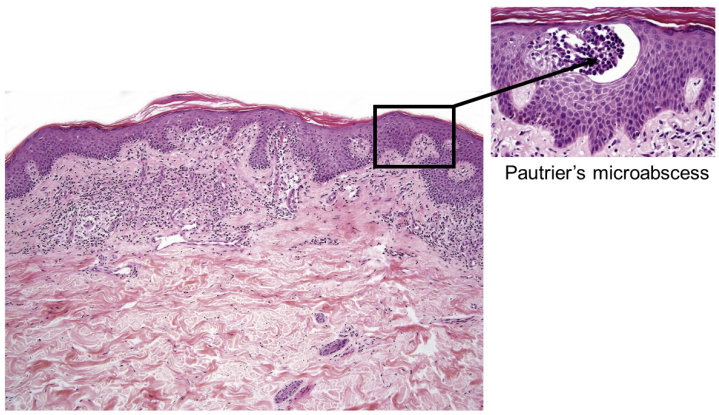

Figure 10.
The histopathological picture of mycosis fungoides is characterized by atypical lymphocytes, epidermotropism and sometimes Pautrier's microabscesses. Photo: Ulrika Hansson, Sahlgrenska University Hospital, Gothenburg.

Since the clinical manifestations in MF can resemble common skin diseases, such as AD and psoriasis, a diagnostic delay up to several years is not uncommon (162). In early stages of MF, the lymphoma is usually limited to the skin, and investigations to detect a possible systemic involvement are not necessary. However, in advanced stages of MF and SS, investigations with CT or PET-CT of neck/thorax/abdomen/pelvis, sometimes lymph node biopsy and bone marrow examinations are recommended (147).

Classification and prognosis of mycosis fungoides and Sézary syndrome

MF and SS are staged according to the same TNMB classification, assessing the involvement of skin, peripheral lymph nodes, blood, and visceral organs (157) (Table 1). Stages IA-IIA are considered early stages of disease and stages IIB-IVB are regarded as advanced stages of disease.

The TNMB stage at diagnosis remains the most important prognostic factor (163, 164). Patients with early stages of MF (stages IA-IIA) have a 5-year disease-specific survival of 89-98% and patients with advanced stages of MF (stages IIB-IVB) have a 5-year disease-specific survival of 18-56% (157, 165). The 5-year disease-specific survival for SS is 36% (157, 164, 166). Around 25% of patients with early stages of disease will progress to advanced stages (147).

Multivariate analyses of cohorts of patients with MF and SS have identified adverse prognostic factors consisting of male gender, age > 60 years, plaques, folliculotropic disease and stage N1/Nx in early disease; and negative prognostic factor for advanced disease consisting of male gender, age > 60 years, stages B1/B2, N2/N3 and visceral involvement (167, 168). The presence of plaques in early stage of disease, large cell transformation in the skin and elevated lactate dehydrogenase have also been reported as adverse prognostic factors (165, 169, 170).

Table 1.
TNMB staging system for mycosis fungoides and Sézary syndrome (157) and 5-year disease-specific survival (165). T1: patches/plaques < 10% body surface area; T1a: patches only; T1b: patches +/- plaques; T2: patches/plaques > 10%; T2a: patches only; T2b: patches +/- plaques; T3: Tumor ≥ 1 cm in diameter, T4: erythroderma ≥ 80% body surface area; N0: No clinically palpable lymph nodes (LN) (nodes < 15 mm); N1: clinically palpable LN with dermatopathic histopathology or scatted atypical cells; N2: clinically palpable LN with histopathological evidence of lymphomatous infiltration; N3: clinically palpable LN with complete effacement of the nodal architecture; Nx: clinically palpable peripheral LN with no histology (nodes > 15 mm); M0: no visceral disease, M1: visceral disease; B0: No significant blood involvement, ≤ 5% peripheral blood lymphoscytes (PBL) are atypical; B0a: clone negative; B0b: clone positive; B1: low tumor burden > 5% of PBL are atypical, but do not meet criteria for B2; B1a: clone negative, B1b: clone positive; B2: high blood tumor burden with ≥ 1000 cells/µl of atypical PBL with positive clone.

Stadium	Tumor (T)	Node (N)	Metastasis (M)	Blood (B)	5-year disease-specific survival (%)
IA	1	0	0	0-1	98%
IB	2	0	0	0-1	89%
IIA	1-2	1-2	0	0-1	89%
IIB	3	0-2	0	0-1	56%
IIIA	4	0-2	0	0	54%
IIIB	4	0-2	0	1	48%
IVA1	1-4	0-2	0	2	41%
IVA2	1-4	3	0	0-2	23%
IVB	1-4	0-3	1	0-2	18%

Treatment of mycosis fungoides and Sézary syndrome

Patients with early stages of MF are primarily treated by dermatologists. In advanced stages of MF and in SS, the patients are treated by dermatologists and an oncologist/hematologist in cooperation, since the treatments range over a broad spectrum. In some hospitals there are joint multidisciplinary out-patient clinics for patients with primary cutaneous lymphomas, and in Sweden there is also a national multidisciplinary conference for these patients.

The therapeutic options for MF and SS range from skin-directed therapy (SDT) to systemic treatment, and the selection of appropriate treatment for a patient is primarily based on the TNMB classification, but individual factors must be considered, such as other diseases and medications, pregnancy and distance to hospital for UV light therapy or ECP. Since MF and SS are uncommon diseases, large randomized controlled trials for treatment are sparse, and current recommendations for treatment are based on international guidelines (145, 147, 164). Often there are several different treatment options for the same patient with MF. Because MF is an indolent disease, patients often live with their disease for a long time, and over the years several different treatments are often needed to keep the disease under control.

In early stages of MF, skin-directed therapy, such as TCS or UV-light therapy with narrowband UVB (NB-UVB) or PUVA or topical chlormethine, may be sufficient. In early stages that are refractory to SDT or in advanced stages of MF and SS, systemic treatments are used, including retinoid derivatives, low-dose methotrexate, and interferon-alfa. Cutaneous lymphomas are highly radiosensitive and local radiation therapy can be given in all stages of MF. In patients with lymphoma cells expressing CD30, targeted chemotherapy with brentuximab-vedotin can be used. Chemotherapy with gemcitabine or liposomal doxorubicin can be employed; however, this often results in only short-term remission. Extracorporeal photopheresis can be used in erythrodermic stages of MF and SS. The CCR4 antibody mogamulizumab can be used in advanced stages of disease, especially when blood involvement is seen. Highly selected patients with advanced stages of MF or SS can be considered for allogenic stem cell transplantation with a curative intention, but this is a treatment option with considerable risk of morbidity and mortality. Histone deacetylase inhibitors are approved by the FDA, but not by the EMA and are seldom used in Sweden. Total skin electron beam therapy (TSEBT) can be used in widespread disease in the skin or prior to allogenic stem cell transplantation. The treatment of SS is similar to the treatment of advanced stages of MF. Treatment responses are often unpredictable and new reliable predictive markers are needed.

For most patients with MF and SS, there are no curative treatments, and the diseases are to be considered as chronic. The only treatments with a possible curative intention or long-term remission are allogenic stem cell transplantation or local radiation therapy of all disease in early stages of MF.

Immunological changes in mycosis fungoides and Sézary syndrome

In early stages of MF, the malignant T-cells only constitute a minor fraction of the cells, and most of the immune cells are benign reactive Th1-cells and cytotoxic CD8+ cells. With advancing clinical stage there is a decline in Th1-cytokines and an increase in the levels of Th2 cytokines (52, 161). The shift in the microenvironment from a Th1 to a Th2-dominated inflammation is thought to contribute to the progression of the disease.

Dysregulation of the JAK/STAT pathway occurs gradually in the progression of MF and SS, and STAT3, STAT5 and STAT6 become activated in the malignant T-cells, which has been shown to promote the expression of Th2 cytokines (171). An increase in the Th2 cytokines and Th2 milieu seems to initiate a complex cascade of signaling between fibroblasts, keratinocytes, and malignant and non-malignant T-cells.

Skin barrier function in mycosis fungoides and Sézary syndrome

Only a handful of studies have examined the skin barrier function in MF and SS.

Suga et al. examined 26 patients with MF and SS, 6 patients with AD and 5 patients with psoriasis regarding TEWL and filaggrin expression, loricrin and AMPs. The patients with MF and SS had a significantly higher TEWL compared to healthy-looking skin and healthy controls. Expression of filaggrin and loricrin, which are important for the skin barrier function, was significantly reduced in the active lesions in patients with MF and SS and in patients with AD. Expression of AMPs was lower in patients with MF and SS and AD compared to those with psoriasis. It was speculated that MF, SS and AD are Th2-driven diseases where IL-4 and IL-13 could inhibit the expression of filaggrin and loricrin and thus affect the skin barrier, and that Th2 cytokines could reduce AMPs and give an increased tendency to infection in MF, SS and AD (172).

Yazdanparast et al. investigated 21 patients with MF, with what was described as early patch/plaques stages of MF, and the measurements of the skin barrier function were performed on affected skin, perilesional uninvolved skin, and symmetrical uninvolved skin. They found no significant difference in TEWL in affected skin compared with perilesional uninvolved skin and symmetrical uninvolved skin (173).

Gluud et al. studied 12 patients with CTCL and measured TEWL in 15 non-lesional and 16 lesional locations. TEWL was higher in lesional skin compared to matched non-lesional skin in all patients and TEWL also correlated with the severity of the lesion (174).

The role of *Staphylococcus aureus* in mycosis fungoides and Sézary syndrome

The pathogenesis of MF and SS is still not fully understood. Since the 1970s there have been speculations that microorganisms in the skin might play a part in the pathogenesis and progression of CTCL (175). Patients with MF and SS have an increased risk of infections and sepsis, contributing to morbidity and mortality (176, 177). The most frequent pathogen is *S. aureus*, colonizing the skin in 44-76% of the patients with MF and SS and thought to contribute to disease progression (15, 178-183). Several studies have investigated the role of *S. aureus* in MF and SS (15, 180, 184, 185).

Jackow et al. performed a prospective study of 42 patients with MF and SS, where 76% had a positive culture of *Staphylococci* from either the skin or blood, and 38% had positive culture of *S. aureus*. Six patients were colonized with *S. aureus* producing toxic shock syndrome toxin (TSST-1), and these patients also had a TSST-1 driven Vβ2 clone of malignant T-cells in their blood. This observation led

to the hypothesis that *S. aureus* superantigens and enterotoxins might contribute to the progression of MF and SS (180).

Nguyen et al. included 50 patients with MF or SS, 25 healthy controls and 25 patients with psoriasis to examine skin colonization with S. aureus. Colonization was defined as a positive culture from affected skin or from the nasal area. 22 patients with MF or SS were excluded due to recent infection or oral. In the remaining patients with MF or SS, 44% were colonized with *S. aureus*, compared to 48% of those with psoriasis and 28% of healthy controls. Colonization with *S. aureus* was associated with an increased body surface area (BSA) with involvement of MF and SS (184).

Talpur et al. examined 106 patients with MF or SS with a bacterial culture from affected skin and the nasal area. Colonization with *S. aureus* was demonstrated in 42%, with colonization in skin in 31% and in the nasal area 31%. Colonization with *S. aureus* varied by TNMB stage, with 15% of the patients colonized in stage IA compared to 48% of the patients with erythrodermic MF or SS. The highest rate of colonization, 54%, was seen in stage IIB (15).

S. aureus produces a wide range of enterotoxins and superantigens and has been shown to exacerbate MF and SS by stimulating malignant T-cells both directly and indirectly by stimulating non-malignant T-cells to produce growth factors and cytokines, which then trigger activation and proliferation of malignant T-cells (51, 52, 178, 182, 183, 185-189).

In vitro studies have shown that staphylococcal enterotoxins can trigger a complex crosstalk between non-malignant and malignant T-cells, leading to increased proliferation, cytokine production, IL-2-receptor alpha-chain expression and STAT3 activation in malignant T-cells. Willerslev-Olsen et al. found that staphylococcus enterotoxin A induced STAT3 activation and IL-17 activation in malignant T-cells from patients with MF and SS. Staphylococcus enterotoxin A (SEA)-producing strains of *S. aureus* from patients with MF, as well as recombinant SEA, were shown to trigger STAT3 activation and IL-17 production in malignant T-cells when co-cultured with non-malignant T-cells, but not when co-cultured with SEA alone (185) (Figure 11).

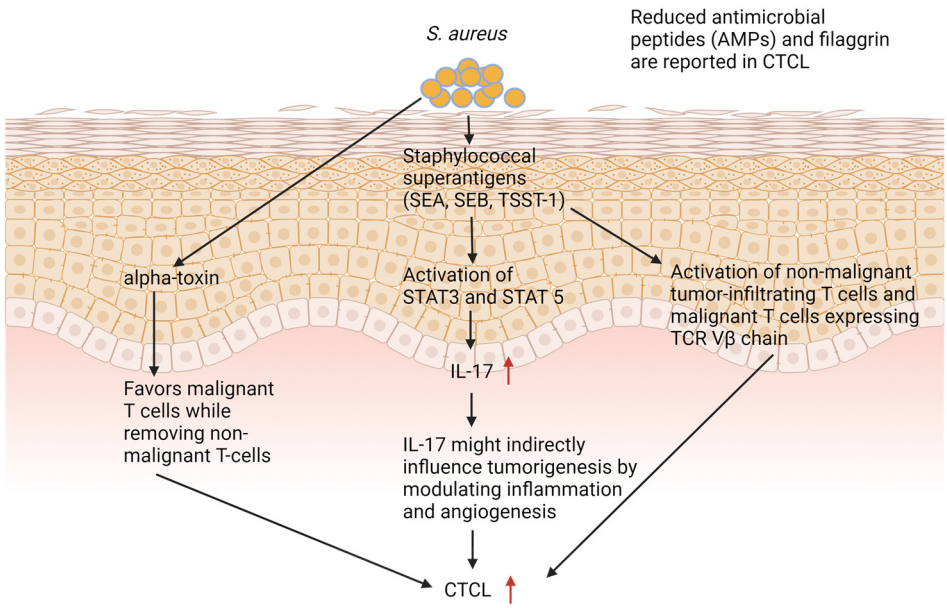

Figure 11.
The proposed role of *S. aureus* in cutaneous T-cell lymphoma (CTCL). Modified from Krejsgaard et al. 2011 (190), Suga et al. 2014 (172), Willerslev-Olsen et al. 2016 (185), Blümel et al. 2019 (178), Lindahl et al. 2019 (181), Lindahl et al. 2022 (191), Jost et al. 2022 (17). Created with BioRender.com

Treatment with antibiotics in mycosis fungoides and Sézary syndrome

Reducing *S. aureus* in the skin with antibiotics in patients with MF and SS has been associated with clinical improvement and a decrease in tumor burden in several studies (15, 181, 182, 192).

Nguyen V et al. recommended sodium hypochlorite bleach baths daily or once per week to reduce colonization with *S. aureus*, but no study was done on the effect of the sodium hypochlorite bleach bath (184).

Talpur et al. conducted a prospective study in patients with MF and SS, where patients with nasal colonization received intranasal and oral antibiotics and patients with only skin colonization received only oral antibiotics. Oral and intranasal antibiotics eradicated *S. aureus* nasally in 85% and in the skin in 91% of the cases after 4 weeks and resulted in a clinical improvement of > 50% was seen in 57% of the patients and a complete response in 23% of the patients (15).

Lewis et al. reported improvement in a patient with SS and methicillin-resistant *S. aureus* (MRSA) who was treated with intravenous antibiotics, whirlpool sessions, wet wraps with TCS and emollient. This was called "the Duvic regimen" (192).

Lindahl et al. conducted a prospective study of eight patients with advanced stage (> IIB) MF with no sign of acute infection, who were treated first with antibiotics intravenously and then oral antibiotics. All patients had a clinical improvement after two months. Punch biopsies before and after treatment showed a reduction in cell proliferation, soluble interleukin 2 receptor (sIL-2R) and phosphorylated-STAT3. One hypothesis was that staphylococcus enterotoxin A stimulates STAT3 activation, sIL-2R expression and proliferation of malignant T-cells. Antibiotics did not appear to have a direct effect on the malignant T-cells, but instead an indirect effect mediated by the inhibition of staphylococcus enterotoxin A and interaction with non-malignant T-cells. Disease progression might also be associated with dysregulation of JAK/STAT signaling (181).

However, in most patients *S. aureus* re-emerges after treatment with antibiotics and life-long antibiotics is not feasible due to the risk of antibiotic resistance and side-effects (191). The 2023 EORTC consensus recommendations for treatment of MF and SS found the current evidence intriguing, but insufficient to recommend antimicrobial treatment of MF and SS when no obvious bacterial infection is present (164).

The skin microbiome in mycosis fungoides and Sézary syndrome

Only a handful of studies have investigated the skin microbiome in MF and SS.

Salava et al. compared the microbiome from affected skin and contralateral healthy-looking skin in 20 patients with stages IA-IIB of MF and found no significant differences at the genus level or in microbial diversity (193).

Harkins et al. analyzed the microbiome in four patients with stages IA-IIIA of MF and two patients with SS stage IVA1 compared to healthy controls and found a non-significant trend towards lower relative abundance of *Cutibacterium acnes* (*C. acnes)* and a higher relative abundance of several staphylococcal species in the skin of patients with MF and SS. Although the trend was not significant, Harkins et al. suggested that a bacterial shift appeared to correlate with stage of disease (194).

Cutibacterium acnes in mycosis fungoides and Sézary syndrome

Cutibacterium acnes (C. acnes), formerly named *Propionibacterium acnes,* is a gram-positive rod bacterium and one of the most frequent commensal bacteria on the skin. In dermatology, *C. acnes* is best known because of its association to acne vulgaris; however, *C. acnes* has recently begun to be considered an important factor

for maintaining the normal skin barrier function and contributing to a healthy skin microbiome (195-197). A loss of abundance or alternations of *C. acnes* in the skin has been shown to be associated with dysbiosis and skin diseases, including AD, psoriasis, and acne (196). In healthy individuals as well as in patients with AD, *C. acnes* is reported to protect against colonization by *S. aureus* (11, 119, 197).

C. acnes has been shown to induce an increase in the synthesis of lipids, including triglycerides, ceramides, cholesterol, and free fatty acids, thus improving the skin barrier function (195). *C. acnes* contributes to the acidic environment in the skin by metabolizing free fatty acids from sebum and producing propionic acid, which helps to inhibit pathogenic bacteria, including *S. aureus*. In patients with acne, *C. acnes* has been reported to induce activation of Th1 cells in vivo (198). In a study on mice with AD, injections with *C. acnes* resulted in an enhanced Th1 response and improvement in AD (199). *C. acnes* also secretes the protein radical oxygenase of Propionibacterium acnes (RoxP), which has been reported to have antioxidant activity (200, 201).

In patients with psoriasis, dysbiosis and a decreased level of *C. acnes* were associated with disease progression (196). Recently, progression of acne was shown to be associated with a decrease in the diversity of *C. acnes*, rather than with a proliferation of *C. acnes* (196). Analysis of the microbiome from basal cell carcinoma and actinic keratosis also revealed diminished *C. acnes* compared to healthy skin (201-203).

Harkins et al. investigated the skin microbiome in MF and SS skin lesions compared to healthy controls and found a non-significant trend of a lower relative abundance of *C. acnes* and a higher relative abundance of *Corynebacterium* and *Staphylococcal* species in skin lesions with MF and SS compared to healthy controls, thus indicating a need for further investigations of the skin microbiome in relation to stage of disease (194).

Soluble interleukin-2 receptor in mycosis fungoides and Sézary syndrome

Interleukin-2 (IL-2) is a cytokine mainly produced by activated Th cells. IL-2 have an autocrine effect, binding to receptors on the same cell, leading to an expansion of clones of antigen-specific T-cells. IL-2 binds to an IL-2-receptor in the cell membrane that consists of three polypeptides, the α subunit, β subunit, and the γ subunit. When there is an increased expression of IL-2R, a soluble form of the IL-2 α subunit, called soluble interleukin 2 (sIL-2R) is seen in serum. The IL-2 receptor is expressed on both T-and B-cells and monocytes (204). sIL-2R is an unspecific marker for activation of the immune system and has been suggested as a possible marker for prognosis and progression in autoimmune diseases, transplant rejection,

infectious disorders, and cancer, including forms of leukemia, lymphomas and CTCL (205-209).

Zachariae et al. examined sIL-2R in 20 patients with MF and found that 3 patients had normal levels with less than 500 kU/L, 9 patients had levels between 500-1,000 kU/L and 5 patients had levels higher than 1,000 kU/L. Four of the five patients with high levels above 1,000 kU/L died within one year. The authors concluded that high levels of sIL-2R could be a prognostic parameter that correlated to disease activity, although it is not disease-specific for MF (205).

Wasik et al. analyzed the concentration of sIL-2R in 101 patients with CTCL. The serum concentration of sIL-2R correlated positively with CTCL tumor burden, by lactate dehydrogenase concentration and by Sézary cell counts in blood erythrodermic disease. The portion of patients with sIL-2R concentrations > 1000 kU/L was 15% in MF with patches, 33% in MF with plaques, 47% in MF with tumors and 90% in erythrodermic variants. The median levels of sIL-2R in erythrodermic CTCL was more than threefold higher than in earlier stages of MF (206).

Vonderheid et al. measured sIL-2R serially in 36 patients with advanced disease and found that sIL-2R correlated with severity of skin manifestations, nodal involvement, and large-cell transformation. During treatment, sIL-2R correlated significantly with the clinical status and pre-treatment levels correlated significantly with survival (207).

Eklund et al. conducted a retrospective study of 44 Swedish cases and found that sIL-2R correlated with stage of disease, with higher levels in stages II-IV compared to stages IA-IB (208).

sIL-2R is not diseases-specific for CTCL and can therefore not be used for diagnostic purposes; however, it may be a prognostic marker for disease activity, although the use of sIL-2R in clinical practice in Sweden is not widespread.

TARC/CCL17 in mycosis fungoides and Sézary syndrome

Thymus and activation-regulated chemokine (TARC/CCL17) is a member of the CC chemokine group and is produced by keratinocytes, fibroblasts, endothelial cells, and dendritic cells. The chemokines are a family of small cytokines with an ability to stimulate migration of cells, mainly leukocytes (210). TARC/CCL17 binds to CC chemokine receptor 4 (CCR4) and CC chemokine receptor 8 (CCR8), expressed mainly on Th2 cells, and functions as a chemoattractant for these cells (211, 212).

Kakinuma et al. measured TARC/CCL17 in a cohort of 20 patients with MF and compared them with 10 patients with psoriasis and 10 healthy controls. The serum levels of TARC/CCL17 were significantly higher in patients with MF compared to

psoriasis and healthy controls. Serum levels of TARC/CCL17 correlated significantly to serum levels of lactate dehydrogenase levels, immunoglobulin E and sIL-2R (213).

Serum TARC/CCL17 has been reported to be significantly higher in patients with AD compared to patients with psoriasis and healthy controls (212, 214, 215). The increased levels of TARC/CCL17 correlated with disease severity of AD and were lower after treatment and clinical improvement. The levels of TARC/CCL17 also correlated to higher eosinophil number in blood and serum sIL-2R (214). There has been a report suggesting that TARC/CCL17 might also be an important chemokine in bullous pemphigoid (213).

Tamaki et al. investigated TARC/CCL17 levels in several skin diseases and high serum levels of TARC/CCL17 were only seen in AD, chronic actinic dermatitis, papulo-erythema syndrome, MF, Sézary syndrome and staphylococcal scaled skin syndrome (216).

Total IgE and sensitization to skin-associated microorganisms in patients with mycosis fungoides and Sézary syndrome

Since the late 1970s it has been debated whether patients with AD have an increased risk of developing MF and SS, and several studies have addressed the subject (217-221).

Some studies have reported increased serum levels of total IgE and eosinophil number in patients with MF compared to healthy controls (222, 223). Kural et al. inferred that atopy was more common in patients with MF compared to healthy controls (222).

Recently, Vonderheid et al. investigated total IgE and specific IgE to staphylococcal enterotoxins in 20 patients with SS and 20 patients in the plaque stage of MF. Total IgE was increased in SS and marginally increased in MF. Specific IgE to staphylococcal enterotoxins was found in 40% of patients with SS and 20% of patients with MF. No correlation was found between personal history of atopic disorder, total IgE levels or specific IgE to staphylococcal enterotoxins with prognosis or overall survival of MF and SS (224).

Vonderheid et al. recently presented a study that did not reveal any increased prevalence of atopy in patients with MF. However, atopy is a factor contributing to increased IgE levels and eosinophil number in patients with MF and SS who do have a pre-existing atopy. Another proposed factor was related to stage of disease of MF, where malignant Th2 cells produce cytokines (IL-4, IL-13, IL-5) promoting the production of total IgE and eosinophils. A third factor might be skin colonization by *S. aureus* that produce staphylococcal superantigens that might enhance the IgE production (225).

Quality of life in patients with MF and SS

MF is a chronic and indolent disease that often evolves slowly over several years. SS can have a more abrupt onset with erythroderma. Itching is a common symptom in MF and SS, and can affect quality of life. Previous studies of quality of life have reported a mild to moderate effect in early stage of MF, and an even greater impact on quality of life in advanced stages of MF and SS (226, 227).

Aims

The general aim of the thesis was to improve our knowledge of factors that might be important for the course of disease in AD, MF and SS. Although AD is a benign skin disease and MF and SS are malignant CTCL, they do have in common that they have a mainly Th2-driven inflammation, and the chronic course of the diseases seems to be affected by interactions with the skin microbiome. This thesis aimed to further explore these factors through patient-related research.

Paper I

- To investigate the skin barrier function in patients with AD colonized with *S. aureus*, in patients with AD not colonized with *S. aureus* and in healthy controls.
- To determine if sensitization to skin-associated microorganisms (*S. aureus, Malassezia, Candida*) can affect the skin barrier function in patients with AD.

Paper II

- To investigate *MBL* gene polymorphism in patients with AD in relation to sensitization to skin-associated microorganisms, including *Candida albicans*.
- To study whether *MBL* gene polymorphism in patients with AD can affect disease severity and skin barrier function.

Paper III

- To find predictive and prognostic biomarkers in skin and blood in patients with MF and SS.

Paper IV

- To investigate whether there are any differences in the quantities of *Staphylococci* and *C. acnes* in the skin microbiome between unaffected and affected skin in patients with early stages of MF, patients with advanced stage of MF and healthy controls.

- To investigate whether there are any differences in the skin barrier function between unaffected and affected skin in patients with early stages of MF, patients with advanced stages of MF and healthy controls.
- To study whether there is a difference in sIL-2R and TARC/CCL17 between patients with early stage of MF and patients with advanced stage of MF.

Materials and methods

Study design and study population

Paper I

This study was a cross-sectional study recruiting adult patients (> 18 years) with AD on their visit to the Department of Dermatology and Venereology, Skåne University Hospital, Lund, Sweden. The diagnosis was confirmed using the UK Working Party's diagnostic criteria for AD (76). Exclusion criteria were patients undergoing topical treatment on the day of examination or ongoing UV light treatment. Healthy adult individuals without a history of AD were included as a control group.

Paper II

This study was a cross-sectional study including adult patients (> 18 years) with AD on their visit to the Department of Dermatology and Venereology, Skåne University Hospital, Lund, Sweden. The diagnosis was confirmed using the UK Working Party's diagnostic criteria for AD (76). Exclusion criteria were patients with other skin diseases, ongoing UV light treatment or systemic treatment against AD.

Paper III

This paper was a study protocol for a prospective translational study, named the BIO-MUSE (Predictive and prognostic biomarker in patients with mycosis fungoides and Sézary syndrome) study, aiming to include 120 adult patients with MF or SS and a control group of 20 healthy adults. Each patient was to be sampled every three months for three years. The healthy control group was to be investigated on three occasions, at least two months apart.

Inclusion criteria for the patient group were patients aged 18-100 years with histologically confirmed MF or SS and a WHO performance status of 0-3. Inclusion criteria for the control group were healthy adults aged 18-100 years with a WHO performance status 0-3 and an absence of any malignant, autoimmune, and infectious disease. For both groups, the exclusion criteria were psychiatric illness and conditions that could interfere with the ability to understand the requirements of the study. The study was non-interventional and participation in the study did not affect the choice of treatments against MF and SS.

Paper IV

Paper IV was a cross-sectional study that was part of the BIO-MUSE study and was based on samples from the baseline visit in the BIO-MUSE study. The inclusion and exclusion criteria were the same as in paper III. The participants were included between May 2021 and June 2023 at Skåne University Hospital, Lund, Sweden.

Assessment of disease severity in atopic dermatitis

In papers I-II, the signs and severity of AD were assessed by using SCORing Atopic Dermatitis (SCORAD) (228). SCORAD combines extent, intensity, and subjective symptoms. The extent and the subjective symptoms account for 20% each of the total score and the intensity items represent 60%.

SCORAD is calculated according to the formula: A/5 + 7B + C

A = A is the extent of disease as a percentage of body area. In adults, the rule of 9 is used to calculate the affected area A as a percentage of the whole body; where head and neck are 9%, the upper limbs are 9% each, the lower limbs are 18% each, the anterior trunk is 18% and the genitals are 1%. The scores of each area are added up and A has a maximum of 100 (%).

B = B is the intensity of the eczema for each of the following signs, assessed as none (0), mild (1), moderate (2), or severe (3) for erythema, oedema/papulation, oozing/crusts, excoriations, skin thickening (lichenification) and dryness (assessed in an area with no inflammation). The intensity scores are added up to give B. B has a maximum of 18.

C = C is the subjective symptoms of itching and sleep disturbance for the last 3 days according to the visual analogue scale (VAS) where 0 is no symptoms and 10 is the worst imaginable symptoms. These scores are added up to give C. C has a maximum of 20.

SCORAD < 25 = mild eczema, SCORAD 25-50 = moderate eczema, SCORAD > 50 = severe eczema. Maximum SCORAD is 103.

In paper II, the SCORAD results were categorized as mild disease with SCORAD < 25, intermediate disease with SCORAD 25-50, and severe disease with SCORAD > 50. The presence of head- and neck dermatitis was also investigated in paper II.

Methodological considerations

There are several scoring instruments used to assess the signs of AD (228-230). Some of these scoring instruments are objective and evaluated by a physician or a nurse, and some of them are subjective and evaluated by the patient or a relative.

In papers I-II, SCORAD was used to evaluate signs and severity of AD, and at that time SCORAD was widely used as a scoring instrument in clinical studies.

The international Harmonizing Outcome Measures for Eczema (HOME) group decided in 2013 that Eczema Area and Severity index (EASI) should be the preferred standardized instrument to measure signs of AD in clinical trials and health care (231). Both SCORAD and EASI measure the signs erythema, papulation, lichenification and excoriation, but EASI was preferred because it only measures signs and not subjective symptoms, and because the extent of signs was of greater importance in EASI compared to SCORAD. In recent years, EASI has become the instrument most often used to measure signs of AD in clinical studies and in health care (229).

TNMB staging of mycosis fungoides and Sézary syndrome

In papers III-IV, the patients were staged according to the TNMB classification of MF and SS (157). In papers III-IV, the patients were grouped as early stage of disease (stages IA-IIA) or advanced stage of disease (stages IIB-IVB).

Assessment of disease severity in mycosis fungoides and Sézary syndrome

In papers III-IV, the extent of MF and SS in the was skin was measured according to the modified Severity-Weighted Assessment Tool (mSWAT) (232-234).

In 2002, Stevens et al. published a quantitative tool to assess the extent of skin involvement in MF and SS, called the Severity-Weighted Assessment Tool (SWAT) (232).

In SWAT, the investigator measures the total body-surface area (TBSA) involvement as a percentage for each type of MF and SS lesion (patches, plaques, or tumors) in 12 regions of the body. The patient's palm and fingers represent 1% of the TBSA. The sum of each type of lesion is then multiplied by a weighting factor, and then the numbers are summed up to derive the SWAT score. The weighting factors in SWAT are 1 for patches, 2 for plaques and 3 for tumors (232). After discussion about the appropriate weighting factor for tumors, this was later changed to a weighting factor of 4 for tumors, and this was called the modified Severity-Weighted Assessment Tool (mSWAT) (233, 234). The mSWAT score ranges from 0 to 400.

According to a consensus statement in 2011, the manifestations of MF and SS in the skin should be measured according to the modified Severity-Weighted Assessment Tool (mSWAT) in clinical trials (233).

Measurement of transepidermal water loss

In papers I-IV, the skin barrier function was measured as transepidermal water loss (TEWL) with a closed chamber TEWL meter, the Vapometer 300 (Delfin Technologies Ltd, Kuopio, Finland) (Figure 12, Figure 13). According to the guidelines, the participants had to rest for 5-15 minutes before the measurements and the environment had a relative humidity of 10-60% and a temperature of 20-22 °C (235). TEWL was measured two times at each location and a mean value was calculated.

In papers I-II, TEWL was measured on the left volar forearm, left dorsal forearm and abdomen to the right of the navel.

In papers III-IV, TEWL was measured on affected skin and on unaffected skin located 5 cm away from affected skin in patients with MF, preferably on the upper part of the body, and on the upper arm in healthy controls.

Methodological considerations

TEWL is an established non-invasive method to evaluate the skin barrier function (7, 236). The method was first described in the 1940s and today several different technologies are available. TEWL measures the quantity of condensed water that diffuses across a fixed area of the stratum corneum to the skin surface per unit of time and is expressed in $g/m^2/h$. TEWL is measured with a probe that is placed in contact with the skin surface and contains sensors that detect changes in water vapor density. An increased TEWL is associated with skin barrier dysfunction and a decreased TEWL is regarded as an intact skin barrier (7, 235, 237, 238).

TEWL is affected by climate conditions, anatomical location, and the accuracy of the measurement devices. Anatomical location seems to be important and in healthy adults TEWL values as low as 2.3 $g/m^2/h$ have been found in breast skin and levels as high as 44 $g/m^2/h$ have been found in the axilla (238). Some studies have found that TEWL decreased with age (239), but other studies found no difference in TEWL with age. There are mixed results on how gender, ethnicity, body temperature, and other factors effect TEWL (238, 240). FDA guidance gives a defined standard limited value of < 15 $g/m^2/h$, but normal reference values for TEWL are still being debated and a consensus is lacking (238, 240, 241). Updated

guidelines for the surrounding conditions while performing TEWL measurements have been suggested (7).

Since TEWL can vary in different anatomical locations in the same patient, we wanted to, as far as possible, measure the same anatomical location in both patients and healthy controls in papers III-IV. All healthy controls were investigated for TEWL on the upper arm and in the patients TEWL was analyzed on the upper body, preferably on the upper arm or on the thorax. In patients, the unaffected skin 5 cm away from affected skin was meant to act as a control of unaffected skin in the same anatomical location within the same patient.

Figure 12.
The closed chamber TEWL meter, the Vapometer 300 (Delfin Technologies Ltd, Kuopio, Finland). Photo: Emma Belfrage

Figure 13.
Measurement of transepidermal water loss. Photo: Emma Belfrage

Bacterial cultures

In paper I, bacterial cultures were taken from the left volar forearm or left antecubital fossa with a dry swab, Venturi Transystem (Copan, Italia S.p.A, Brescia, Italy) and analyzed for qualitative identification of *S. aureus* according to standards methods at the Department of Clinical Microbiology at Skåne University Hospital. Participants with a positive culture for *S. aureus* were regarded as *S. aureus*-culture positive.

In papers III-IV, bacterial cultures were taken with Copan eSwab (Copan Italia S.p.A, Brescia, Italy) (Figure 14) from affected skin and nares in patients, and from nares in healthy controls, and analyzed as described above.

Methodological considerations

Bacterial culture is a widely used method used in clinical practice to detect living bacteria.

Figure 14.
Copan eSwab (Copan Italia S.p.A, Brescia, Italy) used for bacterial cultures and microbiome samples. Photo: Emma Belfrage

Measurement of bacteria with the contact agar disc method

In paper I, bacterial samples were taken with a contact agar disc method (CADM) for a quantitative assessment of *S. aureus* (242, 243). The contact agar disc method was modified and performed by pressing a TGSE agar food stamp (Termometer.se Ltd, Gothenburg, Sweden) (Figure 15) against the antecubital fossa for five seconds and then incubating at room temperature for 48-72 hours. The number of colony-forming units (cfu) were registered.

Methodological considerations

The contact agar disc method (CADM) has not been widely used before. Williams et al. investigated patients with AD and healthy controls with a contact-plate method and compared it with the Williamson and Kligman scrub-wash method and found that the contact-plate agar technique was trustworthy for quantification of *S. aureus* (243).

Figure 15.
TGSE agar food stamp (Termometer.se Ltd, Gothenburg, Sweden). Photo: Emma Belfrage

DNA extraction of skin swabs and absolute quantification of gene copies using ddPCR for analysis of the skin microbiome

In papers III-IV, skin swabs for microbiome analysis were taken from affected skin in patients with MF and from unaffected skin at least 5 cm away, preferably from the upper body. Samples for the microbiome analyses from the healthy control group were taken from the upper arm. An area of 2x2 cm was swabbed with Copan eSwab (Copan Italia S.p.A, Brescia, Italy) (Figure 16) and cellular material was collected through centrifugation (10,000 g, 2 min) from which DNA was extracted using the PureLink Microbiome DNA purification kit (Invitrogen, Thermo Fischer, Waltman, MA, USA). Quantity and quality of DNA extractions were evaluated by NanoDrop.

Absolute quantification of gene copies was performed on a Bio-Rad QX200 Digital Droplet system. The used primers were designed to specifically target *C. acnes* and most of the *Staphylococci* in the skin microbiome, including *S. aureus* and *S.*

epidermidis (Table 2). Amplified products were analyzed in a QX200 Droplet Reader (Bio-Rad) and the data were analyzed with the QuantaSoft analysis software. The results were converted into gene copies per cm^2. All samples were analyzed in technical triplicates.

Methodological considerations

Different methods can be used to analyze the skin microbiome. In this study we used the method digital droplet PCR, also called ddPCR, which enables the measurement of absolute quantifications of gene copies. Another method for analysis of the skin microbiome is 16S ribosomal RNA, also called 16S rRNA, which measure the relative ratio of bacterial genes in the skin, but not the absolute quantification of gene copies. The skin microbiome analyses investigate bacterial genes in the skin microbiome but are not able to separate dead bacteria from live bacteria.

Table 2.
Primers specifically targeted *C. acnes*, as well as most *Staphylococci* (e.g. not *S. argenteus, S. equorum, S. hyicus, S. roterodami, S. schweitzeri, S. argnetis, S. carnosus, S. condiment, S. piscifermentans, S. simulans, S. debuckii.)*

Target	Forward	Reverse	Probe	Fluorophore
C. acnes	CGGCGCTGCT AAGAACTTAA	CTTCTTGC TTGCAGTT GCGA	TGTGGGTTCC TTTCATCGGG AACAATTGC	HEX
Staphylococci	GTGTTGAACG TGGTCAAATCA	ATGTTGTC ACCAGCTT CAGC	GTTACTGGTG TAGAAATGTTC CGTAA	FAM

Figure 16.
An area swabbed with Copan eSwab (Copan Italia, S.p.A, Italy) for microbiome analysis. Photo: Emma Belfrage

Analysis of total IgE and specific IgE to skin-associated microorganisms

In papers I-IV, venous blood samples were taken for the investigation of total immunoglobulin E (IgE) and specific IgE to staphylococcal enterotoxin A (SEA) (m80), staphylococcal enterotoxin B (SEB) (m81), toxic shock syndrome toxin (TSST-1) (Rm226), *Malassezia* (m227) and *Candida albicans* (m5) measured in serum with Immuno-CAP™ system, Phadia AB, Uppsala, Sweden. Specific IgE ≥ 0.35 was considered positive and regarded as sensitization. The analyses were performed at the Department of Clinical Immunology, Skåne University Hospital.

Analysis of MBL in serum

In paper II, venous blood samples were taken and the MBL serum concentrations were analyzed at the Department of Clinical Immunology, Skåne University Hospital. MBL deficiency was defined as serum MBL < 100 µg/l. The normal reference value for serum MBL is > 100 µg/l at the Department of Clinical Immunology, Skåne University Hospital.

Assessment of *MBL* gene polymorphisms

In paper II, mannose-binding lectin gene polymorphisms were analyzed in venous blood samples. Allele-specific PCR amplification was used to determine variants of *MBL* due to mutations at codon 52 (D), 54 (B) and 57 (C) in exon 1 of the *MBL* structural gene and promotor variants at position -550 (H/L) and -221 (X/Y).

The normal wild-type structural allele was designated A, while 0 was the description of the mutant alleles B, C and D.

MBL gene polymorphisms were divided into three groups based on the previously described associations between *MBL* gene polymorphism and the corresponding MBL serum concentrations in 200 healthy controls (29, 30, 40, 41). Group 1 (low MBL) consisted of patients with two structural mutant alleles (0/0) or on one haplotype a structural mutant allele together with another haplotype containing an LX promotor and the wild-type structural allele (ALX/0); this group was expected to have serum MBL deficiency. Group 2 (intermediate MBL) consisted of patients with the promotor LX on both haplotypes, but with normal structural alleles (ALX/ALX); this group was expected to have intermediate levels of serum MBL. Group 3 (high MBL) consisted of patients with the A/A genotype and at least one non-LX promotor; this group was expected to have high levels of serum MBL.

Group 1 (low MBL) was expected to have deficiency of serum MBL, and group 2 (intermediate MBL) and group 3 (high MBL) were expected to have no deficiency of serum MBL (30, 40, 41).

The analyses of *MBL* gene polymorphisms were performed at the Section of Rheumatology, Department of Clinical Sciences, Lund University, Sweden.

Analysis of sIL-2R, interleukins and TARC/CCL17

In papers III-IV, venous blood samples were analyzed for complete blood count, liver and kidney function, lactate dehydrogenase, sIL-2R, interleukin-6 (IL-6), IL-8, IL-10 and TARC/CCL17 in patients, but not in the control group.

In paper IV, venous blood samples were analyzed for sIL-2R, IL-6, IL-8 and TARC/CCL17. The normal reference values were sIL-2R < 700, IL-6 < 8, IL-8 < 60, and TARC/CCL17 had a normal reference value of 71-848. The analyses were performed at the Department of Clinical Immunology, Skåne University Hospital.

Analysis of lymphocyte subpopulations

In paper III, investigation with flow cytometry of peripheral blood was performed in patients to analyze the abundance of different T-cell subpopulations and the analyses were conducted at the Department of Clinical Immunology, Skåne University Hospital.

In paper III, flow cytometry of peripheral blood was also used to detect the presence of malignant clonal T-cells in patients, and the analyses were conducted at the Department of Pathology, Skåne University Hospital (244, 245).

Serum-based profiling of immune-related soluble proteins

In paper III, broad sets of serum-based profiling of immune-related proteins were performed in patients. Using commercially available methods, global protein profiling will allow high-plex analyses of immune-related soluble molecules to be detected, and the analysis will focus on identification of biomarkers that indicate clinical progression (246).

Assessment of tumor microenvironment by digital spatial profiling in skin biopsies and single-cell RNA sequencing in blood

In paper III, two skin biopsies from affected skin and one skin biopsy from unaffected skin, at least 5 cm away, were performed at baseline in patients, and then two biopsies from affected skin were taken once per year and at progression, to investigate the cellular immune microenvironment of the tumor. One skin biopsy from the upper arm was performed in the control group at baseline. The skin biopsies were formalin fixed and paraffin embedded. In skin biopsies, digital spatial profiling will be used to study immune cells' infiltration and changes over time and during progression (247).

In paper III, venous blood was drawn from patients and peripheral blood mononuclear cells were cryopreserved. Single-cell RNA sequencing and T-cell receptor sequencing were applied to follow the malignant T-cell clone and changes over time and during progression.

Patient-oriented life quality measures

In paper III, the Dermatology Life Quality Index (DLQI), the Peak Pruritus Numerical Rating Scale (NRS), the Sleep Disturbance NRS and the Connor-Davidson Resilience Scale was used to measure quality of life in patients (248-250).

The DLQI is a validated questionnaire of 10 questions to assess the impact of a skin disease on quality of life. The questions range over several subjects, including daily activities, symptoms, work, leisure time, treatments, and relationships. For each question, the patient answers how much their skin disease has affected them during the last week with a number, according to "not at all = 0", "a little = 1", "a lot = 2" or "very much = 3". Together, these 10 questions produce a score from 0 being "no impact on quality of life" to 30 being "maximum impairment" (248).

The Peak Pruritus NRS is a patient-reported item to measure peak pruritus for the last 24 hours on a scale from 0 to 10, with 0 being "no itch" and 10 being "worst itch imaginable" (249).

The Sleep Disturbance NRS is a patient-reported item to measure sleep disturbance during the last night on a scale from 0 to 10, with 0 being "no sleep loss" and 10 being "I did not sleep at all".

Ethics

Papers I-II

Studies I-II were approved by the Regional Ethics Examination Board of Lund with registration number 2012/82. Informed written consent was obtained from all participants according to the Helsinki Declaration.

Papers III-IV

The BIO-MUSE study was approved by the Swedish Ethics Committee under registration number 2019-05130 and was registered with Clinicaltrials.gov NCT04904146. Written informed consent was obtained from the participants according to the Declaration of Helsinki and ICH-GCP guidelines.

Ethical considerations

Clinical research is important for the advancement of medicine and mankind, but history has taught us that the ethical principles are important to protect the safety and interest of the participants in a study. After the Second World War, the first international code for ethics in clinical research, the Nuremberg Code, in 1947 stated the first guidelines for research on humans (251). Later the Helsinki Declaration was published in 1964 by the World Medical Association and has since undergone several updates and still serves as the fundamental document regarding clinical research involving humans (252).

The four cardinal ethical principles used in medical ethics are autonomy, non-maleficence, beneficence, and justice. Autonomy refers to the importance of informed consent, where the patients have the right to choose or refuse a treatment or participation in a clinical study. The patient should be given sufficient information and time to understand the purpose of a treatment or a study, as well as the risks and benefits. The participants should also be informed of the right to withdraw their consent at any time. Non-maleficence implies the importance of that the treatments and studies should cause no harm. Beneficence means that the treatment and clinical research must promote the well-being of patients and society. Justice refers to the question of scarcity of health resources and who these resources are distributed to and who are entitled to a treatment. These ethical principles also apply to clinical research.

Most clinical research studies involving humans in Sweden must go through an application and an evaluation by the Swedish Ethical Review Authority.

All papers in this thesis were approved by the Swedish Ethical Review Authority and the registration numbers are documented in Material and Methods. Written informed consent was obtained in all papers, meaning the participants were

informed and understood the risks and benefits of the study as well as the purpose of the study and agreed to participate. The patients were informed they could withdraw their consent at any time.

In papers I-II, the patients with AD were enrolled in the study at their visit at the Department of Dermatology and Venereology, Skåne University Hospital and samples were taken. The patients were informed that participation in the study would not affect their medical care. The investigations performed were considered to do no harm to the patients or the healthy controls.

In papers III-IV, the patients with MF and SS were included in the study at their visit to the out-patient clinic for primary cutaneous lymphomas at the Department of Oncology, Skåne University Hospital. The patients were informed that participation in the study would not affect their medical care. The patients were informed that their visits to the hospital would become more frequent due to sampling every three months for three years. The biggest intrusion for the patients and the healthy controls was probably the skin biopsies. However, these skin biopsies were assessed to be of great value for the study and the harm to the participants was regarded to be minor.

Statistical analysis

Paper I

In paper I, the Wilcoxon-Mann-Whitney rank sum test was used for comparison between two different groups. A *P*-value of < 0.05 was regarded as a significant difference. For comparison of several groups, the Kruskal-Wallis one-way analysis of variance by ranks was used and pairwise multiple comparison procedures (Dunn's method) post-hoc test was used to define groups that differed. The statistical software used was SigmaStat (Systat Software inc., Point Richmond, CA, USA).

Paper II

In paper II, a statistical analysis was performed to study a possible association between the *MBL* gene polymorphism called Group 1 (low MBL) and sensitization to *Candida albicans*. Differences between patients showing positive or negative sensitization to skin-associated microorganisms were evaluated with a chi2 test for categorical independent variables and t-test for continuous independent variables. Logistic regression models were employed to evaluate whether the odds ratio (OR) for Candida albicans sensitization differed between the *MBL* gene polymorphisms, with and without adjustment for SCORAD category, TEWL and presence of head- and neck dermatitis, respectively. The analysis was performed in Stata 14 (StataCorp, 2015) and a *P*-value of < 0.05 was considered statistically significant.

Paper III

After the end of the study, the patients in the BIO-MUSE study will be grouped in early stage of disease (stages IA-IIA) or advanced stage of disease (stages IIB-IVB). About 30% of the patients are estimated to cross over from the early to the advanced stage group; they will be excluded and analyzed both individually and as a group. Translational samples from the group with early stage of disease will be compared to samples from the group with advanced stage, using multivariate analysis with principal component analysis. Individual samples will be compared between the groups using the Mann-Whitney test. Other translational samples will be presented by descriptive statistics.

Paper IV

Statistical analyses were performed using GraphPad Prism 9.4.0 with the Mann-Whitney U test and Wilcoxon for unpaired and paired analyses in a univariate analysis, respectively. Correlative analyses were conducted using Spearman analysis.

Results

Paper I

This study included 30 patients with AD and 10 healthy controls.

The bacterial culture for *S. aureus* was positive in 10 patients with AD and negative in 20 patients with AD. *S. aureus* was not isolated in any of the healthy controls.

S. aureus culture-positive patients with AD had a significantly higher SCORAD compared to patients not colonized with *S. aureus* ($P = 0.002$).

S. aureus culture-positive patients with AD had a significantly higher TEWL compared to patients not colonized with *S. aureus* ($P < 0.05$) (Figure 17).

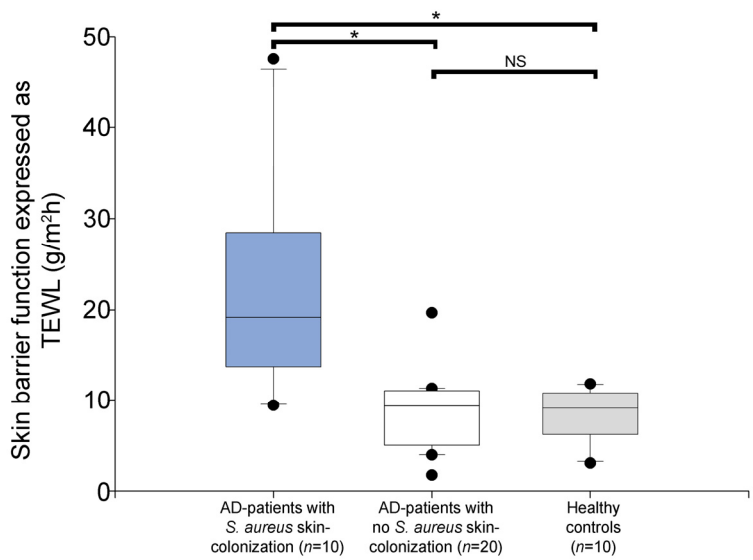

Figure 17.
TEWL level was measured in patients with AD with and without skin colonization by *S. aureus* and in healthy controls. Patients with AD with skin colonization by *S. aureus* had a significantly higher TEWL than patients with AD not colonized or healthy controls. *$P < 0.05$. NS, not significant.

There was a significant association between TEWL and the extent of bacterial skin colonization, quantified by counting the number of colony forming units (cfu) isolated from the forearm ($P = 0.018$) (Figure 18).

In *S. aureus* culture-positive patients, more than 100 cfu were seen in the contact agar disc method compared to only a few or no cfu in *S. aureus* culture-negative patients.

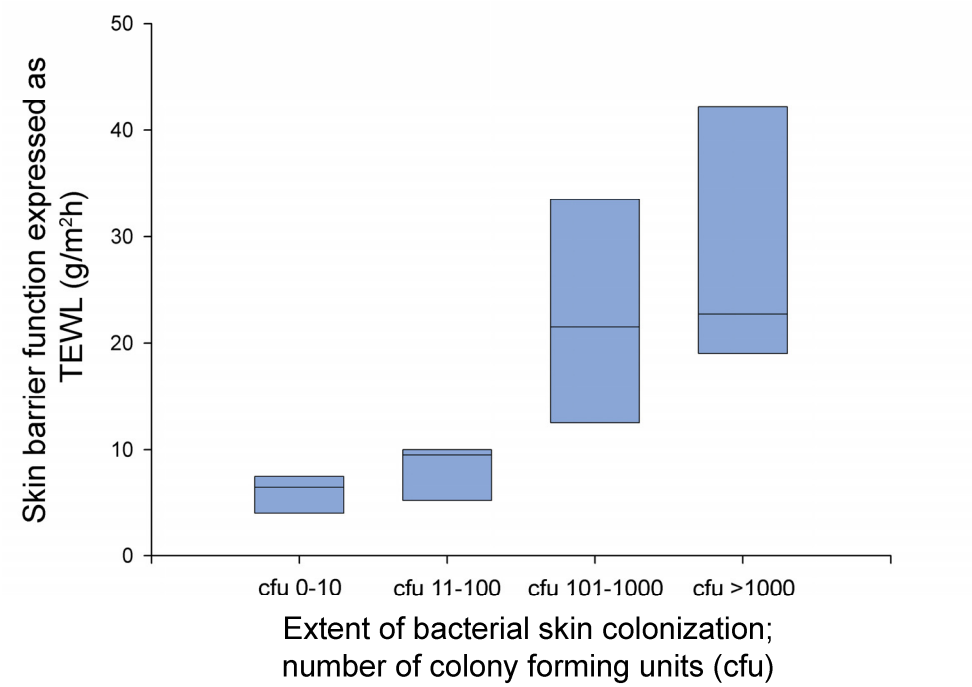

Figure 18.
TEWL was measured in patients with AD in relation to the extent of bacterial colonization, quantified by counting the number of colony forming units (cfu). There was a significant difference in the mean values among the groups ($P = 0.018$).

Patients sensitized to three of the investigated skin-associated microorganisms (*S. aureus*, *Candida albicans* and *Malassezia)* had an increased TEWL (Figure 19), higher SCORAD and total IgE compared to patients sensitized to none, one or two of the investigated skin-associated microorganisms ($P = 0.026$, $P = 0.008$ and $P < 0.001$).

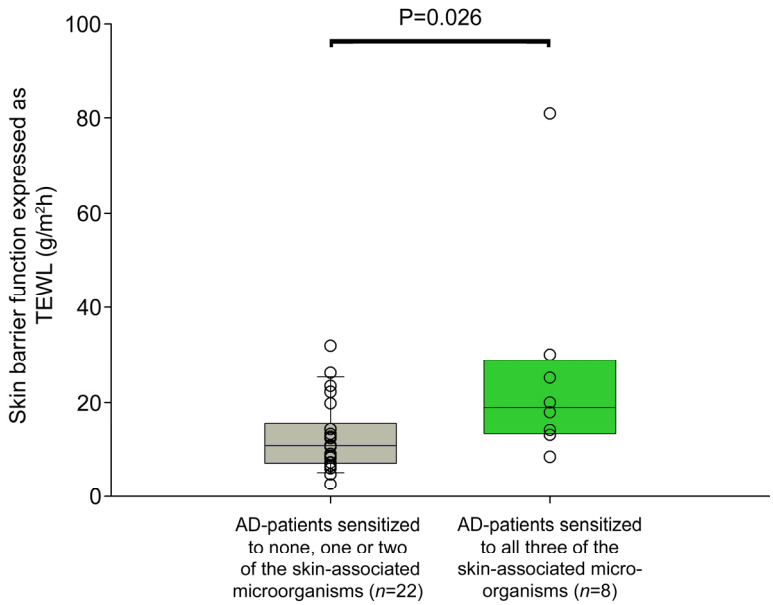

Figure 19.
TEWL was significantly higher in patients with AD sensitized to all three of the investigated skin-associated microorganisms *(S. aureus, Malassezia, and Candida albicans)* compared to patients with AD sensitized to none, one, or two of the skin-associated microorganisms (*P* = 0.026).

Patients sensitized to all five microbial antigens (SEA, SEB, TSST-1, *Malassezia and Candida albicans)* had higher TEWL, total IgE and SCORAD values compared to patients sensitized to less than five microbial antigens.

Paper II

In this study, 65 patients were included and genetic testing for *MBL* gene polymorphism was possible in 60 patients. The remaining 5 patients were excluded from further analysis.

Based on the *MBL* gene polymorphisms, the 60 patients were categorized into three groups, called group 1 (low MBL), group 2 (intermediate MBL) and group 3 (high MBL). Group 1 (low MBL) was patients with an *MBL* gene polymorphism considered to have a corresponding MBL deficiency in serum; eight patients fulfilled these criteria. Group 2 (intermediate MBL) consisted of 22 patients, and group 3 (high MBL) consisted of 30 patients, and both these groups were supposed

to have *MBL* gene polymorphisms corresponding to normal MBL concentrations in serum (Table 3).

In this cohort of patients with AD, the frequency of the *MBL* gene polymorphism associated with MBL deficiency in serum was 13% (8 out of 60 patients).

Table 3.
Results of *MBL* gene polymorphism testing. Analysis of the structural part of the gene; A = wild-type structural allele (normal), 0 = mutant allele B, C or D. Analysis of the promoter part of the gene; HY = normal, LY= intermediate, LX = low. Criteria for group 1 (low MBL) was either two structural allele mutations or one structural mutant allele + wild type structural allele A with an LX promoter mutation. Criteria for group 2 (intermediate MBL) was either two normal structural alleles, but promoter gene mutation LX on both genes or one structural mutant allele + a wild-type structural allele A with a non-LX promoter. Criteria for group 3 (MBL high) was two wild-type structural alleles with at least one promoter allele that is a non-LX-promoter.

***MBL* gene polymorphism**	**Patients, n (%)**
Group 1 (MBL low)	
BLY BLY	4 (6.7)
ALX DHY	2 (3.3)
ALX CLY	1 (1.7)
ALX BLY	1 (1.7)
Group 2 (MBL intermediate)	
AHY BLY	10 (16.7)
ALX ALX	5 (8.3)
AHY DHY	3 (5)
ALY BLY	2 (3.3)
DHY ALY	1 (1.7)
ALY CLY	1 (1.7)
Group 3 (MBL high)	
ALY ALX	9 (15)
AHY ALX	8 (13.3)
AHY AHY	5 (8.3)
AHY ALY	5 (8.3)
ALY ALY	3 (5)

As expected, the median value of MBL serum concentrations of patients in group 1 (low MBL) showed MBL deficiency, even though one patient with polymorphism ALX DHY had an MBL serum concertation > 100 µg/l. In group 2 (intermediate MBL) and group 3 (high MBL), the median values of MBL serum concentrations

showed no MBL deficiency, although three patients with the polymorphism AHY BLY and one patient with ALY BLY had MBL serum concentrations < 100 µg/l (Figure 20).

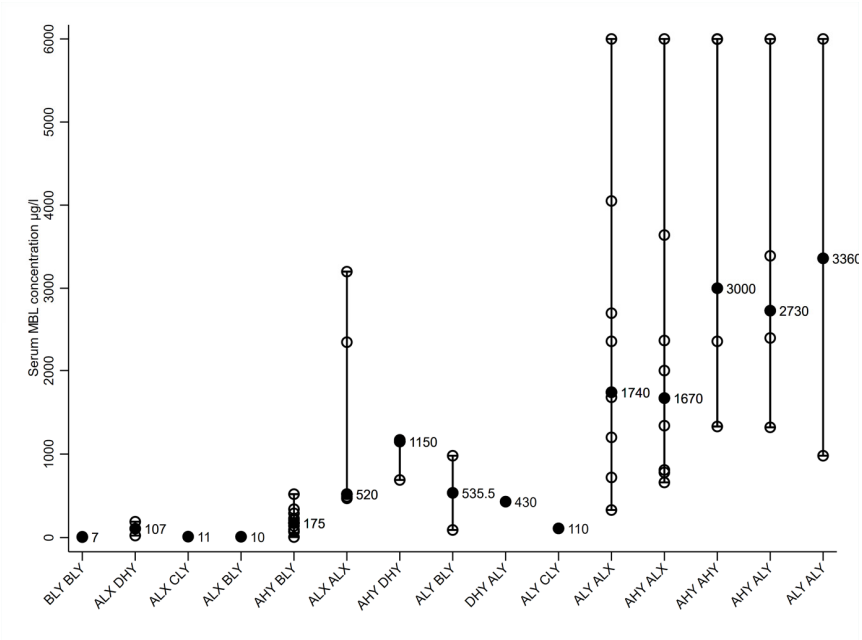

Figure 20.
Black dots indicate the median MBL concentrations for each MBL genotype, which are also printed out in the figure. Each individual MBL serum concentration is represented as white dot and the range is shown as a longitudinal line.

Characterization of the patients in the three *MBL* gene polymorphism groups showed more women than men in all three groups. There was a wide range of total IgE in all groups. The three groups had almost similar median values of TEWL. In group 1 (low MBL) the median of SCORAD was 26, and for group 2 (intermediate MBL) and group 3 (high MBL) the medians were 33.5 and 27, respectively. The prevalence of head and neck-dermatitis was 87.5% in group 1 (low MBL), 77% in group 2 (intermediate MBL) and 60% in group 3 (high MBL) (Table 4).

75% of the patients in group 1 (low MBL) were sensitized to *Candida albicans*, in group 2 (intermediate MBL) 63.3% were sensitized to *Candida albicans*, and in group 3 (high MBL) 33.3% of the patients were sensitized to *Candida albicans.*

Table 4.
Characteristics of the *MBL* polymorphisms groups. SEA = staphylococcal enterotoxin A, SEB = staphylococcal enterotoxin B.

	Group 1 (MBL low) total *n*=8	**Group 2 (MBL intermediate) total *n*=22**	**Group 3 (MBL high) total *n*=30**
Women, *n* (%)	6 (75)	13 (59)	18 (60)
Men, *n* (%)	2 (25)	9 (41)	12 (40)
Age in years, median (range)	27 (21-64)	34.5 (21-73)	29.5 (19-69)
total IgE, kU/l, median (range)	1162.5 (3.6-3102)	372 (16-20220)	232.5 (8.51-13910)
IgE sensitization to *C. albicans, n (%)*	6 (75)	14 (63.6)	10 (33.3)
IgE sensitization to SEA, *n* (%)	2 (25)	6 (27.3)	5 (16.7)
IgE sensitization to SEB, n (%)	2 (25)	3 (13.6)	7 (23.3)
IgE sensitization to TSST, *n* (%)	2 (25)	4 (18.1)	6 (20)
IgE sensitization to *Malassezia, n* (%)	4 (50)	14 (63.6)	14 (46.7)
TEWL g/m^2/h median (range)	13.5 (8-18.8)	14.75 (6.5-80.9)	12.7 (2.6-32)
SCORAD median (range)	26 (14-44)	33.5 (6-85)	27 (1-75)
Head and neck dermatitis, *n* (%)	7 (87.5)	17 (77)	18 (60)

There was a significant difference in the *MBL* gene polymorphism groups concerning sensitization to *Candida albicans* ($P = 0.031$). There were no differences between the *MBL* gene polymorphism groups regarding sensitization to the other skin-associated microorganisms. Logistic regression models showed that patients with AD in group 1 (low MBL) was 6 times more likely to be sensitized to *Candida albicans* compared with group 3 (high MBL) (odds ratio (OR) = 6.00 unadjusted for age and gender, $P = 0.047$) (odds ratio (OR) = 6.34 adjusted for age and gender, $P = 0.045$).

Paper III

Patient inclusion in the BIO-MUSE study started in 2021 and is still ongoing in February 2024 with 18 patients and 20 healthy controls included. As of February 2024, two included patients have declined further participation due to the extra visits

in the study and two patients have died from MF during the study. The patients were included at Skåne University Hospital, Lund, Sweden.

Publication of selected translational findings before the main results of the trial is accepted and has resulted in Paper IV.

Paper IV

In this study, 17 patients and 20 healthy controls were included. The patient group consisted of 7 women and 10 men. The patient group had a median age of 70 years. Fourteen patients had an early stage of disease (stages IA-IIA) and three patients had an advanced stage of disease (stages IIB-IVB). Only patients with MF were included in the study and none of the included patients had SS.

The healthy control group consisted of 16 women and 4 men. The healthy control group had a median age of 48 years.

No participant had ongoing oral antibiotics.

The *Staphylococci* gene copies were significantly increased in unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.05$ and $P < 0.05$, respectively) (Figure 21a).

Gene copies of *C. acnes* were significantly less abundant in the unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.01$ and $P < 0.001$, respectively) (Figure 21b).

The gene copy ratio of *Staphylococci/C. acnes* was significantly higher in unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.01$ and $P < 0.001$, respectively) (Figure 21c).

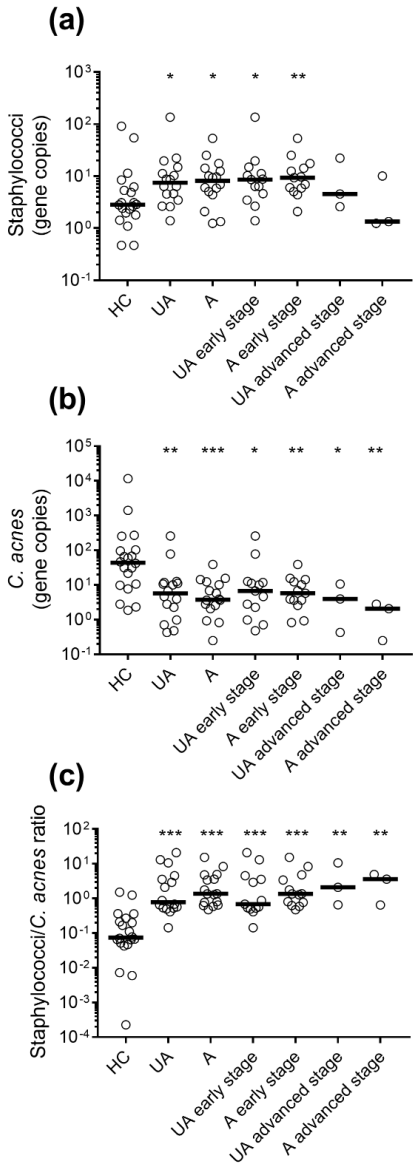

Figure 21.
Gene copies of *Staphylococci* (a) and *C. acnes* (b) and the *Staphylococci/C. acnes* ratio (b) in affected and unaffected skin in patients with mycosis fungoides and in healthy skin of the control group. HC = Healthy control, UA = unaffected skin in patient, A = affected skin in patient, UA early stage = unaffected skin in early stage, A early stage = affected skin in early stage, UA affected advanced stage = unaffected skin in advanced stage, A advanced stage = affected skin in advanced stage. Early stage = IA-IIA, Advanced stage = IIB-IVB. *P*-values were considered significant at $P < 0.05$, with $^{*}P < 0.05$, $^{**}P < 0.01$. and $^{***}P < 0.001$.

TEWL was significantly higher in unaffected and affected skin of patients with MF compared to healthy controls ($P < 0.01$ and $P < 0.001$, respectively) (Figure 22a).

TEWL was significantly higher in affected skin compared to unaffected skin in patients with MF ($P < 0.05$) (Figure 22a). Affected skin in advanced stage of disease had a significantly higher TEWL compared to affected skin in early stage of disease ($P < 0.05$) (Figure 22a).

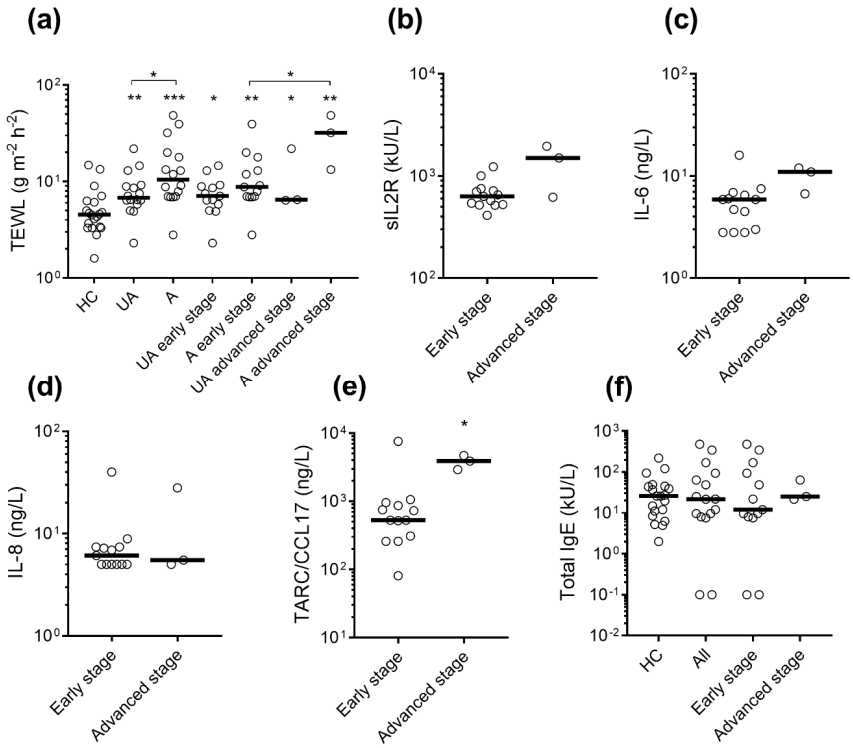

Figure 22.

Transepidermal water loss (TEWL) (a), **S**oluble interleukin-2 receptor (sIL-2R) (b), Interleukin 6 (IL-6) (c), Interleukin 8 (IL-8) (d), Thymus and activation-related chemokine/C-C-Motif-Chemokine-Ligand-17 (TARC/CCL17) (e) and Total IgE (f) in patients with mycosis fungoides. HC = healthy control, UA = unaffected skin in patient, A = affected skin in patient, UA early stage = unaffected skin in early stage, A early stage = affected skin in early stage, UA affected advanced stage = unaffected skin in advanced stage, A advanced stage = affected skin in advanced stage. Early stage = IA-IIA, Advanced stage = IIB-IVB. *P*-values were considered significant at $P < 0.05$, with $*P < 0.05$, $**P < 0.01$. and $***P < 0.001$.

There was no significant difference between early and advanced stage of disease concerning sIL-2R, IL-6, IL-8 and total IgE (Figure 22b, 22c, 22d, 22f). TARC/CCL17 was significantly higher in advanced stage of disease compared to early stage of disease ($P < 0.05$) (Figure 22e).

Discussion

Paper I

The results showed that TEWL was significantly higher in adult patients with AD colonized with *S. aureus* compared to patients not colonized with *S. aureus*, and this also correlated with the degree of colonization. *S. aureus* can impair the skin barrier, which leads to an enhanced penetration of allergens and likely to a sensitization to skin-associated microorganisms and further triggering of inflammation and a worsening of AD. The study highlights the role of *S. aureus* in patients with AD and the negative impact of *S. aureus* on the skin barrier function.

Sensitization to three or more skin-associated microorganisms *(S. aureus, Malassezia and Candida albicans)* further aggravated the disease compared to sensitization to none, one or two skin-associated microorganisms, showing that sensitization to skin-associated microorganisms can affect the course of AD.

In this study 33% of the patients with AD were colonized with *S. aureus*, which is a quite low colonization rate with *S. aureus* compared to what other studies have reported in patients with AD (118-122). This might be explained by the fact that most patients in the study had mild to moderate disease, and *S. aureus* has been reported to be more frequent in severe AD (119).

In paper I, the contact agar disc method (CADM) showed that only patients with a positive bacterial culture for *S. aureus* had a significant bacterial load with > 100 cfu, thus indicating that CADM could be a reliable and simple method for estimating colonization of *S. aureus* in patients with AD (242, 243).

A limitation of the study was the relatively few numbers of patients included in study and that the study also could have had more healthy controls.

Paper II

The prevalence of an *MBL* gene polymorphism associated with a serum MBL deficiency was 13% in this cohort of patients with AD, which is a similar frequency previously reported in other populations (28-30).

The study showed that patients in group 1 (low MBL), with an *MBL* gene polymorphism considered to give an MBL deficiency in serum, had an association with sensitization to *Candida albicans*, compared to patients in group 3 (high MBL).

MBL plays a role in innate immunity and in the defense against *Candida albicans* (26, 28, 45, *46)*. *Candida* species have been reported to be more common in the skin microbiome of AD (139). A study reported that blocking MBL in mice resulted in increased *Candida albicans* colonization of the gut (47). Hypothetically, patients with MBL deficiency may be even more prone to colonization with *Candida albicans* in the skin, mucous membranes and gut, and this colonization may lead to a higher sensitization to *Candida albicans*. Another study showed a significant correlation between sensitization to *Candida albicans* and symptoms of AD in subgroup with gastrointestinal growth of *Candida albicans* (140). Speculatively, a colonization or overgrowth of *Candida albicans* in the skin or gut in patients with MBL deficiency and AD, might stimulate the immune system to a sensitization to *Candida albicans* that could further exacerbate the symptoms of AD. Per oral antifungal treatment might be of importance in patients with MBL deficiency and severe AD, which also are sensitized to *Candida albicans* (114, 128, 134-137).

In paper II, no differences were seen between group 1 (low MBL) and group 3 (high MBL) concerning TEWL and SCORAD. However, patients sensitized to *Candida albicans* had a significantly higher SCORAD compared to patients not sensitized to *Candida albicans*.

Paper III

The aim of the study protocol was to find new predictive and prognostic markers in the skin and blood of patients with MF and SS. Most patients with MF have early stages of disease with a good prognosis. In the study, the patients were categorized as having an early stage of disease (stages IA-IIA) or advanced stage of disease (stages IIB-IVB), based on the clinically relevant fact that the prognosis is poorer for stages IIB-IVB (157, 165). The study will investigate translational samples in skin and blood in relation to stage of disease over time. The advantage of monitoring patients with consecutive sampling over time is the possibility of detecting changes before, during and after progression. The study will be able to compare patients with early stage of disease and patients with advanced stage of disease. It will also be possible to analyze data for MF and SS separately. The translational samplings were chosen to be able to detect changes in the immune system and in tumor cells, as well as changes in the skin barrier function and in the skin microbiome.

Paper IV

The study showed a significantly increased quantity of gene copies of *Staphylococci* in unaffected and affected skin of patients with MF compared to healthy controls. This is in line with earlier studies where the colonization rate of *S. aureus* in MF has been reported to be high, and colonization with *S. aureus* has been postulated to play an important role in the progression of MF and SS (15, 178-183).

In this study, the commensal bacteria *C. acnes* were significantly less abundant in unaffected and affected skin of MF patients compared to healthy controls, confirming a diminished level of this commensal bacteria in this cohort of patients with MF. Low quantity of *C. acnes* has previously been associated with dysbiosis and worsening in other skin diseases, such as AD (196). *C. acnes* seems to be important for maintaining skin homeostasis and in previous studies *C. acnes* has been reported to inhibit *S. aureus* in several ways (196).

Several studies have shown that oral antibiotics can reduce *S. aureus* and can improve the stage of disease (15, 181, 182, 192), but long-term antibiotics have several disadvantages. Instead, other microbiome-modulating strategies with a focus on maintaining the commensals in the skin might be a future treatment option.

TEWL was significantly higher in the affected skin of patients with MF compared to healthy controls, indicating a skin barrier dysfunction in patients with MF. TEWL was also higher in the affected skin of those with an advanced stage of disease compared to the affected skin of those with an early stage of disease, indicating that the skin barrier function deteriorates further in the advanced stage of disease. The impaired skin barrier may be a result of the disease itself or caused by *S. aureus* or by a decreased level of common skin commensals. The skin barrier dysfunction can lead to an increased colonization and infections of *S. aureus*, which may lead to further inflammation and progression of MF. To prevent the effects of *S. aureus* it is important to maintain a normal skin barrier function.

TEWL has in some studies been reported to be lower in healthy populations at the age over 65 years. Although the patient group had a higher median age compared to the healthy controls, the patient group had a higher TEWL.

In this study there was a tendency towards higher levels of serum sIL-2R in the group of advanced stage of disease compared to early stage of disease, but this trend was not significant. This was probably due to the low total number of patients included in the study, and especially the low number of included patients with advanced stage of disease. Previous studies have proposed sIL-2R as a prognostic marker in MF, since higher levels of SIL-2R have correlated to advanced stage of disease (205-209). Future studies of larger cohorts are warranted to investigate the role of sIL-2R as a prognostic marker in MF and SS.

TARC/CCL17 was significantly higher in patients with advanced stage of disease compared to early stage of disease in this study, indicating a role for TARC/CCL17 as a possible prognostic marker, but further studies are needed.

One limitation of the study was the relatively low number of included patients in the cohort. However, MF and SS are rare diseases, and previous studies concerning the skin microbiome have enrolled even fewer patients. Another limitation was that the patient group was older (median age 70 years) compared to the healthy control group (median age 48 years). The patient group consisted of more men than women, and the healthy control group consisted of more women than men, which is also a limitation.

The composition of the skin microbiome and TEWL varies in different parts of the body. Therefore, we wanted to take the samplings from the same anatomical location in both patients and healthy controls as far as possible. A limitation was that it was not feasible to take the samples from the upper arm in all patients due to no disease in the area, and in some patients the samplings were instead taken from another location on the upper body.

Conclusion

Paper I

- Patients with AD and colonization with *S. aureus* had a significantly more severe disease and more impairment of the skin barrier function compared to patients with AD not colonized with *S. aureus* and in healthy controls.
- The contact agar disc method correlated to the results from the bacterial culture of *S. aureus* and may be used as a method to evaluate colonization of *S. aureus* in the skin.
- Patients with AD sensitized to all three of the investigated skin-microorganisms (*S. aureus*, *Candida* and *Malassezia)* had more severe disease, greater impairment of the skin barrier function and higher total IgE compared to patients sensitized to none, one or two of the investigated skin-microorganisms.

Paper II

- In this cohort of patients with AD, the frequency of *MBL* gene polymorphisms associated with MBL deficiency in serum was 13%.
- There was a significant difference between the *MBL* gene polymorphism groups (low MBL, intermediate MBL and high MBL) concerning sensitization to *Candida albicans*, but there were no differences between the *MBL* gene polymorphism groups regarding sensitization to the other skin-associated microorganisms *(S. aureus, Malassezia)*.
- Patients with AD with an *MBL* gene polymorphism associated with MBL deficiency in serum were 6 times more likely to be sensitized to *Candida albicans* compared to patients with *MBL* gene polymorphisms associated with normal serum MBL.

Paper III

- Further knowledge about MF and SS is needed to find new, reliable predictive and prognostic markers in blood and skin.

Paper IV

- *Staphylococci* were significantly more abundant in the skin microbiome in both unaffected and affected skin of patients with MF compared to healthy controls.
- *C. acnes* was significantly less abundant in the skin microbiome in both unaffected and affected skin of patients with MF compared to healthy controls.
- The gene copy ratio of *Staphylococci/C. acnes* was significantly higher in unaffected and affected skin of patients with MF compared to healthy controls.
- The skin barrier function was significantly more impaired in unaffected and affected skin of patients with MF compared to healthy controls. The skin barrier function was significantly more impaired in affected skin compared to unaffected skin in patients with MF. Affected skin in advanced stages of MF (stages IIB-IVB) had significantly greater impairment of the skin barrier function compared to affected skin in early stages of MF (stages IA-IIA).
- TARC/CCL17 was significantly higher in advanced stages of MF (stages IIB-IVB) compared to early stages of MF (stages IA-IIA).

General conclusions

This thesis aimed to investigate biomarkers and skin barrier function in AD and in the cutaneous T-cell lymphomas MF and SS. Although AD is a benign inflammatory skin disease and MF and SS are malignant cutaneous T-cells lymphomas, they have several things in common. The skin barrier impairment facilitates the colonization with *S. aureus* and create an imbalance in the skin microbiome, which promotes the Th2-dominated inflammation that further triggers the diseases and results in an even more affected skin barrier function.

It is difficult to distinguish whether the observed skin barrier dysfunction and skin microbiome dysbiosis is a cause or a consequence of the diseases, but it clearly appears to promote a progression of the diseases (18).

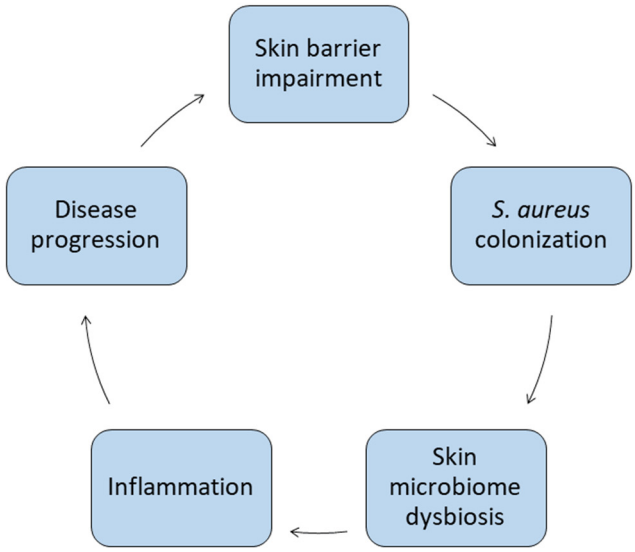

Figure 22.
The skin barrier impairment facilitates the colonization with *S. aureus* and create an imbalance in the skin microbiome, which promotes the Th2-dominated inflammation that further triggers the diseases and results in an even more affected skin barrier function.

Future perspective and Clinical implications

General perspective

The pathogenesis and the progression of the diseases are probably multifactorial in both AD and MF and SS. In the future perspective, there is a need new disease-targeting therapies with limited side effects.

There are many novel treatments in the clinical pipeline for AD. With many new therapies for AD in the last years, there is a need for clinical registries, head-to-head comparisons, analysis of cost-effectiveness and safety, and real-world data (253).

In advanced stages of MF and SS several investigation clinical trials are ongoing, including treatments with JAK-STAT-inhibitors and targeted immunotherapies, such as immune checkpoint inhibitors (anti-PD-1-agents) and lacutamab (anti-KIR3DL2 antibody) (254, 255).

To be able to predict the response of a therapy, finding new reliable predictive biomarkers is of great value.

Based on results from this thesis and previous reports on AD and MF and SS, it seems important for the future to find new ways to restore the skin barrier function, reduce the colonization of *S. aureus*, maintain a healthy skin microbiome, and reduce the Th2-dominated inflammation (17, 126). All these possible strategies together could also contribute to the improvement of the diseases.

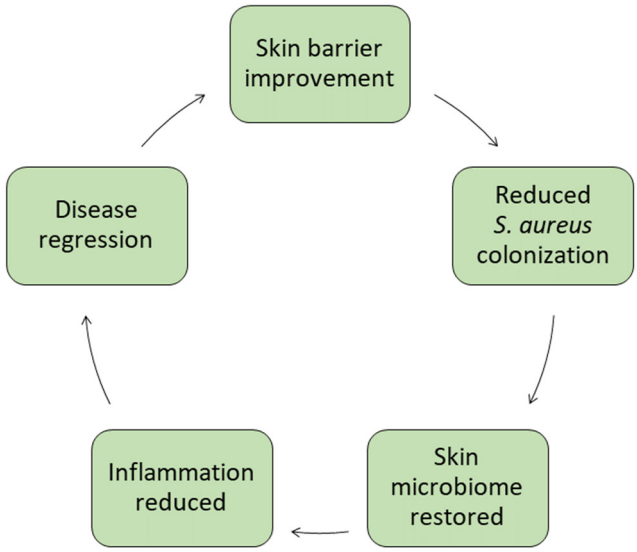

Figure 23.
Skin barrier improvement reduces the colonization with *S. aureus* and contributes to healthy skin microbiome, reduced inflammation and regression of disease, which further improves skin barrier function.

Microbiome-modulating strategies of the skin

Dysbiosis of the skin microbiome may lead to progression of AD and MF and SS, which makes new microbiome-modulating strategies an interesting future field for research and future clinical implications (196). New therapy options and modulation of the skin microbiome that do not require oral antibiotics are warranted.

The gut microbiome is a field where several microbiome-modulating strategies have been studied to change a dysbiotic pattern associated with disease (256). Compared to gut probiotics, studies concerning skin probiotics with skin commensals are relatively sparse (196).

Microbiome modulation can be performed using probiotics (live microorganisms), prebiotics (substrates utilized by health-promoting host microorganisms), synbiotics (probiotics and prebiotics) or postbiotics (non-viable microorganisms) and can be administered oral or topical (196). Transplantation of part of the skin microbiome, analogous to fecal microbiota transplants for the treatment of gastric *Clostridium*

difficile infections, have been reported in some studies (18). In one study, autologous transplant of *S. epidermidis* and *S. hominis,* which produce AMPs, showed a reduction in *S. aureus* in patients with AD (11). Genetic modifications of commensal bacteria to make them produce active biotherapeutics, such as filaggrin, is another method. All these methods have their advantages and disadvantages (18). In the future, microbiome-modulating strategies with skin commensals, such as *C. acnes*, might play a role to reduce *S. aureus* and to maintain a healthy skin microbiome in AD and MF and SS and contribute to inhibiting disease progression (50).

Treatment approaches used to reduce *S. aureus* in AD might also be considered in MF and SS in the future. Sodium hypochlorite bleach baths have been reported to reduce *S. aureus* in patients with AD, and even though the evidence is low, that is sometimes used as a therapy for AD (257-259). Some studies have reported no added beneficial effect of sodium hypochlorite bleach baths, if the patient is treated at the same time with TCS (260, 261). A reduction in *S. aureus* has been reported in patients with AD when treated with TCS, which might indicate that *S. aureus* may be secondary to disease progression and skin barrier impairment (262, 263). However, in a subgroup of patients with moderate to severe AD, sodium hypochlorite bleach baths have been proposed to probably improve disease severity (257). Further studies are needed to study the effect of sodium hypochlorite bleach baths in AD and MF and SS (164).

In the BIO-MUSE study, we will continue to investigate and analyze the skin microbiome and the role of *Staphylococci* and commensal bacteria, such as *C. acnes,* in relation to other factors in the tumor microenvironment and blood. Strains of *S. aureus* has been isolated in the BIO-MUSE study and further studies are planned with whole genome sequencing to characterize differences in *S. aureus* strains related to other factors, such as stages of disease.

Biomarkers

In paper I, colonization with *S. aureus* and sensitization to three or more of the analyzed skin-associated microorganisms was associated with a higher total IgE, skin barrier impairment and more severe AD. These results are in line with earlier studies (67). Since these factors seem to contribute to a worsening of disease, it can be of value in patients with severe disease to analyze skin colonization with *S. aureus*, total IgE and sensitization to skin-associated microorganisms and try to reduce skin colonization with *S. aureus* or other skin-associated microorganisms to avoid triggering of AD.

In paper II, *MBL* gene polymorphism and serum MBL were investigated in patients with AD. Analyzing *MBL* gene polymorphism or serum MBL in patients with AD

doesn't seem to be motivated in clinical practice. The *MBL* gene polymorphism cannot be analyzed in clinical routine in Sweden, but it is possible to analyze serum MBL at the Department of Clinical Immunology. The only rationale for doing so might be in patients with AD with high levels of IgE to *Candida albicans*, to confirm a probable MBL deficiency contributing to colonization with *Candida albicans* and sensitization to *Candida albicans,* which might trigger AD and motivate antifungal treatment. However, in clinical practice, it is more pragmatic to only analyze IgE to *Candida albicans,* since the information of MBL deficiency does not lead to any practical consequences. MBL deficiency has been associated with infections and autoimmune disease, but most patients with an MBL deficiency remain healthy. MBL replacement therapy has been discussed theoretically in special populations but is not performed in clinical practice in Sweden (264).

In papers I and IV, skin barrier function was measured as TEWL, and a higher TEWL correlated with higher colonization of *S. aureus* in patients AD, and with higher quantity of gene copies of *Staphylococci* and disease activity in patients with MF. In the future, TEWL might be used as a method to investigate skin barrier function even in clinical practice, but more studies are needed to establish normal reference values of TEWL.

There is a need for new reliable prognostic and predictive biomarkers in MF and SS. In paper IV, we could not find a significant difference in serum sIL-2R between patients with early stages of MF and patients with advanced stages of MF. Previous studies with more patients found a significant difference and proposed a role of sIL-2R as a prognostic biomarker in MF and SS. Further studies are needed, since the number of patients was probably too few in our study, and there might be a future clinical implication for sIL-2R as a prognostic marker.

In paper IV, there was a significant difference in TARC/CCL17 between early stages of MF and advanced stages of MF, but since only a few patients were included, further studies are needed before any clinical implications of TARC/CCL17 as a prognostic marker in MF can be made.

Quality of life in patients with MF and SS

It is of great importance to further investigate the quality of life in patients with MF and SS. In the BIO-MUSE study, patient-oriented quality of life measurements are conducted, including the Dermatology Life Quality Index (DLQI), the Peak Pruritus Numerical Rating Scale (NRS), the Sleep NRS and the Connor-Davidson Resilience Scale and these will be analyzed over time and in relation to other parameters.

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