Skin Disorders, Atopic Manifestations and Primary Immunodeficiency Diseases

Identifying clinical features and common pathways of immune dysregulation to improve diagnosis and personalized treatment

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Huidaandoeningen, atopische manifestaties en primaire immuundeficiënties

Identificatie van klinische kenmerken en gemeenschappelijke processen van immuundysregulatie ter verbetering van diagnostiek en gepersonaliseerde behandeling

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Chapter 1

General introduction

IMMUNE SYSTEM

The human immune system is comprised of a complex network that involves lymphoid organs, cells, humoral factors and cytokines. The essential function of the immune system in host defense is to protect against invading pathogens, including bacteria and viruses, and foreign bodies. The immune system can be divided in two categories; the innate or nonspecific immunity and the adaptive or specific immunity. The innate immune response forms the host's first line of defense and consists of physical and chemical barriers (skin and mucosa), effector cells (e.g. granulocytes, macrophages and dendritic cells), antimicrobial peptides (e.g. defensins and cathelicidins), soluble mediators (e.g. cytokines and complement) and cellular receptors (e.g. Toll-like receptors (TLRs)) that can provide immediate and non-specific response to a wide array of pathogens.[1] The adaptive or acquired immune response forms the second line of defense and consists of antigen-specific reactions through highly specialized T lymphocytes and B lymphocytes.[2] Whereas the innate response is rapid, the adaptive response may take days to weeks to develop. Moreover, after an initial pathogen encounters, adaptive immune cells can persist in the host for life, providing immunological memory and the capacity for rapid response in the event of re-exposure.

Primary immunodeficiency diseases (PIDs) are characterized by a compromised or entirely absent function of a part of the immune system, which makes people vulnerable for infections. In patients with a PID, the types of infections depend on the underlying immunological defects. For example, patients with a humoral immunodeficiency due to a defect in B lymphocyte function are at increased risk for recurrent infections predominantly caused by extracellular, encapsulated bacterial pathogens, mainly of the upper and lower respiratory tract and gastrointestinal tract. On the other hand, patients with a cellular immunodeficiency, i.e. defect in T lymphocyte function, have an increased risk of infections caused by intracellular pathogens, including Herpes simplex virus, *Mycobacterium*, *Listeria* and intracellular fungal infections.

PRIMARY IMMUNODEFICIENCY DISEASES

PIDs encompass a heterogeneous group of more than 430 inheritable defects of immunity caused by variants in genes encoding functional proteins of human immune cells.[3-5] However, with the increasing power of next-generation sequencing the number of recognized genetic disorders is even expanding.[5] The incidence of symptomatic PIDs is estimated at 1 in 2,000 live births with a prevalence of 1 in 10,000-12,000 in the general population, of which the majority is due to highly consanguineous populations in the Middle East/Northern African region.[4,6,7] PIDs are clinically typically characterized by an increased risk of

recurrent and/or severe infections. In addition, patients may suffer from autoimmune and autoinflammatory complications and have an increased risk of development of (hematological) malignancies and allergic disorders.[8-10] Autoimmune disorders, such as type 1 diabetes mellitus, rheumatoid arthritis and psoriasis, are the result of an immune response directed against normal bodily constituents, called auto-antigens. In autoinflammatory disorders, like familiar mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), the innate immune system is abnormally activated, leading to recurrent episodes of fever and inflammation.[11] Autoimmune as well as autoinflammatory conditions are characterized by disruption of the normal function of the immune system, also called immune dysregulation. Interestingly, various forms of immune dysregulation, both as primary or as accompanying symptoms next to the immunodeficiency, occur in many PIDs and, therefore, PIDs could be considered as immune dysregulation syndromes.[12]

Currently, PIDs are classified into ten main groups of PID according to the predominant immunological mechanisms that are disrupted and their most relevant clinical features.[3] These groups include (i) immunodeficiencies affecting cellular and humoral immunity; (ii) combined immunodeficiencies with associated or syndromic features; (iii) predominantly antibody deficiencies; (iv) diseases of immune dysregulation; (v) congenital defects of phagocyte number, function or both; (vi) defects in intrinsic and innate immunity; (vii) autoinflammatory disorders; (viii) complement deficiencies; (ix) bone marrow failure; and (x) phenocopies of PID. The primary humoral immunodeficiencies are categorized within the predominantly antibody deficiencies (PADs) and are characterized by B lymphocyte abnormalities that result in decreased numbers or impaired function of B lymphocytes, low immunoglobulin (Ig) levels or both. On a global scale, PADs form the largest PID phenotype as more than 60% of the PIDs diagnosed in clinical practice consist of a humoral immunodeficiency.[13-18]

One of the clinical hallmarks of PIDs is an increased susceptibility to infections. Therefore, a PID should be considered when a patient has recurrent, severe, prolonged and/or difficult-to-treat infections. Based on these clinical findings, ten general warnings signs of PID have been composed by the European Society for Immunodeficiencies (ESID), mainly focusing on the presence of infectious complications, to raise the suspicion of a PID.[19] These warning signs include (i) four or more new ear infections within one year; (ii) two or more serious sinus infections within one year; (iii) two or more months on antibiotics with little effect; (iv) two or more pneumonias within one year; (v) failure of an infant to gain weight or grow normally; (vi) recurrent, deep skin or organ abscesses; (vii) persistent thrush in mouth or fungal infection of the skin; (viii) need for intravenous antibiotics to clear infections; (ix) two or more deep-seated infections including septicemia; and (x) a family history of PID. In case of presence of two or more warning signs, the suspicion for a PID should be raised.

However, despite use of these warning signs to improve earlier recognition of an underlying PID, diagnosis of PIDs is still delayed. The diagnostic delay, i.e. the time between onset of the first symptoms and diagnosis, of PIDs in the Netherlands may be up to 14.5 years for defects in innate immunity.[13] As a consequence, these inherited PIDs are diagnosed at a median age of 19.0 years.[13]

Diagnostic delay in PIDs results in persistence of symptoms, irreversible organ damage and dysfunction, recurrent hospitalizations, and functional limitations of patients, which all contribute to a lower quality of life for both mental and physical components as compared with healthy controls and patients with other chronic diseases.[20-24] Therefore, early recognition of PIDs is crucial and the identification of new PID-characteristic symptoms as early warning signs for suspicion of PIDs could aide earlier diagnosis.

Skin disorders in primary immunodeficiency diseases

It has been well recognized that a wide spectrum of both infectious and noninfectious skin disorders are common in PIDs and may be among the presenting clinical manifestations.[25-30] Overall, *Staphylococcus* (*S.*) *aureus*-induced skin infections, such as folliculitis and skin abscesses, are the most common infectious skin disorders reported in PIDs, like leukocyte adhesion defects (LADs), chronic granulomatous disease (CGD), severe congenital neutropenia and hyper IgE syndrome (HIES).[31-33] Known noninfectious skin disorders include autoimmune, autoinflammatory, malignant and allergic manifestations, which could all be attributed to immune dysregulation. Dermatitis is described as one of the most prominent noninfectious skin manifestations in PIDs.[30]

The relation between skin disorders and PIDs has been investigated in few studies. Studies in PID cohorts from Iran and Mexico have demonstrated that skin manifestations preceded and were the basis for PID diagnosis in 31.8% and 78.9% patients, respectively.[26,27] In addition, Aghamohammadi *et al.* have shown that in patients with severe and/or therapy refractory dermatitis an underlying PID could be detected in 8% of the patients, including HIES and Wiskott-Aldrich syndrome (WAS).[34] Although skin conditions seem to be frequently occurring in PIDs and may even precede the diagnosis of a PID, they are currently not considered as one of the warning signs for PIDs.

Atopic manifestations in primary immunodeficiency diseases

Atopic manifestations consist of atopic dermatitis (AD), food allergy (FA), asthma and allergic rhinitis (AR). In general, patients with severe dermatitis frequently have an atopic constitution and tendency towards development of other atopic manifestations.[35,36] The atopic manifestations encompass allergic disorders, which are already known as prevalent comorbidities in various PIDs.[4,30,37] Nonetheless, a narrative review reported occurrence of

these manifestations mainly in immunodeficiencies affecting cellular and humoral immunity, like DOCK8 deficiency, and combined immunodeficiencies (CIDs) with associated or syndromic features, such as Comèl Netherton syndrome.[30] Other original studies reported atopic manifestations most commonly in CIDs and, albeit in lower frequencies, in PADs, like selective IgA deficiency.[38-54] However, original data on atopic manifestations in PIDs are limited, mainly based on small numbers of PID patients and the diagnosis of atopic manifestations is generally not based on diagnostic tests.

ATOPIC SYNDROME

Atopy is the genetic predisposition to produce specific IgE following exposure to allergens. This predisposition results in the development of AD, FA, asthma and AR: the atopic syndrome.[55] The worldwide prevalence of these manifestations in children varies between 15-20%, 1-10%, 3-29% and 9-15%, respectively, and in adults between 1-3%, 3-4%, 2-12% and 7-42%, respectively.[56-60] The atopic march characterizes the course of atopic manifestations over time, generally starting with AD in infancy and followed by FA, asthma and AR later in childhood.[61] However, it is known that the atopic march not always follows the classic sequence and may occur at any age.[62,63] Furthermore, not all atopic patients will develop the complete spectrum of atopic manifestations.[61]

Subgroups of the atopic phenotype, termed endotypes, are possibly responsible for the heterogeneous presentation of the atopic syndrome. These endotypes are the result of variations in physiological, biological, immunological and/or genetic mechanisms, as involved in the multifactorial pathogenesis of atopic manifestations.[64] Various genetic loci associated with multiple atopic manifestations have been identified in recent years based on genome-wide association studies showing common genetic mechanisms involved.[65-74] Additionally, immune dysregulation plays an important role in the pathogenesis of the atopic syndrome. The major immunological abnormality consists of enhanced IgE production against environmental antigens triggering the release of inflammatory mediators, including histamine, in the skin, gastrointestinal tract, lungs and nose.[75] The abnormal regulation of antigen-specific IgE production in patients with atopic manifestations seems to be the result of a preferential presence of CD4+ T lymphocytes producing interleukin (IL)-4 and IL-5, but not interferon γ (IFN-γ), which suppresses IgE synthesis.[76-78]

Interestingly, atopic manifestations are prevalent comorbidities in various (monogenic) PIDs, which may be due to overlapping pathogenic pathways. Therefore, current insights in the pathways involved in PIDs could be used to define the endotypic profile of atopic patients in more detail, contributing to determination of more homogeneous subclasses of

these patients. Subsequently, pathway-targeted or even gene-targeted treatment strategies could be developed to personalize treatment regimens for the atopic syndrome based on endotype profiles.

ATOPIC DERMATITIS

AD is an important cutaneous manifestation within the atopic syndrome and one of the most common chronic inflammatory diseases. It is characterized by intense itch, erythema and scaling. Symptoms generally start in infancy with a relapsing-remitting course, but may occur at any age.[79] Based on genetic and epidemiological data, AD is found to be associated not only with the atopic syndrome but also with systemic immune-mediated inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease. This suggests that AD should be considered as manifestation of systemic inflammation rather than being inflammation limited to the skin.[80,81]

AD has a multifactorial pathogenesis characterized by three major pathophysiological changes consisting of (i) abnormalities of the skin barrier; (ii) changes in the immune response; and (iii) alterations in the skin microbiome.

Abnormalities of the skin barrier

The healthy skin forms the first line of defense of the body against harmful stimuli from the environment, like irritants, allergens, antigens and microorganisms. Furthermore, it prevents the body from excessive water loss. The impaired barrier function in AD enables environmental stimuli to penetrate into the skin and subsequently provoke an immune reaction. Various abnormalities in the skin barrier function, including an increased skin pH, reduced expression of antimicrobial peptides and a breach in epidermal lipids resulting in increased skin permeability, have been associated with development of AD.[82-85] Additionally, a filaggrin deficiency, which is involved in skin hydration and water retention within the epidermis, was found as most important genetic risk factor for AD.[83,86]

Changes in the immune response

Exposure to microorganisms through an impaired skin barrier initiates a rapid innate immune response preventing further invasion of these microorganisms. Both skin tissue damage and invading microorganisms stimulate TLRs, which are expressed by keratinocytes and antigen-presenting cells in the skin.[87] This leads to a release of inflammatory mediators that enhances the strength of tight junctions to limit penetration of allergens and microorganisms. Patients with AD, however, were shown to have decreased function of TLR2 and TLR9, which leads to alterations in the skin microbiome, increased penetra-

tion of microorganisms and more severe inflammation.[87,88] Accordingly, a genome-wide association study in AD identified candidate genes involved in regulation of the innate host defense and T lymphocyte function. This emphasizes the contribution of immunological processes in the pathogenesis of AD.[65]

In AD, the nonlesional skin shows increased numbers of T helper (Th) lymphocytes, like Th2, Th17 and Th22, representing in a pro-inflammatory state.[80] Enhanced penetration of environmental stimuli through the impaired skin barrier stimulates additional Th2 cell migration into the skin and subsequent acute inflammation.[89] These AD lesions are predominated by production of pro-inflammatory cytokines, including IL-4, IL-13 and IL-31, which further modulate the skin barrier function, amongst others, by suppressing filaggrin expression and inhibiting the production of antimicrobial peptides. Chronic inflammation promotes a shift towards a Th1 cell immune response controlled by IL-12 production by dendritic cells, possibly stimulated by *S. aureus*.[90] The Th1 cells in chronic AD lesions produce IFN-γ, which inhibits keratinocyte differentiation resulting in skin hyperplasia.

The humoral immune response is also involved in AD. Penetration of allergens through the skin leads to Th2 cytokine production. These cytokines stimulate IgE production by B lymphocytes. Many patients with AD show high IgE levels against specific allergens, like food allergens or inhalant allergens.[91-93] Moreover, some patients with AD also have increased IgE against microbial antigens, suggesting that microbes act as allergens instead of antigens.[94-99] In addition to the increased IgE levels in AD, IgG antibody production was found to be stimulated in response to contact with food antigens, leading to a pro-inflammatory response and phagocytosis of the antigen.[100] Furthermore, IgG levels against microbial antigens on the skin of AD patients are found to be higher than in controls.[101] Further identification of antibody responses against microbial antigens could help us to better understand how microbes interact with the immune system and potentially induce inflammation in AD.

Alterations in the skin microbiome

Multiple studies have described alterations of the skin microbiome in patients with AD, predominantly consisting of an overgrowth of *S. aureus* on both the lesional and nonlesional skin accompanied by reduced diversity of commensal bacteria.[102,103] Moreover, *S. aureus* colonization was found to be positively correlated with AD severity, with patients having a higher *S. aureus* load during flares.[102,104] A birth cohort study, which aimed to identify the role of the skin microbiome in AD, found that *S. aureus* colonization and lower number of commensal *Staphylococcus* species at the age of two to three months were correlated with development of AD later in life.[105] These findings suggest that cutaneous dysbiosis, including abundance of *S. aureus*, plays a role in initiation of AD. However, a

systematic review found that not only *S. aureus* is involved in the dysbiosis in AD, but also other species, including *S. epidermidis*, *Propionibacterium* and *Malassezia*.[106]

Some mechanisms by which *S. aureus* interacts with the skin barrier and immune system have been unraveled. For example, *S. aureus* can aggravate skin inflammation via the production of enterotoxins that stimulate the release of pro-inflammatory cytokines.[90,102,104,107] Furthermore, *S. aureus* produces α-toxin that induces keratinocyte damage.[108] However, the importance of *S. aureus* colonization in the complex pathogenesis of AD, as compared with the other involved genetic and immunological factors, remains poorly understood.[106]

Interaction between skin barrier, immune system and skin microbiome

The above described pathophysiological components within the multifactorial pathogenesis of AD seem to interact in a multidirectional way. Pro-inflammatory cytokines cause skin barrier impairment, while, on the other hand, an increased skin permeability results in environmental stimuli penetrating through the skin and provoking an immune reaction.[109,110] Both alterations in the immune system and skin barrier impairment might favor *S. aureus* colonization and staphylococcal antigens contrarily seem to interact with the immune system and skin barrier.[103,108,111] However, studies on the interaction between the immune system and *S. aureus* are still scarce. Further evaluation of the antibody response against antimicrobial antigens could provide insights in the antigens that are expressed by the skin microbiome *in vivo* and will reveal how the immune system of AD patients counteracts these antigens. Thereby, the contribution of each of the three factors to the AD phenotype is still unknown.

As previously described, *S. aureus* is abundant in the skin microbiome of AD patients, which could therefore be a target for treatment in AD. Current long-term anti-staphylococcal treatment strategies, like antibiotics, have the disadvantages of affecting the commensal microbiota and inducing bacterial resistance.[112-114] In this context, it would be interesting to study the effect of an endolysin selectively targeting *S. aureus* on AD symptoms in a randomized controlled trial (RCT).

AIMS OF THE THESIS

- To evaluate whether skin disorders and atopic manifestations are prognostic warning signs for PIDs in order to shorten the diagnostic delay.
- To define homogeneous endotypes within the atopic phenotype based on known pathological pathways in PIDs in order to improve patient stratification for future pathway-targeted treatment strategies.

- To provide an overview of the antibody responses against *S. aureus* antigens, as most abundant microorganism in patients with AD, in order to gain insight into the interaction between the immune system and skin microbiome in the pathogenesis of AD.
- To study the effect of a targeted intervention against *S. aureus* on AD symptoms in order to elucidate the contribution of the microbiome within the multifactorial pathogenesis of AD.

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Chapter 2

Skin disorders are prominent features in primary immunodeficiency diseases: A systematic overview of current data

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ABSTRACT

Background

Primary immunodeficiency diseases (PIDs) are characterized by an increased risk of infections, autoimmunity, autoinflammation, malignancy, and allergic disorders. Skin disorders are also common clinical features in PIDs and may be among the presenting manifestations. Recognition of specific PID-associated skin conditions in combination with other clinical features as described in the currently used warning signs could raise suspicion of an underlying PID.

Objective

We aimed to provide a systematically obtained overview of skin disorders and their prevalence in PIDs. Secondary, the prevalence of *Staphylococcus* (*S.*) *aureus*–associated skin disorders and atopy was reviewed, as these are the most prominent skin features in PIDs.

Methods

A systematic search was performed in EMBASE, MEDLINE, Web of Science, Cochrane, and Google Scholar (up to May 9, 2018). All original observational and experimental human studies that address the presence of skin disorders in PIDs were selected. We rated study quality using the Institute of Health Economics Quality Appraisal Checklist for Case Series Studies.

Results

Sixty-seven articles (5030 patients) were included. Study quality ranged from 18.2% to 88.5%. A broad spectrum of skin disorders was reported in 30 PIDs, mostly in single studies with a low number of included patients. An overview of associated PIDs per skin disorder was generated. Data on S. aureus-associated skin disorders and atopy in PIDs were limited.

Conclusion

Skin disorders are prominent features in PIDs. Through clustering of PIDs per skin disorder, we provide a support tool to use in clinical practice that should raise awareness of PIDs based on presenting skin manifestations.

INTRODUCTION

Primary immunodeficiency diseases (PIDs) represent a heterogeneous group of inherited disorders caused by mutations in genes encoding functional proteins of the immune cells. Based on registries and epidemiologic surveys, it has been suggested that six million people are living with a PID worldwide, whereas only 27.000-60.000 patients have been identified to date.[1] PIDs are usually characterized by recurrent and/or severe infections as well as an increased risk of autoimmunity, autoinflammation, malignancy and allergic disorders.[2-4] Moreover, both infectious and noninfectious skin disorders are common in PIDs and may be among the presenting clinical manifestations.[5-8] *Staphylococcus* (*S.*) *aureus* induced skin infections are the most common infectious skin disorders reported in PIDs, including leukocyte adhesion defects (LAD), chronic granulomatous disease (CGD), severe congenital neutropenia and hyper immunoglobulin (Ig) E syndrome (HIES).[9-11] On the other hand, dermatitis is one of the most prominent noninfectious skin manifestations in PIDs and may be part of the atopic syndrome.[12] Patients with an atopic constitution show next to atopic dermatitis (AD) tendency towards development of food allergies, asthma and rhinoconjunctivitis.[13]

Based on previous narrative reviews without a systematic approach, *S. aureus* skin infections, dermatitis and other skin disorders as well as atopy seem to be all fairly common in patients with a PID, but are also frequently described in the general population.[12] Therefore, it is of importance to realize that presence of specific skin symptoms alone does not necessarily point towards a PID. However, recognition of specific skin conditions in combination with other clinical features suggestive of an immunodeficiency should raise awareness to an underlying PID and may facilitate earlier diagnosis of PIDs.[14]

The aim of this review was to provide a systematically obtained overview of skin disorders and their prevalence in patients with PIDs. Focusing on two prevalent skin disorders in PIDs, the relation between PIDs and *S. aureus*-related skin disorders and atopy will be reviewed in more detail.

MATERIALS AND METHODS

Studies

This review with a systematic approach was conducted and reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines, where applicable.[15] All original observational and experimental human studies were included. We selected both articles reporting skin disorders in patients with PIDs and articles present-

ing a differential diagnosis of a specific skin disorder that includes a PID. No restrictions were made with respect to publication date and language. We excluded case reports (<5 patients), conference abstracts, letters and editorials as the quality of these types of articles can be highly variable. Also articles describing acquired immunodeficiencies, articles reporting skin disorders in PIDs that developed after or during treatment/intervention and articles in which the description of skin disorders in PIDs was not part of the results section were excluded. Data on skin disorders were only extracted if at least five patients per PID were reported.

Study participants

Patients of all ages with a PID according to Picard *et al.*[16] from both hospital setting and general population were included.

Study outcomes

The primary outcome is the presence of skin disorders in PIDs. Secondary outcomes include the prevalence of skin disorders in PIDs, *S. aureus*-associated skin disorders in PIDs, and PIDs associated with an atopic constitution (i.e. atopic dermatitis, food allergy, asthma, rhinoconjunctivitis).

Search strategy

The electronic search was conducted in EMBASE, MEDLINE, Web of Science, Cochrane, and Google Scholar up to May 9th 2018 (Appendix 1). The search was composed of terms of the categories primary immunodeficiency, skin disorder, *Staphylococcus aureus* and atopy supplemented by specific PIDs and skin disorders based on recent literature.[12,17-19]

Study selection and data extraction

All studies identified in the systematic search were screened for relevance by title and abstract. Duplicates and studies that did not meet our inclusion criteria were excluded (Appendix 2). Remaining articles were assessed for eligibility by full text review. Furthermore, a cross-reference check was performed to identify other eligible studies based on the reference lists of all included articles and relevant review articles. Translation of non-English studies was conducted officially. Study selection and data extraction were performed independently by two researchers (JdW and JvV, JdW and RB or JvV and RB). Disagreements were resolved and consensus was reached. If one population was described in different articles, we included the study with the most detailed description of the results. The methodological quality of the individual articles was rated using the Institute of Health Economics (IHE) Quality Appraisal Checklist for Case Series Studies (Appendix 3).[20]

Analysis of data

The prevalence of skin disorders in PIDs was extracted from the included studies. If required, the prevalence was calculated with the available raw data. Because the reported number of patients with a PID was mainly low, the proportion of patients with a PID and skin disorders was descriptively presented. Proportions of skin disorders in PIDs were compared with the prevalence of skin disorders in the general population.[21-26] Data from the general population were based on a birth cohort in Finland (n=1932, age 45-47 years) and a Dermatology outpatient clinic in Turkey (n=11 040, age 1-99 years).[21,22] In addition, a nationwide study of Furue *et al*.[23] reported the prevalence of cutaneous disorders in 67 448 Japanese patients of all ages. In the study of Verhoeven *et al*.[24], the skin disease prevalence per 1000 patient-years in family practices in the Netherlands was converted to a point prevalence in the general population (n=501, age 18-97 years). Finally, two studies from the United States of America and the United Kingdom performed in 1978 and 1976 showed the prevalence of skin disorders in community studies in respectively 20749 (age 1-74 years) and 614 (age 15-74 years) patients.[25,26]

RESULTS

Study characteristics

The literature search identified 15 871 studies. Removal of duplicates resulted in 12 834 studies. Screening on title and abstract yielded 86 full-text articles of which 36 articles remained after full-text screening. Finally, after cross-reference check, a total of 67 articles (5030 patients) were included for further analysis (Figure 1). Skin disorders in patients with PIDs were described in 67 articles, and three articles reported PIDs as part of the differential diagnosis of a specific skin disorder. Fifty-seven studies showed a mean percentage of males of 62.2%. Both children and adults were included with a mean age of 15.8 years, reported in 26 articles. The IHE Quality Appraisal Checklist for Case Series Studies ranged from 18.2% to 88.5% (Table S1).

Skin disorders and their prevalence in primary immunodeficiency diseases

Thirty individual PIDs and their related skin manifestations were found. We categorized the skin disorders in 15 main groups and in 20 more specific subgroups (Table 1). The skin disorders per PID were mainly reported in single studies. Therefore, meta-analysis was not possible. The presence of skin telangiectasia, café au lait macules and hypopigmented macules in ataxia-telangiectasia (AT), skin abscesses in HIES, atopic dermatitis in hypogammaglobulinemia, atopic dermatitis, alopecia (areata), vitiligo and psoriasis in selective IgA deficiency (SIgAD), alopecia, vitiligo and nail dystrophy in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) and abscesses and granuloma in CGD were

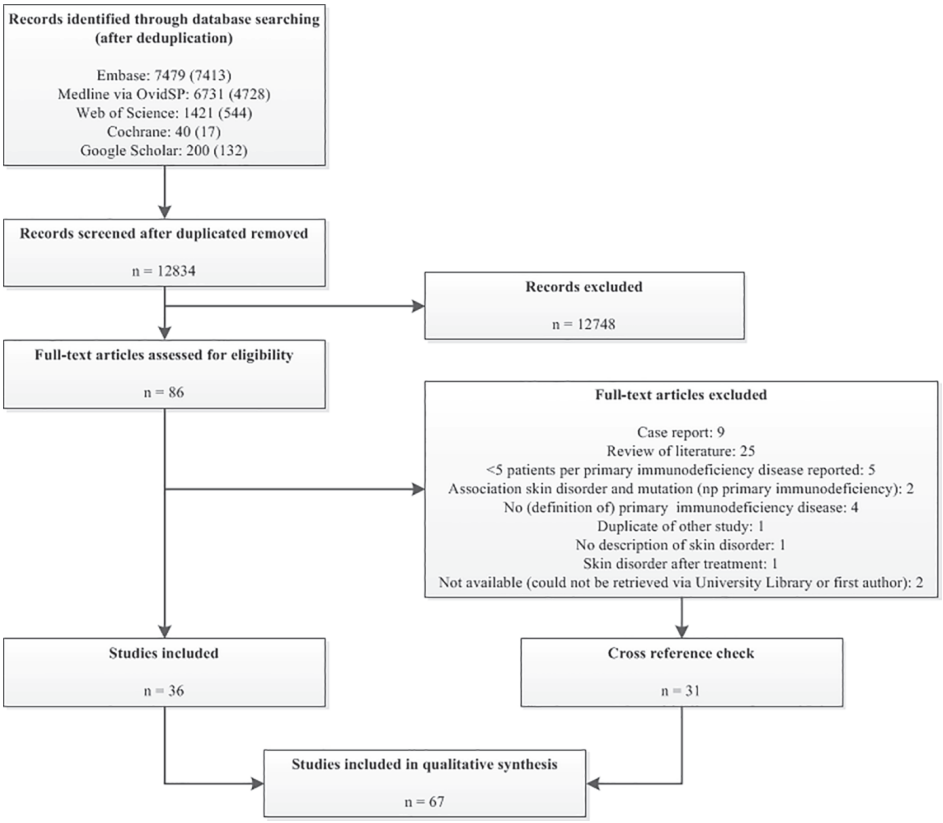

Figure 1. Flow chart of search strategy and study selection

confirmed in at least three articles. All reported skin disorders per PID were used to provide an overview of PIDs per skin disorder group (Figure 2).

Staphylococcus aureus-associated skin disorders in primary immunodeficiency diseases

Skin disorders associated with *S. aureus* in PIDs were reported in six articles (Table S1). In HIES unspecified, 4/7 patients with a papulopustular eruption had a positive *S. aureus* culture.[36] *S. aureus* was also found positive in patients with AD-HIES and a papulopustular rash (2/5), eczematous dermatitis (20/20), cold abscesses (20/20) or wounds (3/4).[39,41] Renner *et al.*[44] described that skin abscesses were frequently due to *S. aureus* infections in autosomal recessive HIES (AR-HIES). In Comèl-Netherton syndrome 8/9 described patients showed recurrent or persistent *S. aureus* skin infections once skin lesions had developed.[49] Lastly, *S. aureus* was isolated in 1/4 patients with CGD and suppurative dermatitis.[80]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases

Primary immunodeficiency disease					General population
Main groups of skin disorders	**Subgroups of skin disorders**	**Skin disorders as reported in included articles**	**Number of reported cases with skin disorder (proportion)**	**Prevalence of skin disorder (%)**	**Prevalence of skin disorder (%)†**
Immunodeficiencies affecting cellular and humoral immunity					
Severe combined immunodeficiency					
Dermatitis-like lesions		Seborrheic dermatitis	2/9[7]	22.2	2.2-11.7[21-25]
Skin infections	Fungal skin infections	Candidiasis	4/9[7]	44.4	0.6-1.0[22,23]
Omenn syndrome					
Hair abnormalities	Hair loss disorders	Severe alopecia	5/7[27]	71.4	0.4-2.5[21-23]
		Alopecia of eyelashes and eyebrows	3/7[27]	42.9	-
Erythematous skin lesions		Exfoliative erythroderma[a]	7/7[27]	100	0.1[23]
Other skin disorders		Skin induration	6/7[27]	85.7	-
Combined immunodeficiencies with associated or syndromic features					
Ataxia-telangiectasia					
Dermatitis-like lesions		Dermatitis	1/62[6]	1.6	32.4[24]
		Eczema	2/22[28]	9.1	9.0-27.4[21,26]
		Nummular eczema	1/22[28]	4.5	1.9-2.2[21,22]
		Seborrheic rash	2/32[29]	6.3	2.2-11.7[21-25]
Hair abnormalities	Excessive hair growth disorders	Hypertrichosis	7/32[29]	21.9	-
		Hirsutism	2/12[30]	16.7	0.4[22]
	Hair pigmentation disorders	Poliosis	5/12[30]	41.7	-

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Skin infections	Fungal skin infections	Oral candidiasis	1/12[30]	8.3	-
		Coccidioidomycosis	1/22[28]	4.5	-
	Viral skin infections	Viral warts	2/32[29], 8/22[28]	6.3-36.4	3.4-4.5[22,23,26]
		Herpes simplex	2/12[30]	16.7	0.8-1.0[22,23]
	Bacterial skin infections	Chronic impetigo	1/22[28]	4.5	0.8-1.6[22,23]
		Impetigo	1/12[30]	8.3	0.8-1.6[22,23]
Erythematous skin lesions		Pinpoint erythematous macules	2/12[30]	16.7	-
Vascular disorders	Telangiectasia	Skin telangiectasia	6/62[6], 16/26[7], 4/12[30]	9.7-61.5	-
		Telangiectasia on cheeks or nose	4/32[29], 18/22[28]	12.5-81.8	-
		Telangiectasia on ears	15/32[29]	46.9	-
		Telangiectasia on back/ shoulders/neck	5/32[29]	15.6	-
	Vasculitis	Allergic vasculitis	1/22[28]	4.5	-
Pigmentation disorders	Hyperpigmentation disorders	Café au lait macules	27/32[29], 3/22[28], 4/12[30]	13.6-84.4	12.4[21]
		Pigmented nevi (>5 mm)	12/32[29]	37.5	-
		Hyperpigmentation	1/62[6]	1.6	-
		Acanthosis nigricans	3/12[30]	25.0	-
	Hypopigmentation disorders	Hypopigmented macules	3/62[6], 14/32[29], 2/12[30]	4.8-43.8	-
		Albinism	1/32[29], 8/22[28]	3.1-36.4	-
		Vitiligo	1/12[30]	8.3	1.2-1.7[21-23]
	Other pigmentation disorders	Blue naevus	1/22[28]	4.5	1.3[21]
		Freckles	1/22[28]	4.5	-
Neoplastic disorders	Others neoplastic disorders	Basal cell carcinoma	1/22[28]	4.5	0.4-0.5[21,23]
		Juvenile melanoma	1/22[28]	4.5	-
Rash		Facial papulosquamous rash	13/32[29]	40.6	-
Nail disorders	Non-infectious nail disorders	Congenital nail dystrophy	2/12[30]	16.7	-
Granulomatous disorders		Skin granulomas	8/8[31]	100	0.3[23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Other skin disorders		Lichen simplex chronicus	1/32[29]	3.1	3.0[22]
		Sclerodermoid changes	1/22[28]	4.5	-
		Senile keratosis (actinic keratosis)	1/22[28]	4.5	0.4-0.6[21,23]
		Aged skin	2/22[28]	9.1	-
		Shagreen patch	1/12[30]	8.3	-
		Lipoatrophy	1/12[30]	8.3	-
		Hydroa vacciniforme	1/12[30]	8.3	-
		Dermatofibroma	1/12[30]	8.3	0.2-22.2[21,23]
		Purpura	5/26[7]	19.2	-
Wiskott-Aldrich syndrome					
Dermatitis-like lesions		Eczema	5/5[7]	100	9.0-27.4[21,26]
Skin infections	Fungal skin infections	Yeast/fungi	18/154[32]	11.7	-
	Viral skin infections	Varicella	25/154[32]	16.2	-
		HSV I/ HSV II	24/154[32]	15.6	0.8-1.0[22,23]
		Molluscum contagiosum	13/154[32]	8.4	0.8-0.9[22,23]
		Warts	10/154[32]	6.5	3.4-4.5[22,23,26]
	Bacterial skin infections	Impetigo	19/154[32]	12.3	0.8-1.6[22,23]
		Abscesses	19/154[32]	12.3	1.7[22]
		Cellulitis	19/154[32]	12.3	0.9-1.5[22,23]
Vascular disorders	Vasculitis	Henoch-Schönlein purpura	8/154[32]	5.2	-
		Skin vasculitis	12/55[33]	7.8	-
Hyper IgE syndrome unspecified					
Dermatitis-like lesions		Atopic dermatitis	5/6[6], 5/5[34], 28/43[35], 5/8[36]	62.5-100	2.2-12.4[21-24]
		Eczema	30/30[37]	100	9.0-27.4[21,26]
Skin infections	Fungal skin infections	Candida	4/6[6], 25/30[37]	66.7-83.3	0.6-1.0[22,23]
	Bacterial skin infections	Skin abscesses	6/6[6], 36/43[35], 3/8[36], 26/30[37]	37.5-100	1.7[22]
	Other skin infections	Skin infection	3/5[34]	60.0	4.6-43.5[21,25,26]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Ulcers	Oral ulcers	Oral aphthous ulcers	1/5[34]	20.0	0.7[22]
		Oral ulceration	8/11[38]	72.7	0.7[22]
Rash		Newborn rash	35/43[35]	81.4	-
		Maculopapular rash	1/6[6]	16.7	-
		Papulopustular eruption	8/8[36]	100	-
Acne-like lesions		Neonatal acne	6/43[35]	14.0	-
Other skin disorders		Coarse face	3/6[6], 2/8[36]	25.0-50.0	-
Autosomal dominant hyper IgE syndrome					
Dermatitis-like lesions		Eczematous dermatitis	20/21[39]	95.2	32.4[24]
		Eczema	47/82[40], 17/17[41]	57.3-100	9.0-27.4[21,26]
Skin infections	Fungal skin infections	Oral candidiasis	4/21[39]	19.0	-
		Genitalia fungal infection	1/17[41]	5.9	-
	Viral skin infections	Varicella-zoster virus infection	9/21[39]	42.9	-
		Herpes simplex virus infection	4/21[39]	19.0	0.8-1.0[22,23]
		Herpes infection	5/67[40]	7.5	0.8-1.0[22,23]
		Molluscum contagiosum	1/21[39], 1/82[40]	1.2-4.8	0.8-0.9[22,23]
	Bacterial skin infections	Cold abscesses	20/21[39], 9/17[41]	52.9-95.2	1.7[22]
		Skin abscesses	61/82[40]	74.4	1.7[22]
		Cellulitis	15/82[40]	18.3	0.9-1.5[22,23]
		Pustulosis	14/17[41]	82.4	-
		Folliculitis	7/17[41]	41.2	1.1-6.0[21,23]
	Other skin infections	Recurrent skin infections	17/17[41]	100	4.6-43.5[21,25,26]
Ulcers	Oral ulcers	Oral ulcer	1/17[41]	2.4	0.7[22]
Neoplastic disorders	Cutaneous lymphomas	Pilotropic cutaneous T-cell lymphoma	1/21[39]	4.8	-
	Other neoplastic disorders	Squamous cell carcinoma	1/82[40]	1.2	0.3-0.7[23,24]
Rash		Papulopustular rash (<2 months)	14/21[39]	66.7	-

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Nail disorders	Infectious nail disorders	Chronic paronychia	8/21[39]	38.1	-
		Onychomycosis	23/82[40], 4/17[41]	23.5-28.0	9.4[21]
Urticaria		Urticaria	13/82[40]	15.9	0.5-8.3[21-23]
Other skin disorders		Lichenification	1/21[39]	4.8	-
		Coarse facies	10/21[39]	47.6	-
		Dry skin	18/21[39]	85.7	-
		Thrush	17/82[40], 6/17[41]	20.7-35.3	-
		Angioedema	9/82[40]	11.0	-
Autosomal recessive hyper IgE syndrome					
Dermatitis-like lesions		Eczema	19/21[42], 7/10[43]	70.0-90.5	9.0-27.4[21,26]
		Atopic dermatitis	7/10[43]	70.0	2.2-12.4[21-24]
Skin infections	Fungal skin infections	Mucocutaneous candidiasis	9/21[42]	42.9	0.6-1.0[22,23]
		Chronic candidiasis of mucosal sites	10/13[44]	76.9	-
	Viral skin infections	Viral warts	13/21[42]	61.9	3.4-4.5[22,23,26]
		Verruca plana	1/10[43]	10.0	3.4-4.5[22,23,26]
		Herpes simplex virus	12/21[42], 8/13[44]	57.1-61.5	0.8-1.0[22,23]
		Recurrent herpes	1/10[43]	10.0	0.8-1.0[22,23]
		Molluscum contagiosum	10/21[42], 4/13[44]	30.8-47.6	0.8-0.9[22,23]
		Severe primary varicella zoster	7/21[42], 2/13[44]	15.4-33.3	-
		Herpes zoster	5/21[42]	23.8	1.4-2.4[22,23]
	Bacterial skin infections	Bacterial skin infection	17/21[42]	81.0	-
		Skin abscesses	11/13[44]	84.6	1.7[22]
		MRSA wound infected eczema	1/10[43]	10.0	-
	Other skin infections	Recurrent stomatitis	1/10[43]	10.0	-
Neoplastic disorders	Cutaneous lymphomas	Cutaneous T-cell lymphoma	1/21[42]	4.8	-
	Other neoplastic disorders	Squamous cell carcinoma	4/21[42]	19.0	0.3-0.7[23,24]
Rash		Severe eczematoid rash	13/13[44]	100	9.0-27.4[21,26]
		Newborn rash	5/21[42]	23.8	-

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Other skin disorders		Immune thrombocytopenic purpura	1/10[43]	10.0	-
Nijmegen breakage syndrome					
Skin infections	Fungal skin infections	Candidiasis	6/21[45]	28.6	0.6-1.0[22,23]
	Viral skin infections	Herpes virus lip infection	2/21[45]	9.5	0.8-1.0[22,23]
	Other skin infections	Angular cheilitis	2/21[45]	9.5	0.1-0.3[22,23]
Vascular disorders	Telangiectasia	Cutaneous telangiectasia	3/32[46]	9.4	-
Pigmentation disorders	Hyperpigmentation disorders	Café au lait spots	18/21[46]	85.7	12.4[21]
	Hypopigmentation disorders	Vitiligo	14/21[46]	66.7	1.2-1.7[21-23]
Granulomatous disorders		Skin granuloma	5/35[47]	14.3	0.3[23]
Other skin disorders		Hyperkeratosis	1/21[45]	4.8	-
		Gingivitis	19/21[45]	90.5	-
DiGeorge syndrome					
Rash		Rash	5/5[48]	100	-
Comèl-Netherton syndrome					
Dermatitis-like lesions		Eczema[b]	8/9[49]	88.9	9.0-27.4[21,26]
Hair abnormalities	Hair loss disorders	Severe alopecia	9/9[27]	100	0.4-2.5[21-23]
		Alopecia of eyelashes and eyebrows	5/9[27]	55.6	-
	Other hair abnormalities	Bamboo hair	9/9[49]	100	-
Skin infections	Bacterial skin infections	Recurrent/persistent *S. aureus* skin infections	9/9[49]	100	-
Erythematous skin lesions		Exfoliative erythroderma[c]	9/9[27]	100	0.1[23]
Other skin disorders		Congenital ichthyosis	9/9[49]	100	0.1[22,23]
Predominantly antibody deficiencies					
X-linked agammaglobulinemia					

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Dermatitis-like lesions		Dermatitis	4/23[6]	17.4	32.4[24]
Hair abnormalities	Hair loss disorders	Alopecia	1/110[50]	0.9	0.4-2.5[21-23]
Skin infections	Bacterial skin infections	Abscesses	3/23[6]	13.0	1.7[22]
		Furunculosis	2/23[6]	8.7	1.7[22]
		Impetigo	2/23[6]	8.7	0.8-1.6[22,23]
Pigmentation disorders	Hypopigmentation disorders	Vitiligo	0/110[50]	0.0	1.2-1.7[21-23]
Rash		Maculopapular rash	3/23[6]	13.0	-
Psoriasis-like lesions		Psoriasis	0/110[50]	0.0	1.4-8.0[21-26]
Urticaria		Urticaria	2/23[6]	8.7	0.5-8.3[21-23]
Other skin disorders		Pyoderma	1/10[7]	10.0	1.0[22]
		Lichen planus	0/110[50]	0.0	0.3-14.1[21,23,25]
Hypogammaglobulinemia					
Dermatitis-like lesions		Atopic dermatitis	28/28[51], 0/12[52], 46/78[53]	0.0-100	2.2-12.4[21-24]
Common variable immunodeficiency disorder					
Dermatitis-like lesions		Dermatitis	6/28[6]	21.4	32.4[24]
		Atopic dermatitis	9/47[54]	19.1	2.2-12.4[21-24]
		Eczema	4/15[55]	26.7	9.0-27.4[21,26]
Hair abnormalities	Hair loss disorders	Alopecia areata	1/28[6], 1/47[54]	2.1-3.6	0.4-2.5[21-23]
		Alopecia	4/244[50]	1.6	0.4-2.5[21-23]
Skin infections	Fungal skin infections	Candida	4/28[6]	14.3	0.6-1.0[22,23]
		Pseudomembraneous candidiasis	4/15[55]	26.7	-
	Viral skin infections	Recurrent herpes labialis	1/15[55]	6.7	0.8-1.0[22,23]
	Bacterial skin infections	Recurrent skin abscesses	2/31[56]	6.5	1.7[22]
	Other skin infections	Skin infections	7/47[54]	14.9	4.6-43.5[21,25,26]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Ulcers	Oral ulcers	Recurrent aphthosis	5/47[54]	10.6	0.7[22]
		Oral ulcers	9/15[55]	60.0	0.7[22]
		Oral aphthae	10/31[56]	32.3	0.7[22]
Pigmentation disorders	Hypopigmentation disorders	Vitiligo	8/244[50], 2/47[54]	3.3-4.3	1.2-1.7[21-23]
Rash		Maculopapular rash	1/28[6]	3.6	-
Psoriasis-like lesions		Psoriasis	2/244[50], 9/47[54]	0.8-19.1	1.4-8.0[21-26]
Acne-like lesions		Acne	6/47[54]	12.8	0.8-13.1[22-24]
Urticaria		Urticaria[d]	1/28[6]	3.6	0.5-8.3[21-23]
Other skin disorders		Pyoderma	2/5[7]	40.0	1.0[22]
		Lichen planus	1/244[50]	0.4	0.3-14.1[21,23,25]
Selective IgA deficiency					
Dermatitis-like		Dermatitis	5/17[6]	29.4	32.4[24]
		Eczema	2/39[57]	5.1	9.0-27.4[21,26]
		Atopic dermatitis	13/13[51], 4/12[52], 59/102[54], 12/159[58], 12/23[59], 9/123[60], 1/8[61], 9/81[62], 16/347[63]	4.6-100	2.2-12.4[21-24]
		Allergic contact dermatitis	11/347[63]	3.2	1.0-8.5[21-25]
		Seborrheic dermatitis	4/347[63]	1.2	2.2-11.7[21-25]
Hair abnormalities	Hair loss disorders	Alopecia	2/60[50], 1/102[54], 1/8[61]	1.0-12.5	0.4-2.5[21-23]
		Alopecia areata	1/8[64], 1/123[60], 2/347[63]	0.6-12.5	0.4-2.5[21-23]
		Infection related alopecia areata	1/8[64]	12.5	0.4-2.5[21-23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Skin infections	Fungal skin infections	Candida	2/17[6]	11.8	0.6-1.0[22,23]
		Pseudomembraneous candidiasis	10/39[57]	25.6	-
	Viral skin infections	Recurrent herpes labialis	10/39[57], 2/123[60]	1.6-25.6	0.8-1.0[22,23]
		Herpes simplex	4/347[63]	1.2	0.8-1.0[22,23]
		Herpes zoster	1/347[63]	0.3	1.4-2.4[22,23]
		Molluscum contagiosum	2/347[63]	0.9	0.8-0.9[22,23]
	Bacterial skin infections	Folliculitis	1/17[6]	5.9	1.1-6.0 [21,23]
		Erysipelas recidivans	1/8[64]	12.5	0.1[23]
		Chronic recurrent furunculosis	1/8[64]	12.5	1.7[22]
		Cellulitis	5/347[63]	1.4	0.9-1.5[22,23]
	Other skin infections	Skin infections	2/102[54]	2.0	4.6-43.5[21,25,26]
		Angular stomatitis	1/39[57]	2.6	-
		Scabies	3/347[63]	0.9	0.2-1.5[22,23]
Ulcers	Oral ulcers	Recurrent aphthosis	5/102[54]	4.9	0.7[22]
		Oral ulcers	24/39[57]	61.5	0.7[22]
		Aphthosis recidivans	1/8[64]	12.5	0.7[22]
Erythematous skin lesions		Erythroderma	1/8[64]	12.5	0.1[23]
		Erythema nodosum	1/123[60]	0.8	0.2-0.3[22,23]
Vascular disorders	Vasculitis	Vasculitis	3/7[7]	42.9	-
		Kawasaki disease	1/8[61]	12.5	-
	Other vascular disorders	Raynaud syndrome	1/8[61]	12.5	-
Pigmentation disorder	Hypopigmentation disorders	Vitiligo	1/7[7], 3/60[50], 3/102[54], 1/8[64], 7/159[58], 3/123[60], 1/81[62], 2/347[63]	0.6-14.3	1.2-1.7[21-23]
Psoriasis-like disorders		Psoriasis	0/60[50], 2/102[54], 7/159[58], 1/123[60], 7/347[63]	0.0-4.4	1.4-8.0[21-26]
Acne-like lesions		Acne	6/102[54], 69/347[63]	5.9-19.9	0.8-13.1[22-24]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Urticaria		Urticaria	5/23[59]	21.7	0.5-8.3[21-23]
		Atopic urticaria	4/123[60]	3.3	0.5-8.3[21-23]
		Chronic spontaneous urticaria	17/347[63]	4.9	0.5-8.3[21-23]
Other skin disorders		Lichen planus	0/60[50], 2/159[58]	0.0-1.3	0.3-14.1[21,23,25]
		Immune thrombocytopenic purpura	1/17[6]	5.9	-
		Idiopathic thrombocytopenic purpura	2/123[60]	1.6	-
		Chronic idiopathic thrombocytopenic purpura	1/81[62]	1.2	-
		Epidermolysis bullosa dystrophica	1/8[64]	12.5	-
		Local skin scleroderma	1/123[60]	0.8	-
		Dermatitis herpetiformis	1/123[60]	0.8	0.2-0.3[21,22]
		Ichthyosis and keratoderma of handpalms and footsoles in epileptic patients (Rud syndrome)	1/123[60]	0.8	-
IgM deficiency					
Dermatitis-like lesions		Atopic dermatitis	14/14[51], 11/53[52]	20.8-100	2.2-12.4[21-24]
IgG deficiency					
Dermatitis-like lesions		Atopic dermatitis	11/11[51]	100	2.2-12.4[21-24]
Diseases of immune dysregulation					
Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy					
Dermatitis-like lesions		Recurrent and troublesome napkin dermatitis	5/18[65]	27.8	-
Hair abnormalities	Hair loss disorders	Alopecia	2/15[61], 6/18[65], 20/68[66], 6/35[67], 6/22[68]	13.3-33.3	0.4-2.5[21-23]
	Hair pigmentation disorders	Poliosis	1/18[65]	5.6	-

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Skin infections	Fungal skin infections	Oral candidiasis	41/68[66]	60.3	-
		Dermal candidiasis	6/68[66], 6/35[67]	8.8-17.1	0.6-1.0[22,23]
		Chronic mucocutaneous candidiasis	18/18[65], 30/35[67]	85.7-100	0.6-1.0[22,23]
		Mucocutaneous candidiasis	21/22[68]	95.5	0.6-1.0[22,23]
		Life-long genital moniliasis	1/18[65]	5.6	-
	Other skin infections	Angular cheilitis	13/18[65]	72.2	0.1-0.3[22,23]
Vascular disorders	Vasculitis	Cutaneous vasculitis	2/68[66]	2.9	-
Pigmentation disorders	Hypopigmentation disorders	Vitiligo	1/15[61], 2/18[65], 9/68[66], 13/35[67], 6/22[68]	6.7-37.1	1.2-1.7[21-23]
		Halo naevi	1/18[65]	5.6	0.8[21]
Nail disorders	Infectious nail disorders	Ungual candidiasis	33/50[66]	66.0	-
		Candidal paronychia and/or onychomycosis	13/18[65]	72.2	9.4[21]
		Nail candidiasis	12/35[67]	34.3	-
	Non-infectious nail disorders	Nail dystrophy	26/50[66], 6/35[67], 4/22[68]	17.1-52.0	-
Urticaria		Urticarial eruption	23/35[67]	8.6	0.5-8.3[21-23]
		Urticarial rash	2/22[68]	9.1	0.5-8.3[21-23]
Other skin disorders		Oral thrush	35/35[67]	100	-
Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome					
Dermatitis-like		Atopic dermatitis	7/10[69]	70.0	2.2-12.4[21-24]
		Severe eczema	5/14[70]	35.7	9.0-27.4[21,26]
		Mild eczema	4/14[70]	28.6	9.0-27.4[21,26]
		Eczema	5/5[71], 2/5[72]	40.0-100	9.0-27.4[21,26]
Hair abnormalities	Hair loss disorders	Alopecia[e]	2/14[70]	14.3	0.4-2.5[21-23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Skin infections	Fungal skin infections	Chronic mucocutaneous candidiasis	4/5[71]	80.0	0.6-1.0[22,23]
	Viral skin infections	HSV skin infection	1/5[71]	20.0	0.8-1.0[22,23]
		Herpes zoster	1/5[71]	20.0	1.4-2.4[22,23]
	Bacterial skin infections	Staphylococcal superinfection of eczema	1/5[71]	20.0	-
	Other skin infections	Cheilitis	4/10[69]	40.0	0.1-0.3[22,23]
Erythematous skin lesions		Exfoliative erythroderma	2/10[69]	20.0	0.1[23]
Nail disorders	Infectious nail disorders	Onychodystrophy[f]	2/10[69]	20.0	-
		(Peri-)onyxis	1/10[69]	10.0	-
Psoriasis-like lesions		Psoriasiform rash	2/10[69]	20.0	1.4-8.0[21-26]
Other skin disorders		Palmar keratoderma	1/10[69]	10.0	-
		Inflammatory oedema (lips and perioral) or Quincke oedema	3/10[69]	30.0	-
		Mild xerosis	1/14[70]	7.1	-
		Acrodermatitis enteropathica	1/5[71]	20.0	-
Adenosine deaminase 2 deficiency					
Dermatitis-like lesions		Eczema	5/9[73]	55.6	9.0-27.4[21,26]
Ulcers	Oral ulcers	Oral aphthae	3/9[73]	33.3	0.7[22]
	Other ulcers	Livedoid vascuclaropathy/ulcers	1/9[73]	11.1	-
Erythematous skin lesions		Erythema nodosum	2/9[73], 1/8[74]	12.5-22.2	0.2-0.3[22,23]
Vascular disorders	Vasculitis	Cutaneous vasculitis	3/9[73]	33.3	-
		Polyarteritis nodosa	1/9[73]	11.1	-
	Other vascular disorders	Raynaud's phenomenon	1/8[74]	12.5	-
Neoplastic disorder	Other neoplastic disorders	AML chloroma	1/9[73]	11.1	-
Rash		Rash	3/9[73]	33.3	-
Urticaria		Urticaria-like rash	1/8[74]	12.5	0.5-8.3[21-23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Other skin disorders		Livedo reticularis	3/9[73], 6/8[74]	33.3-75.0	0.1[23]
		Livedo racemose	1/9[73]	11.1	0.1[23]
		Aspecific skin induration	4/9[73]	44.4	-
Congenital defects of phagocyte number or function					
Leukocyte adhesion defect unspecified					
Skin infections	Bacterial skin infections	Cellulitis	2/6[6]	33.3	0.9-1.5[22,23]
		Folliculitis	2/6[6]	33.3	1.1-6.0[21,23]
Other skin disorders		Periodontitis	2/6[6]	33.3	-
Leukocyte adhesion defect type 1					
Skin infections	Fungal skin infections	Candida infection	8/15[75]	53.3	0.6-1.0[22,23]
	Bacterial skin infections	Skin abscesses	12/15[75]	80.0	1.7[22]
		Cellulitis	4/15[75]	26.7	0.9-1.5[22,23]
Ulcers	Oral ulcers	Oral ulcers	13/15[75]	86.7	0.7[22]
Other skin disorders		Gingivitis	9/15[75]	60.0	-
Chronic granulomatous disease					
Dermatitis-like lesions		Dermatitis	nm/429[76], nm/39[77]	-	32.4[24]
		Eczema	8/48[78]	16.7	9.0-27.4[21,26]
Skin infections	Bacterial skin infections	Abscesses	23/34[6], nm/429[76], nm/39[77], 1/48[78], 156/368[79], 11/49[80]	2.1-67.6	1.7[22]
		Folliculitis	5/34[6]	14.7	1.1-6.0[21,23]
		Impetigo	3/34[6], 16/95[80]	8.8-16.8	0.8-1.6[22,23]
		Cellulitis	18/368[79]	4.9	0.9-1.5[22,23]
		Furunculosis	nm/429[76]	-	-
		Pustular eruption	10/48[78]	20.8	-
	Other skin infections	Chronic cutaneous infections	1/6[81]	16.7	4.6-43.5[21,25,26]
		Cutaneous/subcutaneous infections	22/48[78], 43/84[82]	45.8-51.2	4.6-43.5[21,25,26]
		Skin infection	59/130[80]	45.4	4.6-43.5[21,25,26]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Ulcers	Oral ulcers	Oral ulceration	7/9[38], 2/6[81]	33.3-77.8	0.7[22]
	Other ulcers	Nose ulcers	5/34[6]	14.7	-
Vascular disorders	Vasculitis	Kawasaki disease	2/48[78]	4.2	-
Nail disorders	Infectious nail disorders	Paronychia	2/34[6]	5.9	-
Granulomatous disorders		Granuloma	2/11[61], nm/429[76], 4/48[78]	8.3-18.2	0.3[23]
Acne-like lesions		Acne	nm/429[76]	-	0.8-13.1[22-24]
Urticaria		Urticaria	1/48[78]	2.1	0.5-8.3[21-23]
Other skin disorders		Discoid lupus erythematosus[9]	30/340[79]	8.8	0.1-0.3[21,22]
		Thrush	11/48[78]	22.9	-
Severe congenital neutropenia					
Skin infections	Fungal skin infections	Mucocutaneous candidiasis	5/18[83]	27.8	0.6-1.0[22,23]
	Bacterial skin infections	Cutaneous abscesses	10/18[83]	55.6	1.7[22]
	Other skin infections	Cutaneous infections	7/18[83]	38.9	4.6-43.5[21,25,26]
Ulcers	Oral ulcers	Oral ulcers	13/18[83]	72.2	0.7[22]
Papillon-Lefèvre syndrome					
Nail disorders	Noninfectious nail disorders	Nail changes (mainly slight thickening of nails)	13/47[84]	27.7	3.3-3.4 [24,26]
Psoriasis-like lesions		Extensive psoriasiform plaques	3/47[84]	6.4	1.4-8.0[21-26]
Other skin disorders		Punctate hyperkeratosis on palms and soles	8/47[84]	17.0	-
		Well-demarcated hyperkeratosis of knees and elbows	23/47[84]	48.9	-
		Ichthyosis	2/47[84]	4.3	0.1[22,23]
GATA2 deficiency					
Neoplastic disorders	Other neoplastic disorders	Cutaneous melanoma	1/71[85]	1.4	0.1-1.2[21,23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease					General population
Defects in intrinsic and innate immunity					
Chronic mucocutaneous candidiasis					
Skin infections	Other skin infections	Perleche (angular cheilitis)	3/7[6]	42.9	0.1-0.3[22,23]
Other skin disorders		Thrush[h]	7/7[6]	100	-
Autoinflammatory disorders					
PLCG2 associated antibody deficiency and immune dysregulation					
Ulcers	Other ulcers	Neonatal-onset ulcerative lesions (cold-sensitive regions)	8/36[86]	22.2	2.0[23]
Erythematous skin lesions		Recurrent red papules and patches	1/36[86]	2.8	-
Pigmentation disorders	Hypopigmentation disorders	Vitiligo	1/36[86]	2.8	1.2-1.7[21-23]
Granulomatous disorders		Granulomatous inflammation	4/36[86]	11.1	0.3[23]
Urticaria		Cold urticaria	36/36[86]	100	0.5-8.3[21-23]
Muckle-Wells syndrome					
Ulcers	Oral ulcers	Oral ulcers	7/29[87]	24.1	0.7[22]
Pigmentation disorders	Hyperpigmentation disorders	Hyperpigmented, sclerotic and hypertrichotic plaques	6/6[88]	100	-
Rash		Skin rash	15/15[87]	100	-
Urticaria		Attacks of recurrent urticaria	2/6[88]	33.3	0.5-8.3[21-23]
		Urticaria	8/8[89]	100	0.5-8.3[21-23]
		Cold-induced urticaria	14/29[87]	48.3	0.5-8.3[21-23]
Other skin disorders		Weals caused by cold	16/16[90]	100	-
Neonatal onset multisystem inflammatory disease					
Urticaria		Urticaria	8/8[89]	100	0.5-8.3[21-23]

Table 1. Skin disorders and their prevalence in primary immunodeficiency diseases (continued)

Primary immunodeficiency disease				General population
Complement deficiencies				
C2 deficiency				
Urticaria	Chronic urticaria	2/47[91]	4.3	0.5-8.3[21-23]
Other skin disorders	Subacute cutaneous lupus erythematosus	2/47[91]	4.3	0.3[22]
	Dermatitis herpetiformis	1/47[91]	2.1	0.2-0.3[21,22]

Abbreviations: nm, not mentioned. †Data from the general population were based on a birth cohort in Finland (n=1932, age 45-47 years) and a Dermatology outpatient clinic in Turkey (n=11 040, age 1-99 years).[21,22] In addition, a nationwide study of Furue *et al*.[23] reported the prevalence of cutaneous disorders in 67 448 Japanese patients of all ages. In the study of Verhoeven *et al*.[24], the skin disease prevalence per 1000 patient-years in family practices in the Netherlands was converted to a point prevalence in the general population (n=501, age 18-97 years). Finally, two studies from the United States of America and the United Kingdom performed in 1978 and 1976 showed the prevalence of skin disorders in community studies in respectively 20749 (age 1-74 years) and 614 (age 15-74 years) patients.[25,26 A-H]See Figure S1.

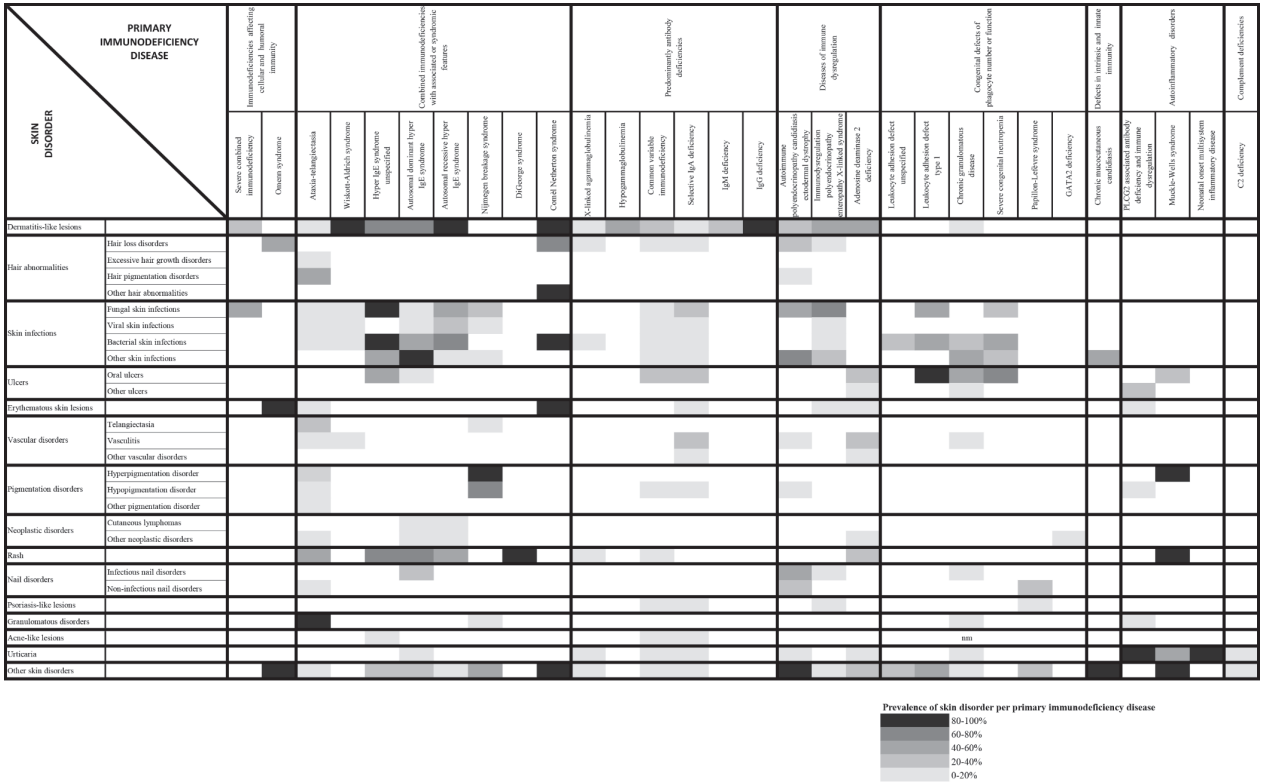

Figure 2. Differential diagnosis of primary immunodeficiency diseases per skin disorder

Abbreviations: nm, not mentioned†. † The exact prevalence of acne-like lesions in chronic granulomatous disease was not reported in the included article. The prevalence of skin disorders per primary immunodeficiency disease was based on the reported proportion of patients with the skin disorders in the total group of patients with the primary immunodeficiency disease. In case of multiple studies reporting a skin disorder in a primary immunodeficiency disease, the general prevalence was calculated by dividing the total number of affected patients (skin disorder) by the total number of patients with the primary immunodeficiency disease.

Primary immunodeficiency diseases associated with atopy

The prevalence of at least two atopic symptoms (i.e. eczema, food allergy, asthma and/or rhinoconjunctivitis) in PIDs was described in 17 articles (Table S1). Cohen *et al.*[30] found no atopy in patients with AT. In HIES unspecified, AD-HIES and AR-HIES, all of the atopic symptoms, if reported, were present in about half of the patients.[35,39-43] Renner *et al.*[49] described the presence of atopy in most Comèl-Netherton patients. The number of patients with eczema, food allergy, asthma, and rhinoconjunctivitis were presented in two studies with hypogammaglobulinemia patients.[52,53] Eight studies reported the prevalence of atopy in a total of 398 patients with SIgAD, in which 11.6% (46/398) patients had eczema, 3.4% (9/263) had food allergy, 37.1% (43/116) had asthma, and 20.0% (55/275) had rhinoconjunctivitis.[52,58-60,62] In IgM-deficient patients, eczema, asthma, and rhinoconjunctivitis were prevalent symptoms.[52] Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) patients were mainly positive for eczema.[70] Eight of 48 CGD patients had eczema and one out of 18 had rhinoconjunctivitis.[78] Finally, Aderibigbe *et al.*[86] have shown that one to three out of eight patients with Phospholipase C Gamma 2 (PLCG2) gene associated antibody deficiency and immune dysregulation (PLAID) had eczema, food allergy, asthma, and/or rhinoconjunctivitis.

DISCUSSION

This review demonstrates that skin disorders are common symptoms in both children and adult patients with PIDs based on data from 67 systematically selected studies. Only a few PIDs related to *S. aureus*-associated skin disorders or atopy were reported in mainly single studies.

This is the first review using a systematic approach without limitations on skin disorders or PIDs. Therefore, we managed to obtain a complete spectrum of skin disorders in PIDs. A recent study of Ettinger *et al.*[92] that focused on PIDs and the respective gene defects included an overview of PIDs per skin disorder in a nonsystematic approach. Although some PIDs are characterized by skin disorders, such as telangiectasia in AT and granuloma in CGD, the novelty of this review is showing an overview of all skin disorders in PIDs including skin disorders of which an association with a PID was not yet known.

Furthermore, we succeeded in composing an overview of PIDs per skin disorder that could serve as a valuable support tool for PID awareness in clinical practice and for registries. In the Netherlands, the diagnostic delay in PIDs (i.e. time period between the date of onset of first symptoms and the date of diagnosis) ranges from 0 to 14.5 years and is dominated by the defects in innate immunity (14.5 years), HIES (10.5 years) and hypogammaglobulinemia

(10.0 years).[93] Moin *et al.*[6] and Berron-Ruiz *et al.*[7] reported that in, respectively, 31.8% and 78.9% of the PIDs the cutaneous alterations preceded and were the basis for the clinical immunological diagnosis. Increased attention for these cutaneous manifestations as signal function of PIDs in combination with presence of the current warning signs for the suspicion of PIDs might improve earlier diagnosis of PIDs. These warning signs include (a) four or more new ear infections within 1 year; (b) two or more serious sinus infections within 1 year; (c) two or more months on antibiotics with little effect; (d) two or more pneumonias within 1 year; (e) failure of an infant to gain weight or grow normally; (f) recurrent, deep skin or organ abscesses; (g) persistent thrush in mouth or fungal infection on skin; (h) need for intravenous antibiotics to clear infections; (i) two or more deep-seated infections including septicemia; and (j) a family history of PID.[94] In addition, narrowing the number of eligible PIDs through clustering of skin disorders could further reduce the diagnostic delay in PIDs. Using the multi-stage diagnostic protocol of de Vries[4] or the phenotypic approach for PID classification and diagnosis by Bousfiha *et al.*[95], the diagnosis of suspected PIDs or PID-classes based on clinical symptoms could be confirmed with laboratory tests. For example, a first diagnostic step in case of a supposed antibody deficiency or neutropenia could be blood count and differentiation, IgG, IgA, IgM and IgE. In case of a possible combined immunodeficiency disease, these tests should be supplemented by lymphocyte subpopulations.

Our review has some limitations. First of all, exclusion of case reports describing fewer than five cases in our analysis might have resulted in loss of information about skin disorders in rare PIDs. However, the quality of case reports is highly variable, potential publication bias plays a role, and thus, exclusion of these case reports might have improved the reliability of this review. Furthermore, through the addition of selected PIDs and skin disorders to our electronic search we could have caused a selection bias. Although we used a cross-reference check, we cannot exclude that we might have missed some articles. Thirdly, demonstrating that specific skin disorders are characteristic for PIDs was not possible. We could only compare the presence of a number of skin disorders in patients with a PID with the prevalence of skin disorders in the general population based on six studies varying in publication year and age of the studied population.[21-26] However, most of these skin disorders were more prevalent in patients with a PID compared with the general population. Lastly, the reliability of the description of skin disorders might be questioned since in only 27 of the 67 included articles the department of Dermatology was involved. Probably, the described skin disorders were not all diagnosed by a dermatologist, but by an immunologist or pediatrician. Moreover, the majority of studies did not use skin biopsy to confirm the diagnosis of the cutaneous manifestations histopathologically. In severe combined immunodeficiency (SCID) patients, it was shown by Denianke *et al*.[96] that clinically comparable skin lesions could demonstrate different histopathological images, possibly due an altered

immune system. Subsequently, the reported clinical diagnosis of skin disorders reported in articles included in this review might not correlate with the corresponding histopathological diagnosis as well.

Because most PIDs are rare, reliable prevalence of skin disorders in PIDs can only be obtained by reporting skin disorders on an international basis. The international PID database of The European Society for Immunodeficiencies (ESID) registers, among others, data on warning signs of PIDs. These warning signs give only attention to infectious skin disorders. Noninfectious cutaneous symptoms are not included. Based on data of this review we suggest to start to collect more detailed data on all skin disorders in the ESID registry.

Future research is needed to validate these data and support an association between specific cutaneous symptoms and PIDs. Given the low number of articles reporting *S. aureus*-associated skin disorders and atopy in PIDs, more data have to be collected to further improve earlier recognition of PIDs. In addition, data on *S. aureus*-associated skin disorders might provide new treatment options for skin disorders, such as targeted therapy directed against *S. aureus*.

Conclusion

This review with a systematic approach shows that skin disorders are a prominent feature in PIDs. Earlier diagnosis of PIDs can be facilitated by recognition of specific skin conditions as signal function of PIDs in combination with the current warnings signs for PIDs or by recognizing PID specific clusters of skin conditions. We provide a support tool to use in clinical practice that should raise awareness of PIDs based on the presenting skin manifestations. Limited data are available on *S. aureus*-associated skin disorders and atopy in PIDs.

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Table S1. Study characteristics per study

	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Gernez 2018[40]	USA	85	58.8	27.3 (med)	5.5/11	Autosomal dominant hyper IgE syndrome	-	47/82 eczema, 31/82 food allergy
Nguyen 2018[85]	USA	71	32.4	-	4.5/11	GATA2 deficiency	-	-
Sahin 2018[74]	Turkey	8	75.0	16.9	7.5	Adenosine deaminase 2 deficiency	-	-
Zhou 2018[80]	China	169	95.9	-	10.5/13	Chronic granulomatous disease	1/4 suppurative dermatitis	-
Azizi 2017[50]	Iran	471	67.3	16.0 (med)	7.5/11	X-linked agammaglobulinemia Common variable immunodeficiency Selective IgA deficiency	-	-
Broides 2017[43]	Israel	10	-	6.4	4.5/11	Autosomal recessive hyper IgE syndrome	-	10/10 eczema, 1/10 food allergy, 4/10 asthma
Deripapa 2017[47]	Russia	35	45.7	13.4 (med)	8.5/13	Nijmegen breakage syndrome	-	-
Erkoçoğlu 2017[62]	Turkey	81	-	10.4	7.5/13	Selective IgA deficiency	-	9/81 eczema, 1/81 food allergy, 28/81 asthma, 22/81 rhinoconjunctivitis
Magen 2017[63]	Israel	347	54.2	24.1	8.5/11	Selective IgA deficiency	-	16/347 eczema
Muşabak 201[56]	Turkey	31	61.3	28 (med)	8/11	Common variable immunodeficiency	-	-
Wolach 2017[82]	Israel	84	79.8	-	5.5/11	Chronic granulomatous disease	-	-
Wu 2017[41]	China	17	47.1	11.8	7.5/11	Autosomal dominant hyper IgE syndrome	3/4 wounds	17/17 eczema, 2/17 food allergy

Table S1. Study characteristics per study (continued)

	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Wu 2017[78]	China	48	91.7	5.8	5.5/11	Chronic granulomatous disease	-	8/48 eczema, 1/18 rhinoconjunctivitis
Zaidi 2017[68]	India	22	-	14 (med)	7/11	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy	-	-
Altun 2016[52]	Turkey	*258*	58.5	59.3 mo	4.5/11	Hypogammaglobulinemia	-	0/12 eczema, 7/12 asthma, 8/12 rhinoconjunctivitis
						Selective IgA deficiency		4/12 eczema, 3/12 asthma, 8/12 rhinoconjunctivitis
						IgM deficiency		11/53 eczema, 19/53 asthma, 35/53 rhinoconjunctivitis
Baris 2016[72]	Turkey	30	83.3	10.6	6.5/11	Immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome	-	2/5 eczema
Blazina 2016[61]	Slovenia	247	59.5	20	8/11	Selective IgA deficiency	-	-
						Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy		
						Chronic granulomatous disease		
Ferre 2016[67]	USA	35	45.7	13.4 (med)	8.5/11	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy		
Mehr 2016[89]	Australia	18	66.7	12 (med)	7.5/11	Muckle-Wells syndrome	-	-
						Neonatal onset multisystem inflammatory disease		
Sobolewska 2016[87]	Germany	37	43.2	-	4.5/11	Muckle-Wells syndrome	-	-

Table S1. Study characteristics per study (continued)

	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Szczawińska-Popłonyk 2016[53]	Poland	78	53.8	17 mo	5/11	Hypogammaglubulinemia		46/78 eczema, 40/78 food allergy
Van Montfrans 2016[73]	The Netherlands, Belgium	9	77.8	17.3	10/13	Adenosine deaminase 2 deficiency	-	5/9 eczema
Aderibigbe 2015[86]	USA	36	-	-	7/11	PLCG2 associated antibody deficiency and immune dysregulation	-	1/8 eczema, 3/8 food allergy, 3/8 asthma, 2/8 rhinoconjunctivitis[d]
Gualdi 2015[54]	Italy	47 (CVID), 102 (SIgAD)	57.4 (CVID), 54.9 (SIgAD)	23.4 (CVID), 8.6 (SIgAD)	9/13	Common variable immunodeficiency	-	9/47 eczema
						Selective IgA deficiency		59/102 eczema
Aghamohammadi 2014[34]	Iran	75	58.7	2 (med)	11.5/13	Hyper IgE syndrome unspecified	-	5/5 eczema
Celiksoy 2014[51]	Turkey	160	61.3	1.2 (med)	11.5/13	Hypogammaglobulinemia	-	28/28 eczema
						Selective IgA deficiency		13/13 eczema
						IgM deficiency		11/11 eczema
						IgG deficiency		14/14 eczema
Greenberger 2013[29]	Israel	32	59.4	11.8	7.5/11	Ataxia-telangiectasia	-	-
Uzel 2013[71]	USA	5	80.0	9.9	10/13	Immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome	-	5/5 eczema
Chu 2012[42]	USA	21	47.6	15.9	4.5/11	Autosomal recessive hyper IgE syndrome	-	19/21 eczema, 14/21 food allergy, 10/21 asthma

Table S1. Study characteristics per study (continued)

	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Olaiwan 2011[39]	France	21	52.4	19	5/11	Autosomal dominant hyper IgE syndrome	2/5 papulopustular rash, 20/20 eczematous dermatitis, 20/20 cold abscesses	20/21 eczema, 5/21 asthma or rhinoconjunctivitis
Aghamohammadi 2009[59]	Iran	23	65.2	8 (med)	8/13	Selective IgA deficiency	-	12/23 eczema, 7/23 food allergy, 12/23 asthma, 8/23 rhinoconjunctivitis
Gregorek 2009[45]	Poland	21	52.4	11.2 (med)	6/11	Nijmegen breakage syndrome	-	-
Halabi-Tawil 2009[69]	France	10	100	-	5.5/11	Immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome	-	7/10 eczema
Renner 2009[49]	Germany	9	66.7	6 (med)	6/11	Comèl-Netherton syndrome	8/9 recurrent or persistent skin infections	9/9 atopy: 8/9 eczema, 7/9 food allergy, 2/9 asthma, 5/9 rhinoconjunctivitis
Van den Berg 2009[76]	The Netherlands	429	81.8	-	7/11	Chronic granulomatous disease	-	eczema
Gambineri 2008[70]	Italy	14	-	range 0-19	7/11	Immunodysregulation, polyendocrinopathy, enteropathy X-linked syndrome	-	11/14 eczema, 1/14 food allergy, 1/14 asthma
Movahedi 2007[75]	Iran	15	66.7	4 (med)	6.5/11	Leukocyte adhesion defect type I	-	-
Rezaei 2007[83]	Iran	18	55.6	8.8	6/11	Severe congenital neutropenia	-	-
Collins 2006[65]	Ireland	18	38.9	14.6	8.5/13	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy	-	-
El-Darouti 2006[88]	Egypt	6	33.3	23.2	2/11	Muckle-Wells syndrome	-	-

Table S1. Study characteristics per study (continued)

	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Moin 2006[6]	Iran	210	58.1	-	5/11	Ataxia-telangiectasia	-	1/62 eczema
						Hyper IgE syndrome unspecified		5/6 eczema
						X-linked agammaglobulinemia		4/23 eczema
						Common variable immunodeficiency		6/28 eczema
						Selective IgA deficiency		5/17 eczema
						Leukocyte adhesion defect		-
						Chronic granulomatous disease		-
						Chronic mucocutaneous candidiasis		-
Eberting 2004[35]	USA	43	37.2	23	5/11	Hyper IgE syndrome unspecified	-	28/43 eczema, 22/43 asthma, 14/43 rhinoconjunctivitis
Haas 2004[90]	Germany	16	43.8	-	6.5/13	Muckle-Wells syndrome	-	-
Markert 2004[48]	USA	5	100	-	9/13	DiGeorge syndrome	-	-
Renner 2004[44]	Germany, USA	13	30.1	-	10/13	Autosomal recessive hyper IgE syndrome	skin abscesses (frequently)	13/13 eczema
Dupuis-Girod 2003[33]	France	55	100	-	5/11	Wiskott-Aldrich syndrome	-	-
Ullbro 2003[84]	Sweden	47	44.7	10 (med)	9/13	Papillon-Lefèvre syndrome	-	-
Chamlin 2002[36]	USA	8	75.0	-	4/11	Hyper IgE syndrome unspecified	4/7 papulopustular eruption	5/8 eczema

Table S1. Study characteristics per study (continued)

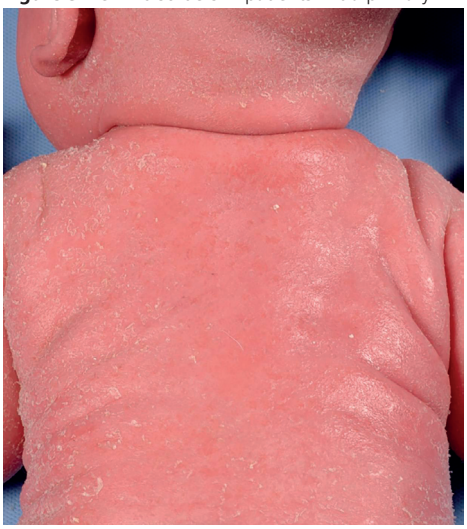
	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Hiel 2001[46]	The Netherlands	55	56.4	-	3/11	Nijmegen breakage syndrome	-	-
Berron-Ruiz 2000[7]	Mexico	130	-	range 0-17	3/11	Severe combined immunodeficiency	-	-
						Ataxia-telangiectasia		-
						Wiskott-Aldrich syndrome		5/5 eczema
						X-linked agammaglobulinemia		-
						Common variable immunodeficiency		-
						Selective IgA deficiency		-
Liese 2000[77]	Germany	39	94.9	-	6/11	Chronic granulomatous disease	-	eczema
Lipsker 2000[91]	France	47	-	-	5/11	C2 deficiency	-	-
Pruszkowski 2000[27]	France	51	47.1	-	5/11	Omenn syndrome	-	-
						Comèl Netherton syndrome		
Winkelstein 2000[79]	USA	368	85.9	-	5/11	Chronic granulomatous disease	-	-
Grimbacher 1999[37]	USA	30	33.3	26.5	5.5/11	Hyper IgE syndrome unspecified	-	30/30 eczema
Koskinen 1996[58]	Finland	159	-	-	8/13	Selective IgA deficiency	-	12/159 eczema, 5/159 food allergy, 17/159 rhinoconjunctivitis
Sullivan 1994[32]	USA	154	100	11	7/11	Wiskott-Aldrich syndrome	-	-
Porter 1993[55]	England	15	86.7	range 3-14	4.5/11	Common variable immunodeficiency	-	4/15 eczema
Porter 1993[57]	England	39	71.8	range 2-13	4.5/11	Selective IgA deficiency	-	2/39 eczema
Patrizi 1992[60]	Italy	142	57.0	6.0	6.5/13	Selective IgA deficiency	-	9/123 eczema

Table S1. Study characteristics per study (continued)

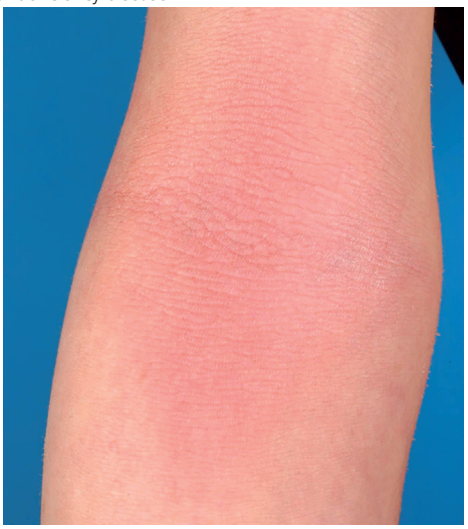
	Country	Patients			IHE Quality Appraisal[a]	Primary immunodeficiency disease	*Staphylococcus aureus*[b]	Atopy[c]
		N	% Male	Mean age (y)				
Paller 1991[31]	USA	8	25.0	10.5	4.5/11	Ataxia-telangiectasia	-	-
Ahonen 1990[66]	Finland	68	54.4	follow-up to age 10 mo-53 y at end of study	10/13	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy	-	-
Charon 1985[38]	USA	27	44.4	20.3	3.5/11	Hyper IgE syndrome unspecified Chronic granulomatous disease	-	-
Cohen 1985[81]	USA	6	66.6	range 17-32	4/11	Chronic granulomatous disease	-	-
Cohen 1984[30]	USA	12	-	-	4.5/11	Ataxia-telangiectasia	-	0/12 atopy
Göring 1981[64]	Germany	8	-	-	3.5/11	Selective IgA deficiency	-	-
Reed 1966[28]	USA	22	63.6	13.4	6/11	Ataxia-telangiectasia	-	2/22 eczema

Abbreviations: N, number of patients; y, year; mo, months; med, median; CVID, common variable immunodeficiency; sIgAD, selective IgA deficiency; IHE, Institute of Health Economics. [a]Prospective studies could reach a maximum score of 13 points and retrospective studies could reach a maximum score of 11 points according to the Institute of Health Economics Quality Appraisal Checklist for Case Series Studies. [b]Number of patients with positive *Staphylococcus aureus* culture / number of patients with reported skin disorder. [c]Number of patients with atopic disorder / number of patients included in the study. [d]Number of patients with atopic disorder / number of patients with a history of cold urticaria (subgroup in original study).

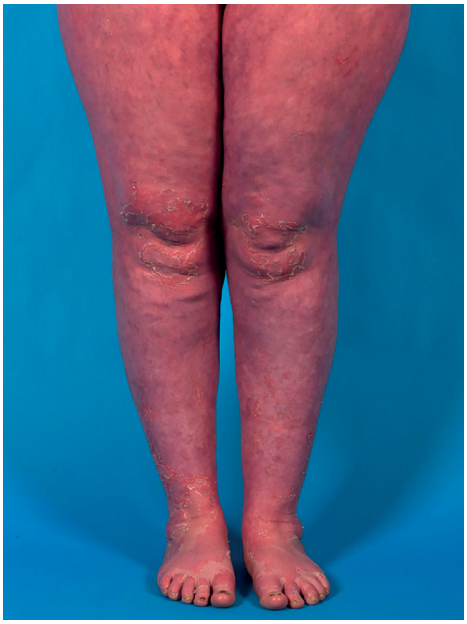
Figure S1. Skin disorders in patients wit a primary immondeficiency disease



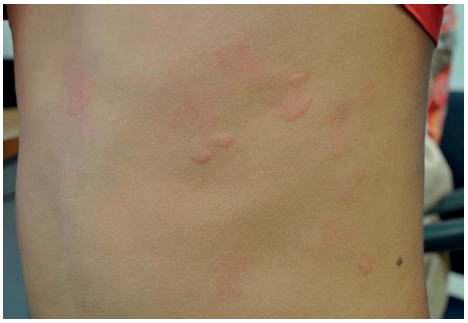
a. Exfoliative erythroderma in Omenn syndrome



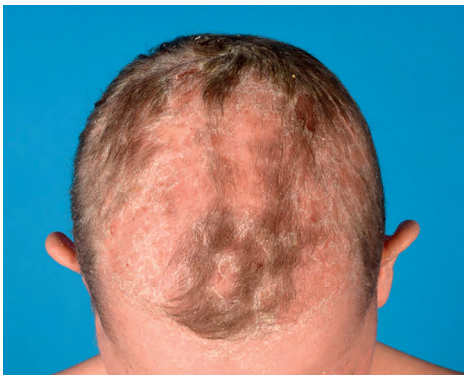
b. Dermatitis in Comèl Netherton syndrome



c. Exfoliative erythroderma in Comèl Netherton syndrome

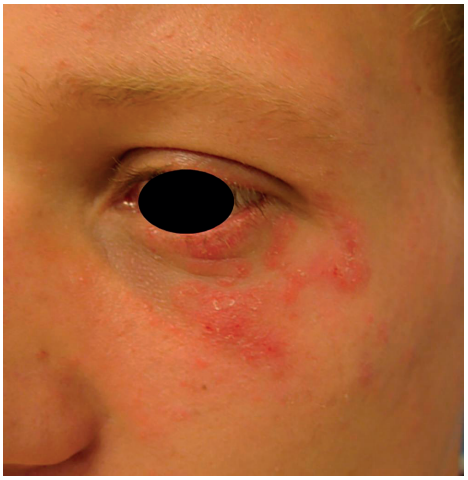


d. Urticaria in common variable immunodeficiency

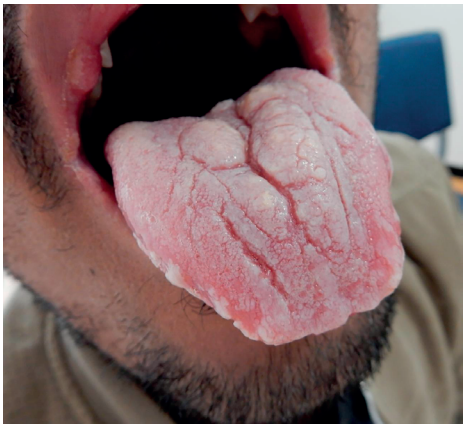


e. Alopecia in immunodysregulation polyendocrinopathy enteropathy X-linked syndrome

f. Onychodystrophy in immunodysregulation polyendocrinopathy enteropathy X-linked syndrome



g. Discoid lupus erythematosus in chronic granulomatous disease



h. Thrush in chronic mucocutaneous candidiasis

Appendix 1. Selected terms of primary immunodeficiency disease and skin disorder used for the electronic search

Primary immunodeficiency disease	Skin disorder
Agammaglobulinemia	Abscess
Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy	Albinism
Ataxia-telangiectasia	Basal cell carcinoma
Chediak-Higashi syndrome	Café-au-lait
Chronic granulomatous disease	Candidiasis
Chronic mucocutaneous candidiasis	Carcinoma
Common variable immunodeficiency	Decubitus
DiGeorge syndrome	Depigmentation disorder
Griscelli syndrome	Dermatitis
Hermansky-Pudlak syndrome	Ecthyma
Hyper IgE syndrome	Eczema
Hyper IgM syndrome	Erythroderma
Hypogammaglobulinemia	Granuloma
Idiopathic CD4+ lymphocytopenia	Hyperkeratosis
IgA deficiency	Hyperpigmentation
IgM deficiency	Hypopigmentation
Interleukin-1 receptor-associated kinase-4 deficiency	Infection
Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome	Lupus erythematosus
Leukocyte adhesion defect	Lymphoma
Comèl-Netherton syndrome	Melanoma
PLCG2 associated antibody deficiency and immune dysregulation	Panniculitis
Severe combined immunodeficiency	Pigment disorder
Transporter-associated-with-antigen deficiency	Pyoderma
Warts, hypogammaglobulinemia, immunodeficiency and myelokathexis syndrome	Small vessel vasculitis
Wiskott-Aldrich syndrome	Squamous cell carcinoma
X-linked agammaglobulinemia	Ulcer
	Verruca
	Vitiligo
	Wart

Appendix 2. Inclusion and exclusion criteria for selecting studies for this review

Types of studies

Inclusion criteria

- Original observational and experimental human studies which assess the presence of skin disorders in patients with a primary immunodeficiency disease.
- Original observational and experimental human studies which report a differential diagnosis of a specific skin disorder that includes a primary immunodeficiency disease.

Exclusion criteria

- Case reports (<5 patients per primary immunodeficiency disease), conference abstracts, letters, editorials and review articles.
- Studies reporting only a genetic mutation (suggestive for a primary immunodeficiency disease) instead of a primary immunodeficiency disease as clinical diagnosis.
- Studies reporting skin disorders in primary immunodeficiency diseases that developed after and/or during treatment or intervention.
- Studies in which the description of skin disorders in primary immunodeficiency diseases was not part of the results section.

Participants

Inclusion criteria

- Patients of all ages with a primary immunodeficiency disease according to Picard et al. both in hospital setting and the general population.[16]

Exclusion criteria

- Patients with an acquired immunodeficiency.

Controls

- No controls.

Outcome measures

- Primary: An overview of the presence of skin disorders described in patients with a PID, in order to compose a differential diagnosis of primary immunodeficiency diseases per skin disorder.
- Secondary: The prevalence of skin disorders in primary immunodeficiency diseases, S. aureus associated skin disorders in primary immunodeficiency diseases and primary immunodeficiency diseases associated with an atopic constitution (i.e. atopic dermatitis, food allergy, asthma, rhinoconjunctivitis).

Appendix 3. Quality assessment score

Institute of Health Economics (IHE) Quality Appraisal Checklist for Case Series Studies

Stars indicate the points allocated if the item criterion is met.[20] A maximum score of 13 can be allocated in each article.

Criteria that are not applicable are excluded.

Study objective

1. **Was the hypothesis/aim/objective of the study clearly stated?**
   Yes: The hypothesis/aim/objective of the study was clearly reported (includes patients, intervention and outcome). ★
   Partial: Only one or two components (patients, intervention, or outcome) were included. ★
   No: The hypothesis/aim/objective was not reported.

Study design

2. **Was the study conducted prospectively?**
   Yes: It was clearly stated that the study was conducted prospectively. ★
   Unclear: Unclear or no information was provided.
   No: The study clearly stated it was a retrospective study.

3. **Were the cases collected in more than one centre?**
   Yes: Cases were collected in more than one centre (multicentre study). ★
   Unclear: Unclear where the patients came from.
   No: Cases were collected from one centre.

4. **Were patients recruited consecutively?**
   Yes: There was a clear statement or it was clear from the context that the patients were recruited consecutively; or the study stated that all eligible patients were recruited. ★
   Unclear: No information was provided about the method used to recruit patients in the study.
   No: The study clearly stated that patients were not recruited consecutively; or the patients were recruited based on other criteria such as access to intervention determined by the distance or availability of resources.

Study population

5. **Were the characteristics of the patients included in the study described?**
   Yes: All of the most relevant characteristics of the patients were reported (for example, number, age, gender, ethnicity, severity of disease/condition, comorbidity, or etiology). ★
   Partial: Some, but not all, of the most relevant characteristics were reported. ½★
   No: Only the number of patients was reported.
   Note: Assessor(s) should decide which aspects are important before using the checklist.

6. **Were the eligibility criteria (i.e. inclusion and exclusion criteria) for entry into the study clearly stated?**
   Yes: Both inclusion and exclusion criteria were reported. ★
   Partial: Either the inclusion or exclusion criteria were reported. ½★
   No: Neither inclusion nor exclusion criteria were reported.
   Note: Assessor(s) should decide which aspects are important before using the checklist.

7. **Did patients enter the study at a similar point in the disease?**
   Yes: It was clear from the baseline data presented in the study (for example, tables of patients' characterises) that the majority (at least 80%) of patients entered the study at a similar point in terms of the duration and severity of the disease/condition and the presence of co-morbidities/complications. ★
   Unclear: There was no baseline information on patients' characteristics to make a judgment.
   No: There was a wide range in the severity of the disease/condition and co-morbidities/complications in patients at baseline.
   Note: Assessor(s) should decide which aspects are important before using the checklist. It might be useful to discuss with specialists to determine the most important aspects that should be considered.

Outcome measures

10. Were relevant outcome measures established a priori?
Yes: All relevant outcome measures were stated in the introduction or methods section. ★
Partial: Some, but not all, of the relevant outcome measures were stated in the introduction or method section. ½★
No: None of the relevant outcome measures were stated in the introduction or method section.

12. Were the relevant outcomes measured using appropriate objective/subjective methods?
Yes: All relevant outcomes were measured with appropriate methods. These measures can be objective (for example, gold standard tests or standardized clinical tests), subjective (for example, self-administered questionnaires, standardized forms, or patient symptoms interview forms), or both. ★
Partial: Some, but not all, relevant outcomes were measured with appropriate methods. ½★
No: The methods used to measure the relevant outcomes were inappropriate.
Note: Assessor(s) should decide which methods are appropriate before using the checklist.

Results and conclusions

15. Was follow-up long enough for important events and outcomes to occur?
Yes: It was clear from the information provided that the follow-up period was long enough for the majority (at least 80%) of patients, to allow for important events and outcomes (for example, changes in clinical status, adverse events) to occur. ★
Unclear: The length of follow-up was not clearly reported.
No: It is clear from the information provided that the follow-up period was not long enough to allow for important events and outcomes to occur.
Note: Assessor(s) should define the appropriate duration of follow-up for each outcome of interest (for example, short-term and long-term adverse events).

16. Were losses to follow-up reported?
Yes: The number or proportion of patients lost to follow-up was clearly reported; the authors reported outcome results on all patients initially included; or the number lost to follow-up can be subtracted from the number of patients enrolled and the number of patients included in the final analysis. ★
Unclear: There was a discrepancy between the number or proportion of patients reported in tables, figures, and text.
No: The number or proportion of patients lost to follow-up was not reported.

19. Were the conclusions of the study supported by the results?
Yes: The conclusions of the study were supported by the evidence presented in the results and discussion sections. ★
Unclear: Unclear conclusion statement that makes it difficult to link the presented evidence to conclusions.
No: The conclusions were not supported by the evidence presented in the results and discussion sections.

Competing interests and sources of support

20. Were both competing interests and sources of support for the study reported?
Yes: Both competing interests and sources of support (financial or other) received for the study were reported; or the absence of any competing interest and source of support was acknowledged. ★
Partial: Either the competing interest or source of support was reported. ½★
No: Neither competing interests nor sources of support were reported.

Chapter 3.1

Skin disorders in primary immunodeficiency diseases: highly prevalent and early presenting clinical features

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ABSTRACT

Background

Skin disorders are common clinical features in primary immunodeficiency diseases (PIDs) and may even precede diagnoses of PIDs for years. Most studies have focused on the prevalence of skin disorders in PIDs in Middle-Eastern countries.

Objective

To determine the prevalence and nature of skin disorders in Dutch children and adults with PIDs compared to partner-controls.

Methods

A partner-controlled questionnaire-based study was performed to evaluate skin disorders in PIDs. In a subsequent observational study in a subgroup of patients with possible *Staphylococcus* (*S.*) *aureus*-related skin disorders we determined the presence of skin disorders by physical examination and *S. aureus* on the lesional skin by culture.

Results

Forty-five children and 207 adults with PIDs, and 56 partner-controls completed the questionnaire. Thirty two (71.1%) children and 166 (80.2%) adults reported a history of skin disorders, compared with 23 (41.1%, $p<0.001$) partner-controls. (Atopic) dermatitis was the most common presenting manifestation in patients and partner-controls. Skin infections and nail disorders were reported more frequently in patients than in partner-controls. Skin disorders in adult patients developed at a mean age of 20.9 (SD 22.0) years; 20.3 (SD 20.5) years before diagnoses of PID, and in partner-controls at 33.7 (SD 26.6) years ($p=0.02$). Skin cultures were positive for *S. aureus* in 40.0% (12/30 cultures, 9/22 adult PID patients).

Conclusion

In PIDs, skin disorders are more prevalent and develop at an earlier age compared with partner-controls. Early recognition of skin disorders in combination with other warning signs could improve earlier diagnosis in PID.

INTRODUCTION

Primary immunodeficiency diseases (PIDs) encompass a heterogeneous group of more than 300 inheritable defects of immunity caused by variants in genes encoding functional proteins of human immune cells.[1,2] The worldwide prevalence of PIDs is estimated at 1 in 2000 live births of which the majority is due to highly consanguineous populations in the Middle-East region.[2,3] PIDs are typically characterized by recurrent and/or severe infections. Additionally, patients may suffer from autoimmunity, autoinflammation, malignancy and allergic disorders.[4-6] A systematic review showed that skin manifestations based on these pathological processes represent an important clinical feature in patients with PIDs.[7] *Staphylococcus* (*S.*) *aureus* induced skin infections were found to be the most common infectious skin disorders reported in PIDs.[8-10] Dermatitis turned out to be one of the most prominent non-infectious skin manifestations in PIDs, in which *S. aureus* has been suggested to play a role in its multifactorial pathogenesis.[11] However, most data originate from Middle-Eastern countries because of their high PID prevalence, and the prevalence and nature of PID-associated skin disorders in the western world remain largely unknown.

The quality of life (QoL) of patients with PIDs is lower for both mental and physical components compared with healthy controls and patients with other chronic diseases. This is mainly caused by the high number of hospitalizations, functional limitations and/or delayed diagnosis.[12-16] Delay in diagnoses and treatment of PIDs can lead to significant morbidity and even mortality.[17] The diagnostic delay of PIDs in the Netherlands ranges from 0 years for T-cell deficiencies, and autoimmune and immune dysregulation syndromes to 14.5 years for defects in innate immunity.[18] As a consequence, these inherited PIDs are diagnosed at a median age of 0 to 19.0 years, respectively.[18] Therefore, warning signs, including occurrence of repetitive (respiratory tract) infections or a family history of PID, have been developed to improve early recognition of an underlying PID.[19]

Skin disorders have been described as prominent clinical feature in PIDs and may even precede the diagnosis of PID. Studies on PID cohorts have demonstrated that skin manifestations preceded and were the basis for 31.8-78.9% of the PID diagnoses.[20,21] In this context, increased attention for skin manifestations as signal function of PIDs in combination with presence of the currently used warning signs of PIDs could improve earlier diagnoses of PIDs.

The aim of this study is to evaluate prevalence and nature of (presenting) skin disorders in a population of Dutch children and adults with a PID compared to partner-controls. Secondly, we assessed the influence of skin disorders on the health-related QoL (HR-QoL) and the prevalence of *S. aureus* on the skin and nose in patients with a skin disorder with possible *S. aureus*-related etiology.

METHODS

Study design

A retrospective adult partner-controlled questionnaire-based study on the prevalence of skin disorders in PIDs and the effect on HR-QoL was followed by a prospective observational clinical study in adult patients suspected of *S. aureus*-related skin disorders to confirm the patient-reported skin disorders by a dermatologist and determine the presence of *S. aureus* on the skin and in the nose. The study was designed and conducted by the department of Dermatology, department of Internal Medicine, division of Clinical Immunology, and department of Pediatrics, division of Infectious Diseases, of the Erasmus MC University Medical Center, Rotterdam, The Netherlands. The study procedures were approved by the institutional review board of the Erasmus MC University Medical Center (MEC-2018-1260 and MEC-2018-1425). All patients aged 16 years or older provided written informed consent themselves. For children below 12 years, both parents or guardians signed, and for children aged 12-16 years both the adolescent and both parents or caregivers signed, in accordance with the Dutch law.

Study population

The questionnaire-based study included patients of all ages with a PID diagnosis according to Picard *et al.*[1]. All patients diagnosed with a PID providing written informed consent are prospectively registered in an ongoing database from the end of 2013 (MEC-2013-026). We selected patients from the database until September 2018. Patients who underwent a curative hematopoietic stem cell transplantation were excluded from this study. Eligible patients or their parent/caregiver (patients <16 years) should have the ability to read and understand the Dutch language. The control group consisted of partners of adult patients who completed the questionnaire and were not deceased in order to correct for environmental factors regardless of genetic influences, which might be involved in development of atopic manifestations in PIDs. Adult patients (≥18 years) who reported to have an active skin disorder with possible *S. aureus*-related etiology, including (atopic) dermatitis, seborrheic dermatitis, nummular eczema, furuncles, impetigo, folliculitis, skin abscesses, erythroderma, cellulitis, perleche, paronychia, and a skin rash with unknown origin, in the questionnaire were eligible for the observational study.

Outcome measurements

The primary outcome of the questionnaire-based study was the self-reported prevalence of skin disorders in children (by parent/caregiver) and adults with PIDs compared to adult partner-controls. Skin disorders evaluated in our cohort included 70 specified skin manifestations, which are frequently reported in PIDs, based on a systematic literature search.[7] Secondary outcomes were the self-reported prevalence of skin disorders with possible *S. aureus*-related etiology, the delay between the first skin disorder and PID diagnosis, and

the influence of skin disorders on the HR-QoL (Appendix 1). In the observational clinical study, skin inspection was performed to confirm the patient-reported skin disorders by a dermatologist. Additionally, the prevalence of *S. aureus* on the lesional skin and nose of patients with an active skin disorder with possible *S. aureus*-related etiology was assessed by semi-quantitative culture.

Study procedures

A questionnaire was sent by mail to 79 pediatric and 360 adult patients with PIDs between October 2017 and September 2018. Adult patients who completed the questionnaire and reported a skin disorder with possible *S. aureus*-related etiology were contacted by telephone to verify the diagnosis. Patients with an active skin disorder at the moment of screening were invited to participate in the observational clinical study. During a subsequently scheduled study visit the skin was systematically inspected by the clinical study physician (JdW and SP) to diagnose and report specific skin disorders. Furthermore, swabs were collected of the lesional skin and nose. Patients with a positive *S. aureus* skin culture were cultured from the same lesional skin location at a second time point, at least two weeks after the first culture, to determine persistent colonization (Appendix 2).

Statistical analysis

The prevalence of skin disorders, diagnostic delay, QoL and presence of *S. aureus* on the skin and in the nose were presented as mean and standard deviation (SD) for normally distributed continuous data or otherwise as median and interquartile range (IQR). The difference in prevalence of skin disorders between adult patients and partner-controls was analyzed using a Chi-Square test or Fisher's Exact test. Basic descriptive statistics and tests were performed using SPSS version 25.0 for windows (IBM Corporation, Armonk, NY). The HR-QoL of pediatric (TAPQOL and Kidscreen-27) and adult (SF-36) patients and partner-controls were compared with adequate reference populations (Appendix 3).[22-24]

RESULTS

Patient characteristics

Questionnaires were completed and returned by 57.4% (45 pediatric and 207 adult) patients (Figure 1). Demographic and disease characteristics were comparable between responders and non-responders, but adult responders had a higher age compared with adult non-responders and more often had a history of skin disorders based on medical records (data not shown). Median age of the included patients was 46.3 (IQR 23.5-61.2) years; children had a median age of 11.2 (IQR 7.1-15.5) years and adults of 53.2 (IQR 37.1-64.6) years. One hundred and twenty (46.9%) patients were male and 248 (98.4%)

were of Caucasian race. The majority of patients (86.6%) had a predominant antibody deficiency (PAD) according to the 2017 international union of immunological societies (IUIS) phenotypic classification for primary immunodeficiencies (Table 1).[25] Mean age at time of diagnosis of the PID was 4.5 (SD 4.2) years in pediatric patients and 41.8 (SD 20.0) years in adult patients. The first classical PID symptom preceded the PID diagnosis 3.2 (SD 3.7) and 15.9 (SD 17.7) years, respectively. Classical symptoms were present at a mean age of 1.4 (SD 2.2) years in children and 25.3 (SD 22.8) years in adults and included mainly upper and lower respiratory tract infections (46.7% and 58.0%, respectively).

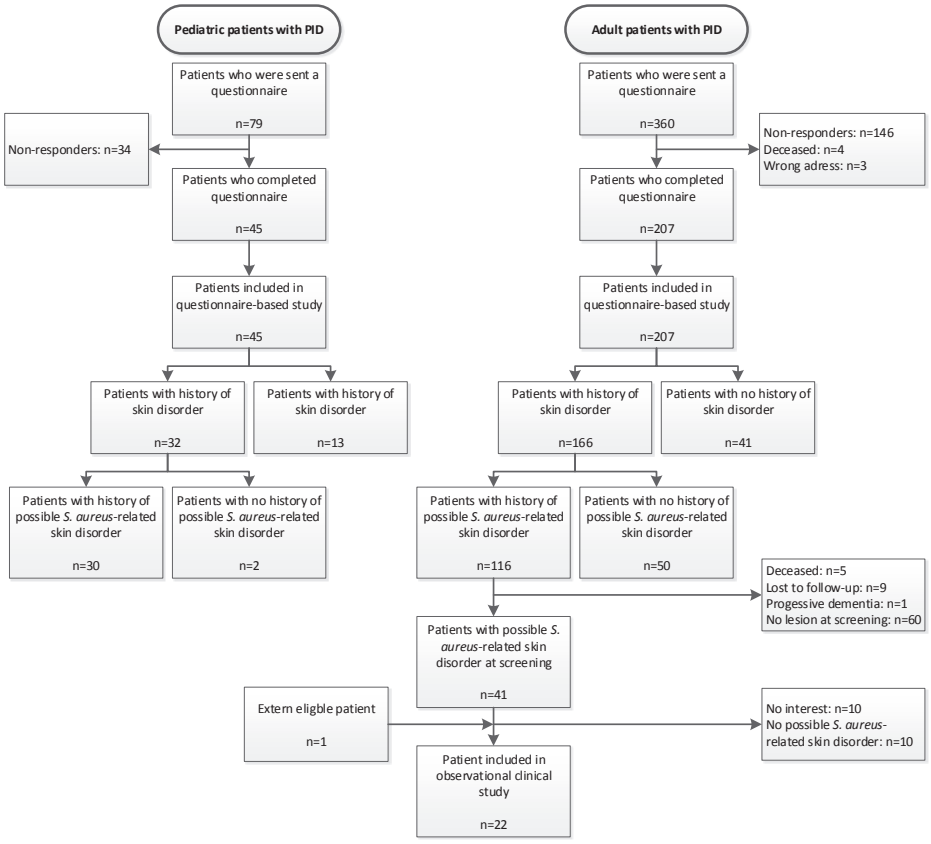

Figure 1. Flowchart of the study design

Partner-control characteristics

Five patients with PIDs died after completing the questionnaire. Therefore, 202 partners of adult patients were sent a questionnaire. A total of 56 (27.7%) questionnaires of partner-controls were completed and included in this study. Partner-controls had a median age of 59.3 (IQR 46.2-69.8) years and 34 (60.7%) were male.

Table 1. General patient demographics

	Questionnaire-based study			Observational clinical study
	Pediatric patients (n=45)	**Adult patients (n=207)**	**Adult partner-controls (n=56)**	**Adult patients (n=22)**
Age				
median (IQR)	11.2 (7.1-15.5)	53.2 (37.1-64.6)	59.3 (46.2-69.8)[1]	48.0 (43.5-56.8)
Sex, male				
n (%)	32 (71.1)	83 (40.1)	34 (60.7)	9 (40.9)
Race, n (%)			Not available	
White	44 (97.8)	204 (98.6)		22 (100)
Black or African American	0 (0)	0 (0)		0 (0)
Asian	1 (2.2)	0 (0)		0 (0)
American Indian or Alaska Native	0 (0)	3 (1.4)		0 (0)
Native Hawaiian or Other Pacific Islander	0 (0)	0 (0)		0 (0)
Unknown	0 (0)	0 (0)		0 (0)
Age PID diagnosis, years			Not applicable	
mean (SD)	4.5 (4.2)[2]	41.8 (20.0)[3]		36.6 (17.0)[4]
IUIS phenotypic classification of PID, n (%)			Not applicable	
Immunodeficiencies affecting cellular and humoral immunity	2 (4.4)	1 (0.5)		0 (0)
CID with associated syndromic features	1 (2.2)	7 (3.4)		1 (4.5)
Predominantly antibody deficiencies	33 (73.3)	182 (87.9)		21 (95.5)
- Common variable immunodeficiency	7 (15.6)	74 (35.7)		8 (36.4)
- IgG subclass deficiency	2 (4.4)	34 (16.4)		7 (31.8)
- Selective IgA deficiency	1 (2.2)	5 (2.4)		0 (0)
- Selective antibody deficiency with normal immunoglobulins	0 (0)	27 (13.0)		2 (9.1)
- X-linked agammaglobulinemia	4 (8.9)	7 (3.4)		2 (9.1)
- Hypogammaglobulinemia	16 (35.6)	23 (11.1)		2 (9.1)
- Hyper IgM syndrome	0 (0)	3 (1.4)		0 (0)
- Combined antibody deficiency	0 (0)	7 (3.4)		0 (0)
- Other	4 (8.9)	2 (1.0)		0 (0)
Diseases of immune dysregulation	0 (0)	1 (0.5)		0 (0)
Congenital defects of phagocyte number, function or both	3 (6.7)	2 (1.0)		0 (0)
Defects in intrinsic and innate immunity	1 (2.2)	5 (2.4)		0 (0)
Auto-inflammatory disorders	2 (4.4)	4 (1.9)		0 (0)
Complement deficiencies	0 (0)	1 (0.5)		0 (0)
Phenocopies of PID	0 (0)	1 (0.5)		0 (0)
Unknown	3 (6.7)	3 (1.4)		0 (0)

Abbreviations: CID, combined immunodeficiency disease; IUIS, International Union of Immunological Societies; IQR, interquartile range; n, number; PID, primary immunodeficiency disease. Missings: [1]n=3 (5.4%), [2]n=1 (2.2%), [3]n=9 (4.3%), [4]n=1 (4.5%).

Skin disorders

Children with primary immunodeficiency disease

Thirty-two (71.1%) pediatric patients reported a history, i.e. lifetime prevalence, of one or more skin disorders, of which 96.9% comprised a history of at least one skin disorder with possible *S. aureus*-related etiology. In general, (atopic) dermatitis (48.9%), varicella zoster virus infection (46.7%) and oral ulcers (35.6%) were the most common skin manifestations (Table S1). Children reported their first skin disorder at a mean age of 1.5 (SD 3.0) years; 3.5 (SD 5.4) years before the PID diagnosis. (Atopic) dermatitis was the most prevalent presenting skin disorder in 17 (37.8%) patients reporting a history of skin disorders.

Adults with primary immunodeficiency disease and partner-controls

A history of one or more skin disorders was reported by 166 (80.2%) adult patients and 23 (41.1%, p <0.001) partner-controls, of which, respectively, 74.1% and 78.3% (p=0.67) included a history of at least one skin disorder with possible *S. aureus*-related etiology. In general, the most frequently noted skin disorders in patients were (atopic) dermatitis (29.5%), oral ulcers (22.7%) and warts (21.7%) (Table S1). (Atopic) dermatitis (25.0%) was most prevalent in partner-controls. Other skin disorders were reported in four or less (≤7.1%) partner-controls (Table S1). Skin infections and nail disorders, including paronychia and onychomycosis, were significantly more prevalent in adult patients compared with partner-controls (p=0.001 and p=0.005, respectively) (Table 2). Patients reported the first skin disorder at a mean age of 20.9 (SD 22.0) years; 20.3 (SD 20.5) years before the PID diagnosis. Partner-controls reported their first skin disorders at a mean age of 33.7 (SD 26.6) years (p=0.02). In adult participants reporting a history of skin disorders, (atopic) dermatitis was the most prevalent first developed skin disorder (15.0% patients and 52.2% partner-controls).

Quality of life

Children with primary immunodeficiency disease

Pediatric patients of all age categories reported a noticeably lower HR-QoL compared with norm data for the KIDSCREEN-27 dimension physical well-being (Table 3). On the other hand, the dimensions autonomy and parents, and social support and peers scored a noticeable better QoL based on the outcomes of the KIDSCREEN-27 proxy questionnaire for children 8-11 years. Children showed a good skin-related QoL (SR-QoL) with a median CDLQI of 1.5 (IQR 0.0-4.0) and IDQOL of 2.0 (Table 3). The influence of the skin disorder on the HR-QoL was limited (median NRS 1.0 (IQR 0.0-6.0)).

Table 2. Skin disorders in primary immunodeficiency diseases

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)	*p*-value[†]
Dermatitis-like lesions, n (%)	24 (53.3)	97 (46.9)	17 (30.4)	0.155
(Atopic) dermatitis	22 (48.9)	61 (29.5)	14 (25.0)[2]	
Seborrheic dermatitis	7 (15.6)	27 (13.0)	1 (1.8)[2]	
Nummular eczema	8 (17.8)	22 (10.6)	1 (1.8)[2]	
Hair abnormalities, n (%)	5 (11.1)	55 (26.6)	5 (8.9)[2]	0.325
Hair loss disorders	3 (6.7)	45 (21.7)	4 (7.1)[2]	
Excessive hair growth disorders	1 (2.2)	15 (7.2)	2 (3.6)[2]	
Hair pigmentation disorders	0 (0.0)	13 (6.3)	0 (0.0)[2]	
Other hair abnormalities	0 (0.0)	1 (0.5)	0 (0.0)[2]	
Skin infections, n (%)	29 (64.4)	127 (61.4)	10 (17.9)	0.001**
Fungal skin infections	11 (24.4)	62 (30.0)	5 (8.9)	
Viral skin infections	24 (53.3)	84 (40.6)	3 (5.4)	
Bacterial skin infections	15 (33.3)	66 (31.9)	1 (1.8)	
Abscesses	4 (8.9)	25 (12.1)	0 (0.0)	
Impetigo	7 (15.6)	12 (5.8)	0 (0.0)	
Folliculitis	3 (6.7)	38 (18.4)	1 (1.8)	
Cellulitis	0 (0.0)	8 (3.9)	0 (0.0)	
Furuncle	3 (6.7)	28 (13.5)	0 (0.0)	
Other skin infections	8 (17.8)	38 (18.4)	1 (1.8)	
Perleche	8 (17.8)	38 (18.4)	1 (1.8)	
Ulcers, n (%)	16 (35.6)	53 (25.6)	2 (3.6)	0.022
Oral ulcers	16 (35.6)	47 (22.7)	2 (3.6)	
Nose ulcers	2 (4.4)	14 (6.8)	0 (0.0)	
Other ulcers	0 (0.0)	3 (1.4)	0 (0.0)	
Erythematous skin lesions, n (%)	5 (11.1)	45 (21.7)	2 (3.6)	0.056
Erythroderma	1 (2.2)	11 (5.3)	1 (1.8)	
Vascular disorders, n (%)	7 (15.6)	50 (24.2)	2 (3.6)	0.031
Telangiectasia	3 (6.7)	32 (15.5)	2 (3.6)	
Vasculitis	2 (4.4)	9 (4.3)	0 (0.0)	
Petechia/purpura	2 (4.4)	12 (5.8)	0 (0.0)	
Other vascular disorders	0 (0.0)	7 (3.4)	1 (1.8)	
Pigmentation disorders, n (%)	15 (33.3)	73 (35.3)	4 (7.1)	0.015
Hyperpigmentation disorders	13 (28.9)	45 (21.7)	3 (5.4)	
Hypopigmentation disorders	4 (8.9)	25 (12.1)	1 (1.8)	
Other pigmentation disorders	7 (15.6)	22 (10.6)	0 (0.0)	
Neoplastic disorders, n (%)	0 (0.0)	20 (9.7)	3 (5.4)	1.000
Rash, n (%)	24 (53.3)	57 (27.5)	4 (7.1)	0.103
Papulosquamous rash	3 (6.7)	9 (4.3)	1 (1.8)	

Table 2. Skin disorders in primary immunodeficiency diseases (continued)

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)	*p*-value[†]
Maculopapular rash	11 (24.4)	15 (7.2)	0 (0.0)	
Papulopustular rash	6 (13.3)	15 (7.2)	1 (1.8)	
Eczematous rash	14 (31.1)	32 (15.5)	2 (3.6)	
Nail disorders, n (%)	12 (26.7)	80 (38.6)	4 (7.1)	0.005*
Non-infectious nail disorders	9 (20.0)	44 (21.3)	3 (5.4)	
Infectious nail disorders	5 (11.1)	61 (29.5)	2 (3.6)	
Paronychia	3 (6.7)	29 (14.0)	0 (0.0)	
Other nail disorders	0 (0.0)	1 (0.5)	0 (0.0)	
Psoriasis-like lesions, n (%)	1 (2.2)	20 (9.7)	1 (1.8)	0.479
Granulomatous disorders, n (%)	1 (2.2)	1 (0.5)	0 (0.0)	1.000
Acne-like lesions, n (%)	5 (11.1)	34 (16.4)	3 (5.4)	0.577
Urticaria, n (%)	6 (13.3)	29 (14.0)	0 (0.0)	0.028
Other skin disorders, n (%)	6 (13.3)	19 (9.2)	0 (0.0)	0.136

[†]Difference in prevalence of 14 main groups of skin disorders between adult patients and partner controls. *Significant after correction for multiple testing using the false discovery rate method (p=0.007). **Significant after correction for multiple testing using the Bonferroni method (p=0.003).

Table 3. Quality of life in pediatric patients with a primary immunodeficiency disease

	Pediatric patients	Range norm data[‡]
Health-related quality of life[†]		
KIDSCREEN-27 self report adolescents 12-18[a] (range 0-100), mean (SD), n=15		
Physical Well-being	41.47 (6.57)[1]*	43.84-53.30
Psychological	37.87 (2.76)*	43.94-53.72
Autonomy & Parents	52.07 (4.31)[2]	44.50-54.32
Social Support & Peers	49.02 (13.05)	44.64-54.60
School Environment	50.67 (7.72)[3]	43.73-53.15
KIDSCREEN-27 proxy children 8-11[b] (range 0-100), mean (SD), n=23		
Physical Well-being	44.57 (8.67)[4]*	47.90-57.40
Psychological	52.99 (10.90)[4]	46.93-56.51
Autonomy & Parents	59.21 (11.33)[5]**	45.93-55.59
Social Support & Peers	57.97 (9.39)[6]**	45.74-55.14
School Environment	54.42 (9.36)[6]	48.02-57.88
KIDSCREEN-27 proxy adolescents 12-18[c] (range 0-100), mean (SD), n=2		
Physical Well-being	25.22 (22.44)*	43.60-53.60
Psychological	42.08[7]*	44.05-54.15
Autonomy & Parents	45.47[7]	44.55-54.71
Social Support & Peers	49.14[7]	44.62-54.92
School Environment	35.35[7]*	43.60-53.34

Table 3. Quality of life in pediatric patients with a primary immunodeficiency disease (continued)

	Pediatric patients	Range norm data‡
TNO-AZL Preschool Children's Quality of Life (range 0-100), mean (SD), n=4		
Stomach problems	75.00 (35.36)[8]*	84.95-98.77
Skin problems	100 (0.00)[8]**	86.37-97.17
Lung problems	91.67 (16.67)	85.50-100
Sleeping problems	75.00 (19.76)	73.69-90.95
Appetite	75.00 (21.52)*	78.02-91.20
Liveliness	100 (0.00)	93.97-100
Positive mood	95.83 (8.33)	95.46-100
Problem behavior	58.93 (22.11)*	60.03-75.35
Anxiety	87.50 (25.00)**	69.33-87.33
TNO-AZL Preschool Children's Quality of Life extended version (range 0-100), mean (SD), n=3		
Social functioning	58.33 (58.93)[9]*	83.64-99.00
Motor function	75.00 (18.75)*	96.27-100
Communication	93.75 (8.49)[9]	86.72-96.60
Skin-related quality of life		
Children's Dermatology Life Quality Index (range 0-30), median (IQR), n=41	1.5 (0.0-4.0)[10]	Not available
Symptoms and feelings, median % (IQR)	16.7 (0.0-16.7)[11]	
Leisure, median % (IQR)	0.0 (0.0-0.0)[10]	
School or holidays, median % (IQR)	0.0 (0.0-33.3)[11]	
Personal relationships, median % (IQR)	0.0 (0.0-0.0)[12]	
Sleep, median % (IQR)	0.0 (0.0-0.0)[11]	
Treatment, median % (IQR)	0.0 (0.0-0.0)[10]	
Infant's Dermatitis Quality of Life Index (range 0-30), median (IQR), n=4	2.0[13]	Not available

Missing: [1]n=1 (6.7%), [2]n=3 (20.0%), [3]n=4 (26.7%), [4]n=3 (13.0%), [5]n=5 (21.7%), [6]n=1 (4.3%), [7]n=1 (50.0%), [8]n=2 (50.0%), [9]n=1 (33.3%), [10]n=3 (7.3%), [11]n=2 (4.9%), [12]n=4 (9.8%), [13]n=2 (50.0%). Note: 2 (4.9%) of the pediatric patients >3 years (n=41) did not completed the KIDSCREEN-27 questionnaire. †High scores indicate a good quality of life, low scores indicate a low quality of life. ‡Norm data thresholds for the TNO-AZL Preschool Children's Quality of Life questionnaires we were calculated using reference data of 340 Dutch children and for the KIDSCREEN-27 questionnaires using international T-values based on Rasch person parameter extracted from the KIDSCREEN questionnaires handbook, and fixed at a value of the mean minus half and plus half a standard deviation to mean.[22,24] A study population mean lower or higher than the norm data thresholds indicate a noticeable lower or better QoL, respectively, compared to the reference population. *Noticeable lower health-related quality of life compared to norm data. **Noticeable better health-related quality of life compared to the norm data. [a]Norm data based on "European Normdata KIDSCREEN females & males Adolescents 12-18".[22] [b]Norm data based on "European Normdata proxy KIDSCREEN females & males Children 8-11" .[22] [c]Norm data based on "European Normdata proxy KIDSCREEN females & males Adolescents 12-18".[22]

Adults with primary immunodeficiency disease and partner-controls

The HR-QoL of adult patients was significantly lower as compared with norm data for most of the SF-36 scales, except for emotional well-being (Table 4). The HR-QoL of partner-controls was comparable to norm data. Patients had a good SR-QoL with a median DLQI of 1.0 (IQR 0.0-3.0) (Table 4). Skin disorders had almost no influence on the HR-QoL (median NRS 1.0 (IQR 0.0-4.0)). All partner-controls had a good SR-QoL (median 0.0 (IQR 0.0-0.0)) and skin disorders had no influence on the HR-QoL (median NRS 0.0 (IQR 0.0-0.0)).

Table 4. Quality of life in adult patients with a primary immunodeficiency disease and partner-controls

	Adult patients n=207	Adult partner-controls n=56	Range norm data‡
Health-related quality of life			
Short Form 36 (range 0-100)†, mean (SD)			
Physical functioning	62.90 (30.57)[1]*	86.27 (21.09)[2]	70.30-93.50
Social functioning	59.70 (35.27)[1]*	94.58 (25.77)[3]	76.65-97.15
Role limitations due to physical health	42.08 (45.02)[4]*	79.63 (37.33)[2]	61.65-97.15
Role limitations due to emotional problems	65.82 (43.57)[5]*	88.68 (29.19)[3]	67.95-100
Emotional well-being	72.04 (18.89)[1]	76.07 (18.28)[6]	67.60-86.00
Energy/fatigue	49.88 (22.86)[1]*	71.39 (22.29)[6]	57.45-77.25
Pain	56.57 (44.53)[1]*	88.06 (21.32)[3]	66.70-92.30
General health	27.28 (18.58)[7]*	69.09 (20.69)[2]	61.35-84.05
Skin-related quality of life			
Dermatology Life Quality Index (range 0-30)†, median (IQR)	1.0 (0.0-3.0)[8]	0.0 (0.0-0.0)	Not available
Symptoms and feelings, median % (IQR)	0.0 (0.0-33.3)[8]	0.0 (0.0-0.0)	
Daily activities, median % (IQR)	0.0 (0.0-0.0)[1]	0.0 (0.0-0.0)	
Leisure, median % (IQR)	0.0 (0.0-0.0)[1]	0.0 (0.0-0.0)	
Work and school, median % (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	
Personal relationships, median % (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	
Treatment, median % (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	

Missing: [1]n=2 (1.0%), [2]n=1 (1.8%), [3]n=3 (5.4%), [4]n=5 (2.4%), [5]n=10 (4.8%), [6]n=3 (3.6%), [7]n=7 (3.4%), [8]n=3 (1.4%). †High scores indicate a good quality of life, low scores indicate a low quality of life. ‡Norm data thresholds for the Short Form 36 questionnaire were calculated using reference data of 1063 Dutch adults (mean age 44.1 years, age range 18-89 years, 65% female) and fixed at a value of the mean minus half and plus half a standard deviation.[23] A study population (adult patients or adult partner-controls) mean lower or higher than the norm data thresholds indicate a significantly lower or better QoL, respectively, compared to the reference population. *Significantly lower health-related quality of life compared to norm data.

Observational clinical study

Twenty-two adult patients were included in the observational clinical study (Figure 1 and Table 1). Most patients (95.5%) were diagnosed with a PAD. Dermatitis (57.1%), folliculitis (31.8%) and rosacea (22.7%) were the most prevalent skin disorders during physical examination (Table 5). Twenty-five (43.9%) of the 57 clinically observed skin disorders were also reported by the patients. Observed skin disorders were not specific to each of the PID diagnoses. Thirty skin lesions with possible *S. aureus*-related etiology were cultured, of which 40.0% (10 patients) were positive for *S. aureus*. Additionally, 50.0% of the 22 nose cultures were positive for *S. aureus.* After a median of 4.1 (IQR 3.1-6.0) weeks, 69.2% (7 patients) of the 12 skin cultures were still positive for *S. aureus* at the second time point.

Table 5. Case series of adult patients with a primary immunodeficiency disease and a *Staphylococcus aureus*-related skin disorder

Study number	Sex	Age, years	PID diagnosis	Reported history of skin disorders in questionnaire	Observed skin disorder	Semiquantitative *S. aureus* culture
01	Female	45	IgG_1 subclass deficiency	(Atopic) dermatitis*, seborrheic dermatitis*, hair loss scalp, papulosquamous rash*, maculopapular rash*, eczematous rash*, red spots	1. Seborrheic dermatitis scalp and face* 2. (Atypical) naevi back and mamma left 3. Xerosis cutis	Skin 1. Glabella – Nose –
02	Female	44	CVID	(Atopic) dermatitis*, seborrheic dermatitis*, oral herpes simplex infection, genital herpes simplex infection, oral ulcers, cafe au lait macula	1. Seborrheic dermatitis scalp and nasolabial* 2. Hyperpigmented macula flank left	Skin 1. Nasolabial left ++ Nose +
03	Female	58	IgG_1 and IgG_2 subclass deficiency	Dermatitis herpetiformis, hair loss scalp, eyelashes and eyebrows, hirsutism, oral candidiasis, genital candidiasis, abscesses*, folliculitis*, red spots, telangiectasia on the face, squamous cell carcinoma, maculopapular rash*, paronychia*	1. Erythrasma inguinal 2. Folliculitis upper leg left 3. Dermatitis lower arm left 4. Dermatitis/ intertrigo inframammary left	Skin 1. Inguinal right + 2. Upper leg left – 3. Lower arm left +++ Nose +
04	Male	46	SADNI	(Atopic) dermatitis*, hair loss eyelashes and eyebrows, oral candidiasis, genital candidiasis, dermal candidiasis, folliculitis*, furuncle*, perleche, nose ulcers, telangiectasia on shoulder, back or neck, cafe au lait macula, naevus > 5mm, freckles, neonatal skin rash, maculopapular rash*, papulopustular rash*, acne vulgaris	1. Erythrasma/ folliculitis inguinal left 2. Folliculitis chest, beard, pubis 3. Seborrheic dermatitis scalp 4. (Atypical) naevus back 5. Xerosis cutis	Skin 1. Inguinal left – 2. Chest left – Nose –
05	Male	50	Hyper IgE syndrome	Unknown	1. Dermatitis frontal 2. Dermatitis/ folliculitis coeur, abdominal, back, legs 3. Onychomycosis toe nails	Skin 1. Frontal – 2. Back - (*S. pin* +) Nose - (*S. pin* ++)

Table 5. Case series of adult patients with a primary immunodeficiency disease and a *Staphylococcus aureus*-related skin disorder (continued)

Study number	Sex	Age, years	PID diagnosis	Reported history of skin disorders in questionnaire	Observed skin disorder	Semiquantitative *S. aureus* culture
06	Male	42	CVID	Hypertrichosis, folliculitis*, cellulitis*, furuncle*, naevus flammeus, naevus >5mm, papulopustular rash*, onychomycosis, acne vulgaris	1. Psoriasis guttata/ dermatitis/ dermatomycosis flank left, back, arms, feet left 2. Rosacea papulopustulosa check 3. Folliculitis coeur, legs 4. (Atypical) naevi back 5. Naevi papillomatosus scalp	Skin 1. Flank right – (*S. cap* +) 2. Cheek left – Nose –
07	Female	64	IgG_2 and IgG_3 subclass deficiency	Seborrheic dermatitis*, nummular eczema*, dermatitis herpetiformis, hair loss scalp, hirsutism, bamboo hair, oral candidiasis, genital candidiasis, dermal candidiasis, varicella zoster virus infection, herpes zoster virus infection, warts, mollusca contagiosum, folliculitis*, oral ulcers, red spots, erythema nodosum, telangiectasia on eyes, cheek, nose, ears and chest, oral purpura/petechiae, hyperpigmented lesions, vitiligo, freckles, papulopustular rash*, maculopapular rash*, papulopustular rash*, eczematous rash*, onychomycosis, acute urticaria	1. Rosacea (telangiectasia/ papulopustulosa)/ folliculitis face	Skin 1. Temporal left – Nose +++
08	Female	57	CVID	Nummular eczema*, hair loss eyelashes, silver hair color, genital candidiasis, abscesses*, perleche*, telangiectasia on nose, hematomas, freckles, eczematous rash*, psoriasis, acne vulgaris, acute urticaria	1. Rosacea telangiectasia/ dermatitis periorbital 2. Furuncle	Skin 1. Periorbital left – 2. Back – Nose –
09	Female	46	Hypogamma-globulinemia	(Atopic) dermatitis*, seborrheic dermatitis*, nummular eczema*, dermatitis herpetiformis, oral candidiasis, genital candidiasis, dermal candidiasis, herpes zoster virus infection, oral herpes simplex virus infection, warts, oral ulcers, erythroderma, red spots, rosacea, telangiectasia on cheek or nose, brittle nails	1. Rosacea papulopustulosa face 2. Verruca vulgaris lower arm left 3. Dermatofibroma upper leg left	Skin 1. Cheek left + Nose –

Table 5. Case series of adult patients with a primary immunodeficiency disease and a *Staphylococcus aureus*-related skin disorder (continued)

Study number	Sex	Age, years	PID diagnosis	Reported history of skin disorders in questionnaire	Observed skin disorder	Semiquantitative *S. aureus* culture
10	Male	57	CVID	(Atopic) dermatitis*, hair loss scalp, perleche*, neonatal rash, eczematous rash*, acne vulgaris, acute urticaria	1. Dermatitis lower legs 2. Superficial basal cell carcinoma chest 3. Blue naevus hand right 4. Dermatofibroma upper arm and upper leg right	Skin 1. Tibia right – Nose –
11	Male	33	CVID	Folliculitis*, acne vulgaris	1. Folliculitis/ acne vulgaris scalp, neck, coeur, back, abdominal	Skin 1. Upper back – Nose ++
12	Female	35	IgG_4 subclass deficiency	Dermatitis herpetiformis, hair loss scalp, genital candidiasis, impetigo*, vitiligo, maculopapular rash*, papulopustular rash*	1. Ecthyma mamma right	Skin 1. Mamma right +++ Nose +
13	Female	58	IgG_1 and IgG_3 subclass deficiency	(Atopic) dermatitis*, seborrheic dermatitis*, nummular eczema*, dermatitis herpetiformis*, oral candidiasis, genital candidiasis, dermal candidiasis, varicella zoster virus infection, warts, abscesses*, folliculitis*, furuncle*, perleche*, oral ulcers, nasal ulcers, erythroderma*, red spots, erythema induratum, erythema nodosum, telangiectasia on legs, purpura/petechiae on arms and legs, naevus > 5mm, vitiligo, hypopigmented lesions, freckles, papulosquamous rash*, maculopapular rash*, papulopustular rash*, eczematous rash*, color change nails, paronychia*, onychomycosis, acne vulgaris, acute urticaria	1. Dermatitis of hands, feet and scalp	Skin 1. Palmar side hand right – Nose –
14	Female	36	SADNI	(Atopic) dermatitis*, hair loss scalp, eyelashes and eyebrows, dermal candidiasis, red spots, eczematous rash*, acute urticaria	1. Dermatitis ears 2. Alopecia areata of scalp	Skin 1. Ear right ++ 1. Ear left ++ Nose ++

Table 5. Case series of adult patients with a primary immunodeficiency disease and a *Staphylococcus aureus*-related skin disorder (continued)

Study number	Sex	Age, years	PID diagnosis	Reported history of skin disorders in questionnaire	Observed skin disorder	Semiquantitative *S. aureus* culture
15	Female	49	IgG_1 subclass deficiency	Folliculitis*, furuncle*, perleche*, oral ulcers, papulopustular rash*, onychomycosis, acne vulgaris	1. Dermatitis dorsal side hands and feet 2. Onychomycosis toe nails 3. Verruca vulgaris knee right	Skin 1. Dorsal side hand right ++ Nose –
16	Male	52	X-linked agamma-globulinemia	Warts, furuncle*, oral ulcers	1. Rosacea papulopustulosa face 2. Folliculitis abdominal 3. Orthostatic dermatitis legs	Skin 1. Nose – Nose –
17	Male	23	X-linked agamma-globulinemia	(Atopic) dermatitis*, seborrheic dermatitis*, hair loss scalp, oral candidiasis, genital candidiasis, dermal candidiasis, herpes zoster virus infection, impetigo*, folliculitis*, perleche*, erythroderma*, red spots, vitiligo, freckles, papulosquamous rash*, maculopapular rash*, papulopustular rash*, eczematous rash*, loss of toe nails, paronychia*	1. Erythroderma/ dermatitis 2. Folliculitis lower legs	Skin 1. Hand left ++++ 2. Lower leg right ++ 2. Lower leg left ++ Nose +++
18	Female	68	IgG_2 subclass deficiency	Seborrheic dermatitis*, nummular dermatitis*, red spots, eczematous rash*	1. Dermatitis face 2. Actinic keratosis frontal, lower arms, dorsal side of hands 3. Xerosis cutis	Skin 1. Frontal ++ Nose +++
19	Male	50	CVID	Skin infection tibia and inguinal*, hyperpigmented lesions, paronychia*	1. Dermatitis face 2. Postinflammatory hypo- and hyperpigmentation lower legs 3. Atrophy blanche ankles	Skin 1. Frontal – Nose ++++
20	Female	65	Hypogamma-globulinemia	(Atopic) dermatitis*, seborrheic dermatitis*, nummular eczema*, red spots, purpura/petechiae on feet, eczematous rash*, onychomycosis, loss of toe nails	1. Dermatitis scalp and face 2. Orthostatic changes legs	Skin 1. Frontal + Nose +++

Table 5. Case series of adult patients with a primary immunodeficiency disease and a *Staphylococcus aureus*-related skin disorder (continued)

Study number	Sex	Age, years	PID diagnosis	Reported history of skin disorders in questionnaire	Observed skin disorder	Semiquantitative *S. aureus* culture
21	Male	38	CVID	Abscesses*, impetigo*, telangiectasia chest, acne vulgaris	1. Folliculitis upper legs 2. Dermatitis neck and face	Skin 1. Upper leg right ++ 2. Neck left +++ Nose ++++
22	Female	29	CVID	Eczematous rash*	1. Seborrheic dermatitis scalp and face 2. Keratosis pilaris upper arms and legs	Skin 1. Frontal – Nose –

Abbreviations: CVID, common variable immunodeficiency disease; Ig, immunoglobulin; *S. cap*, *Staphylococcus capitis*; *S. pin*, *Staphylococcus pseudointermedius*; SADNI, selective antibody deficiency with normal immunoglobulins. * Skin disorders with possible *Staphylococcus aureus*-related etiology.

DISCUSSION

This study demonstrates that skin disorders, including skin infections and nail disorders, are more prevalent in children (71.1%) and adults (80.2%) with PIDs as compared with adult partner-controls (41.1%, $p<0.001$). The first skin disorder, of which (atopic) dermatitis was most commonly reported, developed 3.5 (SD 5.4) years in children and 20.3 (SD 20.5) years in adults before the PID diagnosis.

This is the first study evaluating the nature and prevalence of skin disorders in a mainly Caucasian population of both pediatric and adult patients with PIDs, of which the majority had a PAD. Our findings regarding the prevalence of skin disorders in PIDs are in accordance with a systematic review on skin manifestations in PIDs, which included mainly data from Middle-Eastern countries.[7] However, our population reported more often viral and bacterial skin infections, erythematous skin lesions and skin rashes.

Surprisingly, current literature appointed eczematous dermatitis as common finding and presenting clinical manifestation among PIDs.[26] Although a high frequency of (atopic) dermatitis was reported in PID patients included in this study, the prevalence in adult patients was not significantly different from the prevalence in partner-controls (29.5% vs. 25.0%, respectively) and even less adult patients reported (atopic) dermatitis as first developed skin disorder compared with partner-controls (15.0% vs. 52.2%, respectively). Moreover, the prevalence of a history of (atopic) dermatitis in both adult patients and partner-controls included in this study corresponds to the lifetime prevalence of atopic dermatitis in the Dutch population, which is up to 25% in children and 1-7% in adults.[27,28] Therefore, we feel that (atopic) dermatitis is not a specific skin condition related to PIDs and hypothesize that (a combination of) other skin disorders, like skin infections and nail disorders, are more useful in recognizing a possible underlying PID, additional to the presence of warning signs for PIDs.[19]

Recognition of PID-associated skin disorders might shorten the diagnostic delay of PIDs, which is 3.2 (SD 3.7) years in pediatric and 15.9 (SD 17.7) years in adult patients from the first classical PID symptom, such as respiratory tract infections, and even 3.5 (SD 5.4, $p=0.96$) and 20.3 (SD 20.5, $p=0.23$) years, respectively, from the first cutaneous manifestation. Although the first skin disorder did not precede the first classical PID symptom in pediatric patients for years, this was most likely due to the limited range of age in this population and because in particular PIDs with a long diagnostic delay are diagnosed in adulthood. Moreover, skin disorders might be a useful diagnostic feature in PIDs as patients with a PID appear to develop skin disorders at a younger age compared with people without a PID (adult patients reported their first skin disorder at the age of 20.9 (SD 22.0) years

and partner-controls at 33.7 (SD 26.6) years (p=0.02)). To further explore the usefulness of skin symptoms as warning signs of PIDs, registration of skin disorders on an international basis, for example in the PID database of The European Society for Immunodeficiencies registry, is recommended.

The influence of skin disorders on the HR-QoL was minimal; patients reported in general a lower HR-QoL than norm data and partner-controls, and a good SR-QoL. Other PID-related complaints and symptoms, like fatigue and infections, possibly have a greater influence on the HR-QoL than skin symptoms. Nonetheless, the HR-QoL could indirectly be positively influenced through reducing the diagnostic delay by early recognition of PID-associated skin disorders.[12]

The skin disorders diagnosed during physical examination of 22 adult patients were partly overlapping with those who were reported in the questionnaire. Skin cultures indicated 30.0% of the skin lesions that were persistent colonized with *S. aureus*; seven cultures from a dermatitis lesion, one from ecthyma, and one from folliculitis. The prevalence of lesional and nasal *S. aureus* carriage in patients with PIDs and skin disorders with possible *S. aureus*-related etiology was slightly lower than single culture data of patients with atopic dermatitis, but higher as compared with partner-controls.[11] In a recent study examining the skin microbiome in three rare monogenic PIDs colonization with *S. aureus* was found to be significantly correlated with skin disease severity.[29] Therefore, identification of PID-associated skin disorders with an increased risk of *S. aureus* colonization is necessary as patients with these PIDs could benefit from *S. aureus*-targeting treatments.[30,31]

This study has some limitations. Firstly, the majority of patients (86.6%) had a PAD according to the 2017 IUIS classification, which is in accordance with previous national and international studies.[18,25,32-35] Due to the skewed phenotype distribution and limited numbers of patients with rare PIDs we were not able to present data of skin disorders per specific PID (category) as shown in a systematic review.[7] Secondly, the responder population of adult patients was skewed with regard to age. This might have resulted in a self-selection bias and a subsequent overestimation of the prevalence of skin disorders in PIDs in this study as patients with a skin disorder were more likely to respond to the questionnaire. Lastly, in the observational clinical study we only cultured *S. aureus*, as most common skin pathogen in PIDs. However, an increased representation of the bacteria *Serratia macescens* and *Clostridium* species as well as the opportunistic fungi *Candida* and *Aspergillus* was also found in the skin microbiome of patients with PIDs, which indicates the need for more extensive study of the microbiome.[29] Nonetheless, these microorganisms were not correlated with skin disease severity and, therefore, antimicrobial treatment targeting these microorganisms may not be beneficial.

In conclusion, this questionnaire-based and observational clinical study shows that skin disorders are more prevalent and develop at an earlier age in patients with PIDs as compared with partner-controls. Reduction of the diagnostic delay could be achieved by recognition of non-dermatitis-like skin disorders, like skin infections and/or nail disorders, in combination with the warning signs for PIDs. Therefore, more awareness and detailed registration of skin disorders on an international basis is recommended in order to improve the diagnostic and therapeutic processes in patients with PIDs. Additionally, collection of large numbers of data within homogeneous groups of patients might result in identification of skin disorders specific per PID.

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SUPPLEMENTARY MATERIAL

Appendix 1. Quality of life outcome measurements

HR-QoL was assessed using the TNO-AZL Preschool Children's Quality of Life questionnaire (TAPQOL) for patients <4 years, KIDSCREEN-27 by parents for patients 4-11 years, KIDSCREEN-27 by child for patients 12-17 years, and Short Form 36 (SF-36) for adults. The skin-related QoL (SR-QoL) was measured using Infant's Dermatitis Quality of Life Index (IDQOL) for patients <4 years, Children's Dermatology Life Quality Index (CDLQI) for patients 4-17 years, and the Dermatology Life Quality Index (DLQI) for adults. The influence of the skin disorder on the HR-QoL was assessed using an 11-point Numeric Rating Scale (NRS).

Appendix 2. Detailed sample and laboratory procedures

Sampling procedures were based on the 'Manual of Procedures' for microbiome sampling of the Human Microbiome Project.[1] All samples were obtained by a clinical study physician wearing gloves. Sterile Copan 490CE.A swabs were used to sample the lesional skin and anterior nasal cavity. The skin surface was swabbed during 30 seconds. The mucosal surfaces of both the anterior nares were gently rubbed going round the area during 10 seconds. The swabs were sent to the laboratory at the day of collection using mail. Bacterial cultures were performed using routine diagnostic culture procedures, using blood agar plates and specific S. aureus culture plates (ChromID S. aureus Elite agar (SAIDE), Biomérieux, France) for overnight incubation at 37 °. Subsequently, species were determined by MALDI-TOF (Bruker Daltonics, Bremen, Germany).

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Appendix 3. Reference population KIDSCREEN-27

Norm data thresholds for all scales were based on the KIDSCREEN-27 instructions and fixed at a value of the mean minus half and plus half a standard deviation. A study population mean that was lower or higher than the norm data thresholds was considered as noticeable or significant (depending on study population sample size ≤50 or >50) lower or better QoL, respectively, compared to the reference population.[22]

Table S1. Skin disorders in primary immunodeficiency diseases

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)
Dermatitis-like lesions, n (%)	24 (53.3)	97 (46.9)	17 (30.4)
(Atopic) dermatitis	22 (48.9)	61 (29.5)	14 (25.0)[2]
Seborrheic dermatitis	7 (15.6)	27 (13.0)	1 (1.8)[2]
Nummular eczema	8 (17.8)	22 (10.6)	1 (1.8)[2]
Dermatitis herpetiformis	9 (20.0)	24 (11.6)	2 (3.6)[2]
Other dermatitis-like lesions	0 (0.0)	17 (8.2)	4 (7.1)[2]
Hair abnormalities, n (%)	5 (11.1)	55 (26.6)	5 (8.9)[2]
Hair loss disorders	3 (6.7)	45 (21.7)	4 (7.1)[2]
Hair loss scalp	3 (6.7)	32 (15.5)[3]	4 (7.1)[2]
Hair loss eyelashes and eyebrows	2 (4.4)	11 (5.3)[3]	0 (0.0)[2]
Other	0 (0.0)	4 (1.9)[3]	0 (0.0)[2]
Excessive hair growth disorders	1 (2.2)	15 (7.2)	2 (3.6)[2]
Hypertrichosis	1 (2.2)	7 (3.4)[4]	1 (1.8)[2]
Hirsutism	1 (2.2)	11 (5.3)[4]	1 (1.8)[2]
Other	0 (0.0)	0 (0.0)[4]	0 (0.0)[2]
Hair pigmentation disorders	0 (0.0)	13 (6.3)	0 (0.0)[2]
Local depigmentation	0 (0.0)	5 (2.4)	0 (0.0)[2]
Silvery hair	0 (0.0)	6 (2.9)	0 (0.0)[2]
Other	0 (0.0)	2 (1.0)	0 (0.0)[2]
Other hair abnormalities	0 (0.0)	1 (0.5)	0 (0.0)[2]
Bamboo hair	0 (0.0)	1 (0.5)	0 (0.0)[2]
Skin infections, n (%)	29 (64.4)	127 (61.4)	10 (17.9)
Fungal skin infections	11 (24.4)	62 (30.0)	5 (8.9)
Oral fungal infection	8 (17.8)	38 (18.4)[4]	2 (3.6)
Genital fungal infection	3 (6.7)	37 (17.9)[4]	3 (5.4)
Cutaneous fungal infection	4 (8.9)	40 (19.3)[4]	4 (7.1)
Other	1 (2.2)	1 (0.5)[4]	0 (0.0)
Viral skin infections	24 (53.3)	84 (40.6)	3 (5.4)
Varicella zoster virus infection	21 (46.7)	21 (10.1)	1 (1.8)
Herpes zoster virus infection	3 (6.7)	32 (15.5)	2 (3.6)
Oral herpes simplex infection	4 (8.9)	32 (15.5)	0 (0.0)
Genital herpes simplex infection	0 (0.0)	16 (7.7)	0 (0.0)
Warts	11 (24.4)	45 (21.7)	0 (0.0)
Molluscum contagiosum	13 (28.9)	15 (7.2)	0 (0.0)
Other	0 (0.0)	1 (0.5)	0 (0.0)
Bacterial skin infections	15 (33.3)	66 (31.9)	1 (1.8)
Abscesses	4 (8.9)	25 (12.1)	0 (0.0)
Impetigo	7 (15.6)	12 (5.8)	0 (0.0)

Table S1. Skin disorders in primary immunodeficiency diseases (continued)

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)
Folliculitis	3 (6.7)	38 (18.4)	1 (1.8)
Cellulitis	0 (0.0)	8 (3.9)	0 (0.0)
Furuncle	3 (6.7)	28 (13.5)	0 (0.0)
Other	1 (2.2)	4 (1.9)	0 (0.0)
Other skin infections	8 (17.8)	38 (18.4)	1 (1.8)
Perleche	8 (17.8)	38 (18.4)	1 (1.8)
Other	1 (2.2)	5 (2.4)	0 (0.0)
Ulcers, n (%)	16 (35.6)	53 (25.6)	2 (3.6)
Oral ulcers	16 (35.6)	47 (22.7)	2 (3.6)
Nasal ulcers	2 (4.4)	14 (6.8)	0 (0.0)
Other ulcers	0 (0.0)	3 (1.4)	0 (0.0)
Ecthyma	0 (0.0)	0 (0.0)	0 (0.0)
Erythematous skin lesions, n (%)	5 (11.1)	45 (21.7)	2 (3.6)
Erythroderma	1 (2.2)	11 (5.3)	1 (1.8)
Red spots	3 (6.7)	28 (13.5)	2 (3.6)
Erythema induratum	1 (2.2)	3 (1.4)	1 (1.8)
Erythema nodosum	0 (0.0)	3 (1.4)	1 (1.8)
Other	1 (2.2)	12 (5.8)	0 (0.0)
Vascular disorders, n (%)	7 (15.6)	50 (24.2)	2 (3.6)
Telangiectasia	3 (6.7)	32 (15.5)	2 (3.6)
Telangiectasia on eyes	2 (4.4)	4 (1.9)	0 (0.0)
Telangiectasia on cheeks and/or nose	1 (2.2)	21 (10.1)	0 (0.0)
Telangiectasia on ears	0 (0.0)	0 (0.0)	0 (0.0)
Telangiectasia on shoulders, back and/or neck	0 (0.0)	4 (1.9)	0 (0.0)
Telangiectasia on total body	0 (0.0)	2 (1.0)	0 (0.0)
Other	1 (2.2)	10 (4.8)	1 (1.8)
Vasculitis	2 (4.4)	9 (4.3)	0 (0.0)
Allergic vasculitis	0 (0.0)	0 (0.0)	0 (0.0)
Other	1 (2.2)[4]	9 (4.3)	0 (0.0)
Petechia/purpura	2 (4.4)	12 (5.8)	0 (0.0)
Oral petechia/purpura	0 (0.0)	2 (1.0)	0 (0.0)
Other	2 (4.4)	10 (4.8)	0 (0.0)
Other vascular disorders	0 (0.0)	7 (3.4)	1 (1.8)
Pigmentation disorders, n (%)	15 (33.3)	73 (35.3)	4 (7.1)
Hyperpigmentation disorders	13 (28.9)	45 (21.7)	3 (5.4)
Cafe au lait maculae	6 (13.3)	18 (8.7)	1 (1.8)
Naevus >5mm	8 (17.8)	23 (11.1)	2 (3.6)
Acanthosis nigricans	0 (0.0)	4 (1.9)	0 (0.0)

Table S1. Skin disorders in primary immunodeficiency diseases (continued)

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)
Other	1 (2.2)	11 (5.3)	0 (0.0)
Hypopigmentation disorders	4 (8.9)	25 (12.1)	1 (1.8)
Vitiligo	0 (0.0)	21 (10.1)	0 (0.0)
Albinism	0 (0.0)	0 (0.0)	0 (0.0)
Halo naevus	0 (0.0)	2 (1.0)	0 (0.0)
Hypopigmented spots	4 (8.9)	3 (1.4)	0 (0.0)
Other	0 (0.0)	0 (0.0)	1 (1.8)
Other pigmentation disorders	7 (15.6)	22 (10.6)	0 (0.0)
Blue naevus	0 (0.0)	1 (0.5)	0 (0.0)
Freckles	7 (15.6)	22 (10.6)	0 (0.0)
Other	0 (0.0)	2 (1.0)	0 (0.0)
Neoplastic disorders, n (%)	0 (0.0)	20 (9.7)	3 (5.4)
Cutaneous lymphoma	0 (0.0)	1 (0.5)	0 (0.0)
Basal cell carcinoma	0 (0.0)	10 (4.8)	1 (1.8)
Squamous cell carcinoma	0 (0.0)	4 (1.9)	0 (0.0)
Melanoma	0 (0.0)	2 (1.0)	1 (1.8)
Other neoplastic disorders	0 (0.0)	6 (2.9)	1 (1.8)
Rash, n (%)	24 (53.3)	57 (27.5)	4 (7.1)
Newborn rash	6 (13.3)	6 (2.9)	0 (0.0)
Papulosquamous rash	3 (6.7)	9 (4.3)	1 (1.8)
Maculopapular rash	11 (24.4)	15 (7.2)	0 (0.0)
Papulopustular rash	6 (13.3)	15 (7.2)	1 (1.8)
Eczematous rash	14 (31.1)	32 (15.5)	2 (3.6)
Other	2 (4.4)	10 (4.8)	0 (0.0)
Nail disorders, n (%)	12 (26.7)	80 (38.6)	4 (7.1)
Non-infectious nail disorders	9 (20.0)	44 (21.3)	3 (5.4)
Congenital nail disorders	2 (4.4)	2 (1.0)	0 (0.0)
Acquired nail disorders	2 (4.4)	11 (5.3)	2 (3.6)
Thickening	3 (6.7)	24 (11.6)	1 (1.8)
Other	5 (11.1)	20 (9.7)	1 (1.8)
Infectious nail disorders	5 (11.1)	61 (29.5)	2 (3.6)
Paronychia	3 (6.7)	29 (14.0)	0 (0.0)
Onychomycosis	3 (6.7)	39 (18.8)	2 (3.6)
Other	0 (0.0)	3 (1.4)	0 (0.0)
Other nail disorders	0 (0.0)	1 (0.5)	0 (0.0)
Psoriasis-like lesions, n (%)	1 (2.2)	20 (9.7)	1 (1.8)
Psoriasis	0 (0.0)[5]	17 (8.2)	1 (1.8)
Other psoriasis like-lesions	0 (0.0)[5]	2 (1.0)	0 (0.0)

Table S1. Skin disorders in primary immunodeficiency diseases (continued)

	Pediatric patients (n=45)	Adult patients (n=207)[1]	Adult partner-controls (n=56)
Granulomatous disorders, n (%)	1 (2.2)	1 (0.5)	0 (0.0)
Acne-like lesions, n (%)	5 (11.1)	34 (16.4)	3 (5.4)
Neonatal acne	2 (4.4)	1 (0.5)	0 (0.0)
Acne	4 (8.9)	34 (16.4)	3 (5.4)
Other	0 (0.0)	0 (0.0)	0 (0.0)
Urticaria, n (%)	6 (13.3)	29 (14.0)	0 (0.0)
Acute urticaria	4 (8.9)	16 (7.7)[4]	0 (0.0)
Chronic urticaria	1 (2.2)	6 (2.9)[4]	0 (0.0)
Cold induced urticaria	0 (0.0)	3 (1.4)[4]	0 (0.0)
Other	2 (4.4)	7 (3.4)	0 (0.0)
Other skin disorders, n (%)	6 (13.3)	19 (9.2)	0 (0.0)

Missing: [1]n=3 (1.4%), [2]n=1 (1.8%), [3]n=8 (3.9%), [4]n=4 (1.9%), [5]n=1 (4.5%).

Chapter 3.2

Atopic manifestations are underestimated clinical features in various primary immunodeficiency disease phenotypes

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ABSTRACT

Background

Atopic manifestations are described as clinical feature of various primary immunodeficiency disease (PID) phenotypes and in particular frequently reported in the combined immunodeficiencies. The prevalence of atopic manifestations in other PIDs remains largely unknown. Therefore, we aimed to evaluate the prevalence of atopic manifestations in other PIDs and to identify in which PIDs atopic manifestations are most common in order to improve patient care.

Methods

A partner-controlled questionnaire-based study was performed in pediatric and adult PID patients. Subsequently, data of diagnostic tests for atopic manifestations (i.e. diagnostic criteria for AD, spirometry, specific IgE against food and inhalant allergens) were collected in adult patients to confirm patient-reported atopic manifestations.

Results

Forty-seven children and 206 adults with PIDs, and 56 partner-controls completed the questionnaire. Thirty-five (74.5%) pediatric and 164 (79.6%) adult patients reported to have ever experienced one or more atopic manifestations compared with 28 (50.0%) partner-controls. In adult patients vs. partner-controls, prevalence of atopic dermatitis was 49.5% vs. 27.3% (p=0.003), food allergy 10.7% vs. 1.9% (p=0.031), asthma 55.7% vs. 14.8% (p<0.001) and allergic rhinitis 49.8% vs. 21.8% (p<0.001). The frequency of current atopic manifestations reported by patients was higher than the prevalence based on diagnostic tests (atopic dermatitis 11.2%, food allergy 1.9%, asthma 16.4% and allergic rhinitis 11.5%).

Conclusion

Atopic manifestations are prevalent clinical features in a large spectrum of PIDs and in our cohort frequently present in patients with combined immunodeficiencies and predominant antibody deficiencies. Evaluation of atopic manifestations should be considered in patients with PIDs.

INTRODUCTION

Primary immunodeficiency diseases (PIDs) encompass a heterogeneous group of more than 300 inheritable defects of immunity caused by variants in genes encoding functional proteins of human immune cells.[1,2] The incidence of symptomatic PIDs is estimated at 1 in 2,000 live births with a prevalence of 1 in 10,000-12,000 in the general population.[2-4] PIDs are typically characterized by recurrent and/or severe infections. Additionally, patients may suffer from autoimmunity, autoinflammation, malignancy and allergic disorders.[5-7] Allergic manifestations may be part of the so-called atopic syndrome, which is characterized by atopic dermatitis (AD), food allergy (FA), asthma and allergic rhinitis (AR).

Atopic manifestations are described as clinical feature of various PID phenotypes.[2,8,9] Nonetheless, a narrative review reported presence of these manifestations mainly in immunodeficiencies affecting both cellular and humoral immunity (combined immunodeficiencies; CIDs), like DOCK8 deficiency, and CIDs with associated or syndromic features, such as Comèl Netherton syndrome.[8] Other original studies also reported atopic manifestations most commonly in patients with CIDs and in lower frequencies, comparable to prevalence in the general population, in predominant antibody deficiencies (PADs), like selective immunoglobulin (Ig) A deficiency.[9] However, original data on atopic manifestations in PIDs are limited, as studies comprise small patient samples, and the diagnosis of atopic manifestations is generally not based on diagnostic tests, but on medical records or the data source was not described.

The development of atopic manifestations within the atopic syndrome is the result of a genetic predilection to produce specific IgE (sIgE) following exposure to allergens.[10] In this process, presentation of processed allergen by antigen-presenting cells to T lymphocytes leads to activation of B lymphocytes and subsequent production of sIgE.[11] Although the pathogenesis of atopic manifestations is complex and multifactorial, its pathogenic pathway could overlap with pathways involved in (certain) PIDs.[12] For example, mutations in the *SPINK5* gene can cause both the Comèl Netherton syndrome and atopy. This genetic overlap might, therefore, explain the presence of atopic manifestations in patients with specific PIDs.

Early recognition and treatment of atopic manifestations in patients with PIDs could prevent clinical deterioration. Currently, diagnostic delay for asthma is still 3.3 years.[13] Airways of untreated asthma patients become chronically swollen and persistence of AR can lead to sleep loss and secondary decreased overall cognitive functioning.[14,15] Furthermore, long-term existence of untreated atopic manifestations may contribute to development of other related disease processes, including sinusitis, and a lower quality of life.[15]

The aim of this study is to evaluate the prevalence of atopic manifestations in children and adults with a PID compared to partner-controls by using a questionnaire and diagnostic tests in order to identify specific PIDs with a higher chance of developing the atopic syndrome.

METHODS

Study design

We performed a retrospective partner-controlled questionnaire-based study on the prevalence of atopic manifestations in patients with PIDs. Furthermore, standard care data on diagnostic tests for AD, FA, asthma and AR were retrospectively and prospectively collected in adult patients to confirm the patient-reported atopic manifestations. The study was designed and conducted by the department of Dermatology, department of Internal Medicine, division of Clinical Immunology, and department of Pediatrics, division of Infectious Diseases, of the Erasmus MC University Medical Center, Rotterdam, The Netherlands. The study procedures were approved by the institutional review board of the Erasmus MC University Medical Center (MEC-2018-1260). All patients aged 16 years or older provided written informed consent themselves. For children below 12 years, both parents or guardians signed, and for children aged 12-16 years both the adolescent and both parents or caregivers signed, in accordance with the Dutch law.

Study population

Patients of all ages with a PID diagnosis according to Picard *et al.* were included.[1] We selected patients from an ongoing database of the department of Internal Medicine, division of Clinical Immunology, and department of Pediatrics, division of Infectious Diseases, Erasmus MC University Medical Center, that prospectively registers all patients diagnosed with a PID (MEC-2013-026). We included patients enrolled in this database between 2013 and September 2018. Patients who underwent a curative hematopoietic stem cell transplantation and patients (or their parent(s)/caregiver(s)) who were not able to read and understand the Dutch language were excluded from this study. The control group consisted of partners of adult patients who completed the questionnaire and were not deceased in order to correct for environmental factors regardless of genetic influences, which might be involved in development of atopic manifestations in PIDs.

Outcome measurements

The primary outcome of the study was the self-reported prevalence of current and ever experienced atopic manifestations in children (by parent/caregiver) and adults (≥18 years) with a PID compared to adult partner-controls. Secondary outcomes were the age of onset

of the first atopy-associated symptoms and verification of atopic manifestation using diagnostic criteria or tests. To assess the self-reported or parent-reported (patients <12 years) prevalence of AD, asthma and AR, the Phase Three Core Questionnaire of the International Study of Asthma and Allergies in Childhood (ISAAC) was used (Appendix 1).[16] Asthma data from patients <5 years were not taken into account for further analysis. The prevalence of FA was estimated based on a doctor diagnosis or double-blind, placebo-controlled food challenge (both reported by the patient).

Data from the questionnaires of adult patients were verified using retrospectively and prospectively collected standard care data. The diagnosis of AD was confirmed based on the United Kingdom Working Party Diagnostic Criteria for Atopic Dermatitis by a dermatologist or immunologist at the outpatient clinic.[17-19] Spirometry with either a bronchodilator reversibility test or a bronchial challenge test with histamine was used to confirm the diagnosis of asthma. Asthma was defined according to Global Initiative for Asthma (GINA) guidelines as at least once during the diagnostic process the FEV1/FVC ratio was below the lower limit of normal, the presence of symptoms, and an increase of ≥12% and ≥200 mL from baseline in FEV1 after inhaling a bronchodilator, or a positive provocation test.[20] Additionally, asthma was classified as allergic in case of both sIgE against a panel of inhalant allergens of ≥0.35 kU/L and sIgE against at least one specific inhalant allergen of ≥0.35 kU/L. For verification of the diagnoses FA and AR we used sIgE against a panel of food allergens and a panel of inhalant allergens, respectively. As presence of sIgE to a specific allergen does not necessarily equate to a clinically relevant allergic response to that substance, FA was confirmed if a patient reported a FA that was diagnosed by a doctor or with a double-blind, placebo-controlled food challenge combined with sensitization for food allergens, i.e. sIgE against the panel of allergens of ≥0.35 kU/L and sIgE against at least one specific allergen of ≥0.35 kU/L. Additionally, AR was confirmed if a patient reported ever having hayfever combined with sensitization for inhalant allergens, i.e. sIgE against the panel of allergens of ≥0.35 kU/L and sIgE against at least one specific allergen of ≥0.35 kU/L.

Study procedures

A questionnaire was sent by mail to 80 pediatric and 359 adult patients with PIDs between October 2017 and September 2018. Standard care data on the diagnosis of atopic manifestations were collected from the electronic patient record until August 2019. Biochemical data, i.e. sIgE against inhalant allergens and food allergens, collected before August 2009 were not used.

Statistical analysis

The prevalence of atopic manifestations were presented as mean and standard deviation (SD) for normally distributed continuous data or otherwise as median and interquartile range (IQR). The difference in the ever experienced prevalence of atopic manifestations between adult patients and partner-controls was analyzed using a Chi-Square test. Basic descriptive statistics and tests were performed using SPSS version 25.0 for Windows (IBM Corporation, Armonk, NY).

RESULTS

Study population characteristics

Two hundred and fifty-three patients completed and returned the questionnaire (57.6%; 47 children and 206 adults). Responders and non-responders had comparable demographic and disease characteristics, but adult responders had a higher age compared with adult non-responders (data not shown). The majority of the responding patients (n=218, 86.2%) had a PAD according to the 2017 International Union of Immunological Societies (IUIS) Phenotypic Classification for Primary Immunodeficiencies, including 37 (78.7%) children and 181 (87.9%) adults (Table 1).[21] Median age of the included patients was 47.9 (IQR 24.1-61.1) years; children had a median age of 11.9 (IQR 7.3-15.7) years and adults of 53.6 (IQR 37.5-64.6) years. One hundred and sixteen (45.8%) patients were male. Mean age at time of diagnosis of the PID was 4.6 (SD 4.1) years in pediatric patients and 41.9 (SD 19.9) years in adult patients.

Control group characteristics

A questionnaire was sent to partners of 201 adult patients as five patients with a PID died after completing the questionnaire. Fifty-six (27.7%) questionnaires of partner-controls were completed, returned and included for further analysis. Partner-controls had a median age of 59.3 (IQR 46.2-69.8) years and 34 (60.7%) were male.

Atopic manifestations in children with a primary immunodeficiency disease

Current atopic manifestations

Twenty-nine (61.7%) pediatric patients reported to suffer from one or more atopic manifestations at the moment of completing the questionnaire. AD had a prevalence of 19.1%, FA of 25.0%, asthma of 30.4% and AR of 34.8%.

Table 1. General patient demographics

	Pediatric patients (n=47)	**Adult patients (n=206)**	**Adult partner-controls (n=56)**
Age			
median (IQR)	11.9 (7.3-15.7)	53.6 (37.5-64.6)	59.3 (46.2-69.8)[1]
Sex, male			
n (%)	33 (70.2)	83 (40.3)	34 (60.7)
Age PID diagnosis, years			Not applicable
mean (SD)	4.6 (4.1)	41.9 (19.9)	
IUIS phenotypic classification of PID, n (%)			Not applicable
Immunodeficiencies affecting cellular and humoral immunity	0 (0.0)	1 (0.5)	
Combined immunodeficiencies with associated or syndromic features	1 (2.1)	7 (3.4)	
Predominantly antibody deficiencies	37 (78.7)	181 (87.9)	
- Common variable immunodeficiency	10 (21.3)	74 (35.7)	
- IgG subclass deficiency	2 (4.3)	34 (16.5)	
- Selective IgA deficiency	1 (2.1)	5 (2.4)	
- Selective antibody deficiency with normal immunoglobulins	0 (0.0)	26 (12.6)	
- X-linked agammaglobulinemia	4 (8.5)	7 (3.4)	
- Hypogammaglobulinemia	12 (25.5)	23 (11.2)	
- Hyper IgM syndrome	3 (6.4)	3 (1.5)	
- Combined antibody deficiency	0 (0.0)	7 (3.4)	
- Other	5 (10.6)	2 (1.0)	
Diseases of immune dysregulation	0 (0)	1 (0.5)	
Congenital defects of phagocyte number or function	3 (6.4)	2 (1.0)	
Defects in intrinsic and innate immunity	2 (4.3))	5 (2.4)	
Autoinflammatory disorders	2 (4.3)	4 (1.9)	
Complement deficiencies	0 (0)	1 (0.5)	
Phenocopies of inborn errors of immunity	0 (0)	1 (0.5)	
Unknown	2 (4.3)	3 (1.5)	

Abbreviations: IUIS, International Union of Immunological Societies; IQR, interquartile range; n, number; PID, primary immunodeficiency disease. Missings: [1]n=3 (5.4%).

Ever experienced atopic manifestations

At least one ever experienced atopic manifestation was reported by 35 (74.5%) pediatric patients, of which AD (60.0%) had the highest prevalence. Ever experienced FA, asthma and AR were reported by 25.0%, 34.8% and 30.4% of the children, respectively (Tables 2). Atopic manifestations were reported in a large spectrum of PIDs across the various phenotypes. However, the most important conclusion on the prevalence of atopic manifestations could be drawn within the group of PADs due to the large number of patients in this phenotype group (Table 3). The complete spectrum of atopic manifestation was present in 6.4% of the patients. Three, two or one manifestation were reported by 12.8%, 21.3% and 34.0% of the patients, respectively. Mean age of onset of the first atopy-associated symptom was 2.0 (SD 3.1) years for AD, 2.6 (SD 3.5) years for asthma and 5.1 (SD 4.1) years for AR. FA was diagnosed at a mean age of 1.8 (SD 2.3) years.

Table 2. Atopic manifestations in primary immunodeficiency diseases

	Pediatric patients (n=47)	**Adult patients (n=206)**	**Adult partner-controls (n=56)**	***p*-value***
Atopic dermatitis				
Current, n (%)	9 (19.1)	22 (10.8)[5]	3 (5.5)[15]	
Ever experienced, n (%)	27 (60.0)[1]	100 (49.5)[6]	15 (27.3)[15]	0.003
Diagnostic criteria[†], n (%)		11 (11.2)[7]		
Food allergy				
Ever diagnosed, n (%)	9 (25.0)[2]	18 (10.7)[8]	1 (1.9)[16]	0.031
sIgE against food allergens ≥0.35 kU/L, n (%)		5 (4.8)[9]		
Asthma				
Current, n (%)	14 (30.4)[4]	93 (45.1)[5]	7 (13.0)[17]	
Ever experienced, n (%)	16 (34.8)[4]	113 (55.7)[10]	8 (14.8)[17]	<0.001
Positive spirometry with bronchodilator reversibility test[‡], n (%)		10 (16.4)[11]		
Positive bronchial challenge test with histamine, n (%)		6 (24.0)[12]		
Allergic rhinitis				
Current, n (%)	16 (34.8)[4]	91 (45.1)[5]	9 (16.4)[15]	
Ever experienced, n (%)	14 (30.4)[4]	102 (49.8)[13]	12 (21.8)[15]	<0.001
sIgE against inhalant allergens ≥0.35 kU/L, n (%)		25 (19.2)[14]		

Missings: [1]n=2 (4.3%), [2]n=11 (23.4%), [3]n=6 (12.8%), [4]n=1 (2.1%), [5]n=2 (1.0%), [6]n=4 (1.9%), [7]n=108 (52.4%), [8]n=37 (18.0%), [9]n=101 (49.0%), [10]n=3 (1.5%), [11]n=145 (70.4%), [12]n=181 (87.9%), [13]n=1 (0.5%), [14]n=76 (36.9%), [15]n=1 (1.8%), [16]n=3 (5.4%), [17]n=2 (3.6%). [†]United Kingdom Working Party's Diagnostic Criteria for Atopic Dermatitis applied by a dermatologist or immunologist at the outpatient clinic.[1-3] [‡]Positive was defined according to Global Initiative for Asthma (GINA) guidelines as a FEV1/FVC ratio below the lower limit of normal and an increase of ≥12% and ≥200 mL from baseline in FEV1 after inhaling a bronchodilator.[4] *Difference in prevalence of ever experienced atopic manifestations between adult patients and partner-controls.

Atopic manifestations in adults with a primary immunodeficiency disease and partner-controls

Current atopic manifestations

At least one atopic manifestation at the moment of completing the questionnaire was reported by 134 (65.0%) adult patients and 17 (30.4%) adult partner-controls. AD had a prevalence of 10.8%, FA of 10.7%, asthma of 45.1% and AR of 45.1% (Tables 2). The prevalence of current atopic manifestations in partner-controls was 5.5% for AD, 1.9% for FA, 13.0% for asthma and 16.4% for AR (Tables 2).

Ever experienced atopic manifestations

A total of 164 (79.6%) patients reported to have ever experienced one or more atopic manifestations. AD showed a prevalence of 49.5%, FA of 10.7%, asthma of 55.7% and AR of 49.8% (Tables 2). Fifty percent (n=28) of partner-controls reported to have ever experienced at least one atopic manifestation. Adult patients generally had a significantly higher prevalence of atopic manifestations as compared with partner-controls. Partner-controls showed AD in 27.3% (p=0.003), FA in 1.9% (p=0.031), asthma in 14.8% (p<0.001) and AR in 21.8% (p<0.001) (Tables 2). Atopic manifestations were reported across the various PID phenotypes. However, the most important conclusions on the prevalence of atopic manifestations could be drawn in the CIDs with associated or syndromic features and PADs because of the large number of patients (Table 3). The complete spectrum of atopic manifestation was present in 4.4% of the patients. Three, two or one manifestation was reported by 23.3%, 23.8% and 28.3% of the patients, respectively. None of the partner-controls reported the complete spectrum of atopic manifestations. Three, two or one atopic manifestations were reported by 5.4%, 5.4% and 39.3% of the partner-controls, respectively. The first atopy-related symptom in patients was observed at a mean age of 23.4 (SD 23.7) years in AD, 24.1 (SD 20.9) years in FA, 20.2 (SD 18.9) years in asthma and 19.2 (SD 11.7) years in AR. In controls, corresponding ages were 28.9 (SD 27.5), 24, 16.0 (SD 15.8) and 23.0 (SD 13.1) years, respectively.

Diagnostic criteria and tests on atopic manifestations

Data on inspection of the skin was available from 98 patients, of which 11 (11.2%) patients were diagnosed with AD. Data on sIgE against a panel of food allergens was known for 105 patients. Five (4.8%) patients had elevated sIgE levels (≥0.35 kU/L) against the panel. Within these patients, sIgE levels for 17 specific allergens were ≥0.35 kU/L, suggesting sensitization. Six food allergens within two (1.9%) patients were also reported by patients with a FA based on doctor diagnosis or double-blind, placebo-controlled food challenge (both patient-reported), which indicates a true food allergy. Bronchodilator reversibility tests were performed in 61 patients and bronchial challenge tests with histamine in 25 patients. Based on the diagnostic tests, 11 (16.4%) patients had confirmed asthma, of

Table 3. Ever experienced atopic manifestation according to IUIS phenotypic classification for primary immunodeficiency diseases

	Pediatric patients (n=47)	**Adult patients (n=206)**
Immunodeficiencies affecting cellular and humoral immunity, n (%)	n=0	n=1
Atopic dermatitis	-	0 (0.0)
Food allergy	-	0 (0.0)
Asthma	-	1 (100)
Hayfever	-	0 (0.0)
Combined immunodeficiencies with associated or syndromic features, n (%)	n=1	n=7
Atopic dermatitis	0 (0.0)	4 (57.1)
Food allergy	0 (0.0)[1]	2 (33.3)[5]
Asthma	0 (0.0)	5 (71.4)
Hayfever	0 (0.0)	5 (71.4)
Predominantly antibody deficiencies, n (%)	n=37	n=181
Atopic dermatitis	21 (60.0)[2]	91 (51.4)[6]
Food allergy	7 (25.9)[3]	17 (11.6)[7]
Asthma	15 (41.7)[4]	104 (58.1)[8]
Hayfever	11 (29.7)[4]	95 (52.8)[9]
Diseases of immune dysregulation, n (%)	n=0	n=1
Atopic dermatitis	-	0 (0.0)
Food allergy	-	0 (0.0)
Asthma	-	0 (0.0)
Hayfever	-	0 (0.0)
Congenital defects of phagocyte number or function, n (%)	n=3	n=2
Atopic dermatitis	2 (66.7)	1 (50.0)
Food allergy	1 (33.3)	1 (50.0)
Asthma	1 (33.3)	1 (50.0)
Hayfever	3 (100.0)	0 (0.0)
Defects in intrinsic and innate immunity, n (%)	n=2	n=5
Atopic dermatitis	2 (100)	2 (40.0)
Food allergy	0 (0.0)	0 (0.0)[10]
Asthma	0 (0.0)	1 (20.0)
Hayfever	0 (0.0)	1 (20.0)
Autoinflammatory disorders, n (%)	n=2	n=4
Atopic dermatitis	2 (100)	0 (0.0)
Food allergy	1 (50.0)	0 (0.0)
Asthma	0 (0.0)	0 (0.0)
Hayfever	0 (0.0)	1 (25.0)

Table 3. Ever experienced atopic manifestation according to IUIS phenotypic classification for primary immunodeficiency diseases (continued)

	Pediatric patients (n=47)	Adult patients (n=206)
Complement deficiencies, n (%)	n=0	n=1
Atopic dermatitis	-	0 (0.0)
Food allergy	-	0 (0.0)
Asthma	-	0 (0.0)
Hayfever	-	0 (0.0)
Phenocopies of inborn errors of immunity, n (%)	n=0	n=1
Atopic dermatitis	-	0 (0.0)
Food allergy	-	0 (0.0)
Asthma	-	0 (0.0)
Hayfever	-	0 (0.0)
Unknown, n (%)	n=2	n=3
Atopic dermatitis	2 (100)	2 (66.7)
Food allergy	0 (0.0)	0 (0.0)
Asthma	0 (0.0)	1 (50.0)[11]
Hayfever	0 (0.0)	0 (0.0)

Missings: [1]n=1 (100%), [2]n=2 (5.4%), [3]n=10 (27.0%), [4]n=1 (2.7%), [5]n=1 (14.3%), [6]n=4 (2.2%), [7]n=34 (18.8%), [8]n=2 (1.1%), [9]n=1 (0.6%), [10]n=2 (20.0%), [11]n=1 (33.3%).

which half of the patients reported to have ever experienced asthma as well. Data on sIgE against a panel of inhalant allergens was known for 130 patients, of which 25 (19.2%) patients had elevated sIgE levels (≥0.35 kU/L). Most prevalent specific inhalant allergens were house dust mite (*Dermatophagoides pteronyssinus*) (n=14), followed by grass pollen (n=12), birch tree pollen (n=9), cat dander (n=8), dog dander (n=7), mugwort pollen (n=5), rabbit dander and horse dander (both n=1) (Tables 2). Fifteen (11.5%) of these patients also reported ever experienced hayfever in the questionnaire, indicating true AR.

DISCUSSION

This study demonstrates that all atopic manifestations, including AD, FA, asthma and AR, are prevalent in children and adults with PIDs. In adult patients, patient-reported ever experienced atopic manifestations were significantly more common when compared with adult partner-controls. The atopic manifestations were reported in a large spectrum of PIDs across the various phenotypes, of which the most important conclusions can be drawn within the CIDs and PADs (prevalence ranging from 11.6% for FA to 71.4% for asthma and AR) because of the high number of patients.

This is the first study evaluating the prevalence of atopic manifestations in a cohort of children and adults with a PID using both questionnaire data and diagnostic criteria or tests. Compared to previous reports, in which diagnosis of atopic manifestations was generally based on patient records or the data source was not described, we found a significantly higher prevalence of atopic manifestations in patients with a PAD and comparable numbers of patients with atopic manifestations in CIDs.[9]

The pathogenic pathway involved in development of the atopic syndrome could be characterized by autoallergy, in which atopy seems to stand at the boundary between allergy and auto-immunity, given the presence of IgE antibodies against self-proteins.[22-24] Based on this pathway, in which T lymphocytes play a central role, patients with a PID affecting cellular immunity, such as CIDs, might be more predisposed to developing atopic manifestations.[12] PADs, on the other hand, are generally characterized by a primary antibody production failure. A significant number of patients with common variable immunodeficiency disorder (CVID), the most prevalent PAD (38.5% of all PADs in our cohort), shows also disturbed T lymphocyte function in addition to their primary humoral immunodeficiency, which could contribute to development of the atopic syndrome.[25] Moreover, approximately one third of the CVID patients have a clinical phenotype with autoimmunity, which is inverse correlated with CD8 cell proportions, indicating a T lymphocyte dysfunction as well.[26] However, the exact mechanism underlying the development of atopic manifestations in PIDs remains to be elucidated.

De Wit *et al.* previously identified 22 genes that are related to development of atopy, but are also involved in PIDs.[12] These genes included mainly disease-causing genes resulting in CIDs (n=10), defects in intrinsic and innate immunity (n=5) and diseases of immune dysregulation (n=5). Only two atopy-related genes were also associated with PADs. Furthermore, the Th lymphocyte-mediated genetic pathway was identified to be involved in atopy, which could explain the predominance of atopic manifestations in cellular immunodeficiencies.

Several aspects should be taken into account when interpreting the results of this study. Firstly, according to the atopic march, in which the course of atopic manifestations over time is characterized (generally starting with AD in infancy and followed by FA, asthma and AR later in childhood), the prevalence of asthma and AR in pediatric patients with a PID might be underestimated due to the age of these patients.[27] Secondly, the prevalence of current FA, asthma and AR reported by adult patients was significantly higher than the prevalence based on diagnostic test results. This discrepancy could be due to over-reporting clinical symptoms related to atopy, as is known from FA, in which only half of the patients that believe they are allergic to food actually have a proven intolerance, or because PID patients commonly have asthma-like airway complaints regardless of a

positive diagnostic test.[28] Furthermore, atopic manifestation have a relapsing-remitting course and a specific progression over time, characterized by the atopic march, resulting in a time-varying prevalence. Moreover, a number of patients with chronic obstructive pulmonary disease could have incorrectly reported presence of current asthma as a result of comparable symptoms between both pulmonary conditions. This could also be the case in the discrepancy between current and ever experienced AR in children as current atopic manifestations were extracted based atopy-associated symptoms and ever experienced manifestations based on the atopy diagnosis. Lastly, data from this study might not be applicable to all PID phenotypes because mainly patients with PADs were included in our cohort. However, PADs represent the largest group of PIDs worldwide and, therefore, our results are relevant to a large number of patients with PIDs. Moreover, symptoms of the atopic syndrome were frequently reported in patients with CIDs, which is in accordance with current literature, despite the low number of patients in this PID group. Additionally, as only patients in a tertiary referral center were included in this study, data might not be applicable for all patients with atopic manifestations.

In this study, atopic manifestations seem to develop earlier in life than the age of PID diagnosis (4.6 years in pediatric and 41.9 years in adult patients). As atopic manifestations generally start in childhood, the high age at which the first atopy-associated symptoms were observed in this study (both in adult patients and partner-controls) could be considered an overestimation as result of a recall bias or because data were based on patient-reported outcomes.

In conclusion, this questionnaire-based study shows that patient-reported ever experienced atopic manifestations are more prevalent in adult patients with PIDs as compared with partner-controls. In particular, patients with CIDs and PADs were shown to have a higher chance of developing atopic manifestations. We propose to consider evaluation of patients with CIDs and PADs for atopic manifestations, including asthma, to prevent clinical deterioration. Future studies should pay attention to identifying specific characteristics of atopic manifestations in PIDs that may increase awareness of an underlying PID.

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Chapter 4

Molecular clustering of genes related to the atopic syndrome: towards a more tailored approach and personalized medicine?

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ABSTRACT

Background

The atopic syndrome consists of heterogeneous manifestations, in which multiple associated genetic loci have recently been identified. It is hypothesized that immune dysregulation plays a role in the pathogenesis. In primary immunodeficiency diseases (PIDs), which are often monogenic immunodysregulation disorders, the atopic syndrome is a frequently occurring comorbidity. Based on the genetic defects in PIDs, novel gene/pathway-targeted therapies have been evaluated, which could be relevant in the atopic syndrome as well.

Objective

We aimed to define subclasses within the atopic syndrome based on the expression profiles of immune cell lineages of healthy mice.

Methods

Overlap between known atopy-related genes as described in the Human Gene Mutation Database and disease-causing genes of monogenic PIDs was evaluated. Clusters of atopy-related genes were based on the overlap in their co-expressed genes using the gene expression profiles of immune cell lineages of healthy mice from the Immunological Genome Project. We analyzed pathways involved in the atopic syndrome using Ingenuity Pathway Analysis.

Results

Twenty-two (5.3%) genes were overlapping between the atopy-related genes (n=160) and PID-related genes (n=278). We identified seven distinct clusters of atopy-related genes. Functional pathway analysis of all atopy-related genes showed relevance of T helper cell-mediated pathways.

Conclusion

This study shows a model to define clusters within the atopic syndrome based on gene expression profiles of immune cell lineages. Our results support the hypothesis that both genetic mechanisms and immune dysregulation play a role in the pathogenesis. It also opens up the possibility for novel therapeutic targets and a more tailored approach towards personalized medicine.

INTRODUCTION

Atopy is the genetic predilection to produce specific immunoglobulin (Ig) E following exposure to allergens. This predisposition results in the development of atopic dermatitis (AD), food allergy (FA), asthma, and allergic rhinitis (AR): the atopic syndrome.[1] The worldwide prevalence of these manifestations in children varies between 15-20%, 1-10%, 3-29%, and 9-15%, respectively, and in adults from 1-3%, 3-4%, 2-12%, and 7-42%, respectively.[2-6] Atopic manifestations share a common mechanism involving allergen-specific IgE, which triggers the release of inflammatory mediators, like histamine, in the skin, gastrointestinal tract, lungs and nose. The course of these manifestations over time is characterized by the atopic march, generally starting with AD in infancy and followed by FA, asthma, and AR later in childhood.[7] However, it is known that the atopic march not always follows the classic sequence and may occur at any age.[8,9] Furthermore, not all atopic patients will develop the complete spectrum of atopic manifestations.[7] Despite its heterogeneous presentation, patients with atopic manifestations are mostly uniformly treated with topical or systemic immunosuppressive agents and/or antihistamines resulting in varying therapeutic responses as well.[10-13]

Subgroups of the atopic phenotype, termed endotypes, are possibly responsible for the differences in disease manifestations and treatment responses. These endotypes are the result of variations in physiologic, biologic, immunologic and/or genetic mechanisms.[14] Various genetic loci associated with both inflammation and multiple atopic manifestations have been identified in recent years based on Genome-Wide Association Studies (GWAS), showing common genetic mechanisms involved in the atopic syndrome.[15-24] Nevertheless, the genetics of the atopic syndrome remain complicated for different reasons. For example, gene polymorphisms in different genes might cause the atopic syndrome independent of each other, and bearing a predisposing gene polymorphism does not necessarily result in development of the atopic syndrome.[24] The genetic complexity in the atopic syndrome possibly results in its heterogeneous clinical phenotype. Defining the endotypic profile of atopic patients in more detail contributes to determination of more homogeneous subclasses of patients. Subclasses are currently defined based on clinical and immunological characteristics, like the type of immune response involved.[25] However, stratification of atopic patients based on their genetic defect or polymorphism linked to their expression profile of immune cell lineages has not yet been investigated. This endotyping approach could be of interest as immune dysregulation may play an important role in the pathogenesis of the atopic syndrome. Interestingly, the atopic syndrome is a prevalent comorbidity in primary immunodeficiency diseases (PIDs), for example in hyper IgE syndrome (HIES), Comèl Netherton syndrome, and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, which suggests that the atopic syndrome could be caused by a genetic

defect in pathways that are also involved in these monogenic PIDs.[26,27] This is supported by the hypothesis of autoallergy, in which atopy seems to stand at the boundary between allergy and auto-immunity, given the presence of IgE antibodies against self-proteins.[28-30]

Several gene-targeted and/or pathway-targeted treatment strategies for PIDs have recently been under clinical evaluation, which could be of clinical benefit in atopy as well. Identification of genetic pathways for these targeted and personalized treatment modalities is therefore essential.

We hypothesized that subclasses within the atopic syndrome exist based on genes that act in the same molecular pathway. Additionally, genetic defects in pathways that cause a PID might also be involved in the atopic syndrome.

Therefore, the aim of this study was to define subclasses within the atopic syndrome via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages. We first evaluated the overlap between atopy-related genes and monogenic PID genes. Secondly, we clustered the atopy-related genes based on their expression profile of immune cell lineages of healthy mice. Finally, we analyzed the pathways in which the atopy-related genes are involved.

MATERIALS AND METHODS

Data collection and content – overlap atopy/primary immunodeficiency disease genes

We obtained a complete list of all mutated genes responsible for atopic manifestations by performing a comprehensive search in the Human Gene Mutation Database (HGMD, HGMD® Professional, https://portal.biobase-international.com) up to August 21th 2018.[31] Genes were searched using the phenotype terms "atopy", "increased IgE", "atopic dermatitis", "eczema", "food allergy", "allergy", "asthma", and "allergic rhinitis" (Table S1). Atopy-related genes and the number of mutations per gene were extracted. Additionally, disease-causing genes of monogenic PIDs were obtained from the phenotypic classification for PIDs of the International Union of Immunological Societies (IUIS).[32] We performed a cross-check on atopy-related mutations in PID genes using HGMD. Overlapping genes between both the HGMD and PID lists were identified to select atopy-related with a defect in the same gene as a PID.

Clustering and visualization of atopy-related genes

The atopy-related genes were clustered to identify more homogeneous subclasses of the atopic syndrome. Clusters were made based on their gene expression profiles of immune cell lineages. Therefore, gene expression data from the Immunological Genome Project (ImmGen, http://www.immgen.org) was downloaded from the Genome Expression Omnibus (GEO) database accession number GSE15907 and GSE37448. The ImmGen datasets comprise the gene expression of a large amount of immune cell lineages (both hematopoietic and mesenchymal), that were grouped into 12 cell-populations. Currently, there is limited data on the gene expression signatures of human immune cell types. Therefore, immune cell lineages of healthy mice were used, which might give insights in atopic processes also applicable in human. All atopy-related genes selected via the HGMD query were searched in the ImmGen dataset. The top 40 co-expressed genes in mice were extracted per atopy-related gene. These genes are of biological interest as co-expressed genes are controlled by the same transcriptional regulatory program, functionally related, or members of the same pathway or protein complex as our atopy-related genes of interest.[33] We overlaid the co-expressed genes to identify genes that occurred in the top 40 lists of multiple atopy-related genes. Based on the overlap in co-expressed genes, indicating the degree of similar expression of atopy-related genes, the atopy-related genes were clustered in an unsupervised manner. Accordingly, it is likely that the clustered atopy-related genes act in the same molecular pathway. The clusters were visualized by constructing a correlation network plot using the "qgraph" package in RStudio version 3.4.1.[34] The lines between the genes were weighted and only correlations with a minimum correlation coefficient of 0.65, indicating a strong (positive) relationship, were visualized. If the top 40 list of an atopy-related gene did not contain a single overlapping gene, this atopy-related gene was labeled as an unclustered "bin" gene.

To visualize the gene expression profiles of the clusters, a heat map of the gene expression per cell lineage was constructed. Therefore, gene expression data were imported into Omniviz software version 6.1.13.0. Using Omniviz, the geometric mean of each probeset was calculated and transcriptomic data was log2 transformed to normalize the data. Changes in gene expression were constituted by deviations from the geometric mean to visualize whether genes of immune cell lineages were higher or lower expressed. These deviations are visualized in a heat map by a gradient from red (high expression) to blue (low expression) and ordered per cluster.

Functional pathway analysis

We validated whether the extracted genes from HGMD were atopy-related through analysis of the pathways containing these atopy-related genes. As the separate clusters included small numbers of genes, all clustered atopy-related genes from HGMD with and without unclustered "bin" genes were analyzed using Ingenuity Pathway Analysis (IPA,

Qiagen©) software.[35] The most important pathways, in which the atopy-related genes were involved, were extracted from IPA. The pathways were ranked according to their *p*-value (-log transformed) and the ratio of the atopy-related genes found in each pathway over the total number of molecules in that pathway, indicating the significance of the association between the atopy-related genes and the identified pathways. A *p*-value was calculated using a Fisher's Exact test to determine the probability of the association between the atopy-related genes and the pathways is explained by a random chance alone. A –log (*p*-value) equal to or greater than 1.3, corresponding to a *p*-value of 0.05, was considered statistically significant.

RESULTS

Content of data

The search in HGMD on atopic manifestations retrieved 159 atopy-related genes known in human (Table S1). Based on the overview of the IUIS, 278 disease-causing genes of monogenic PIDs were obtained.[36] During the cross-check on atopy-related mutations in PID genes, *TRAF3IP2* was identified in which mutations were described that might result in an eczema phenotype. This gene did not appear in the search results of HGMD and was therefore added to the list of atopy-related genes, resulting in a total of 160 genes for further analysis. The top three genes with the highest number of atopy-related mutations included *STAT3* (n=107), *FLG* (n=62) and *DOCK8* (n=45). Other genes had six or less atopy-related mutations per gene (Table S1). Twenty-two (5.3%) genes of the atopy (n=160) and PID (n=278) lists were overlapping, including *ARPC1B*, *BTK*, *CASP8*, *CFTR*, *CTLA4*, *DOCK8*, *ICOS*, *IL10*, *IL12B*, *IL12RB1*, *IL17F*, *IL21*, *IL21R*, *IL7R*, *ITK*, *ORAI1*, *PGM3*, *SPINK5*, *STAT3*, *TNFRSF13B*, *TRAF3IP2* and *TYK2* (Figure 1 and Table S1).

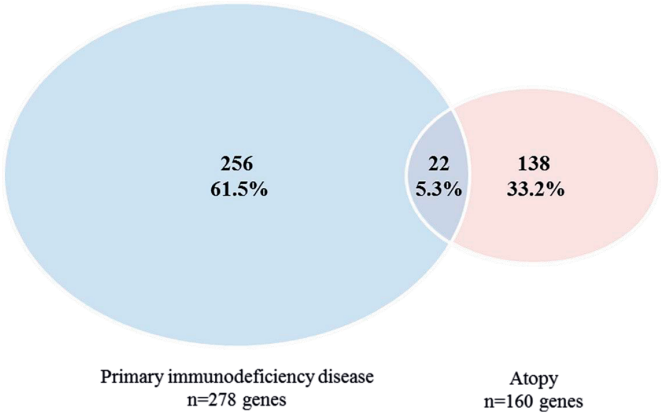

Figure 1. Venn diagram illustrating the overlap of the primary immunodeficiency disease-related genes and the atopy-related genes identified in the Human Gene Mutation Database

Clustering of genes

Fifteen (9.4%) of the 160 atopy-related genes were not expressed in the mouse immune system, of which immune cell lineages were used in the ImmGen dataset, and were therefore excluded from further analysis. As some genes had multiple transcripts and appeared more than once in the gene expression dataset, the complete list for clustering resulted in 153 probes. Eleven clusters were identified, of which seven clusters included five or more genes (clusters A, C, D, F, H, J and K), and 37 non-correlated genes remained as "bin" (Figure 2 and 3, Table S1). Based on the gene expression profiles, we identified one pair of anti-correlated clusters (clusters D and F), i.e. opposite expression profiles between clusters D and F (Figure 3). The 22 overlapping genes between the atopy-related genes and monogenic PID genes were localized in two of the seven atopy-related gene clusters, including cluster F (n=8) and cluster D (n=3) (Table S1).

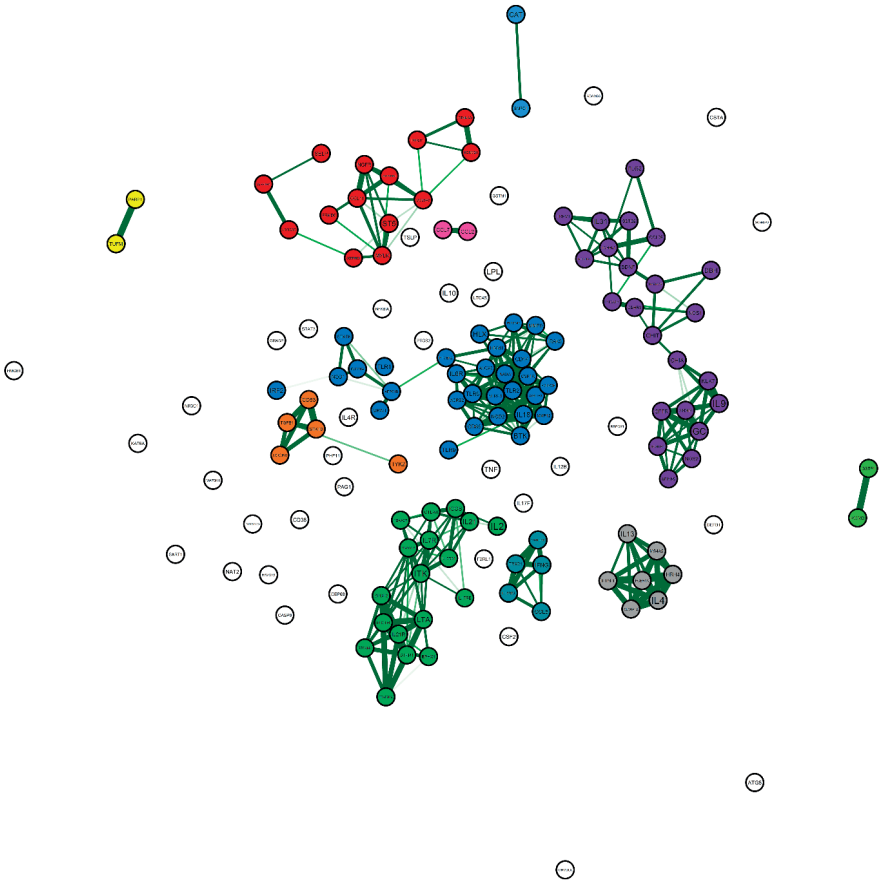

Figure 2. Genetic correlation network plot of atopy-related genes
The line width between the atopy-related genes indicate the overlay in the top 40 co-expressed gene lists per atopy-related gene and is proportional to the strength of correlation within the clusters.

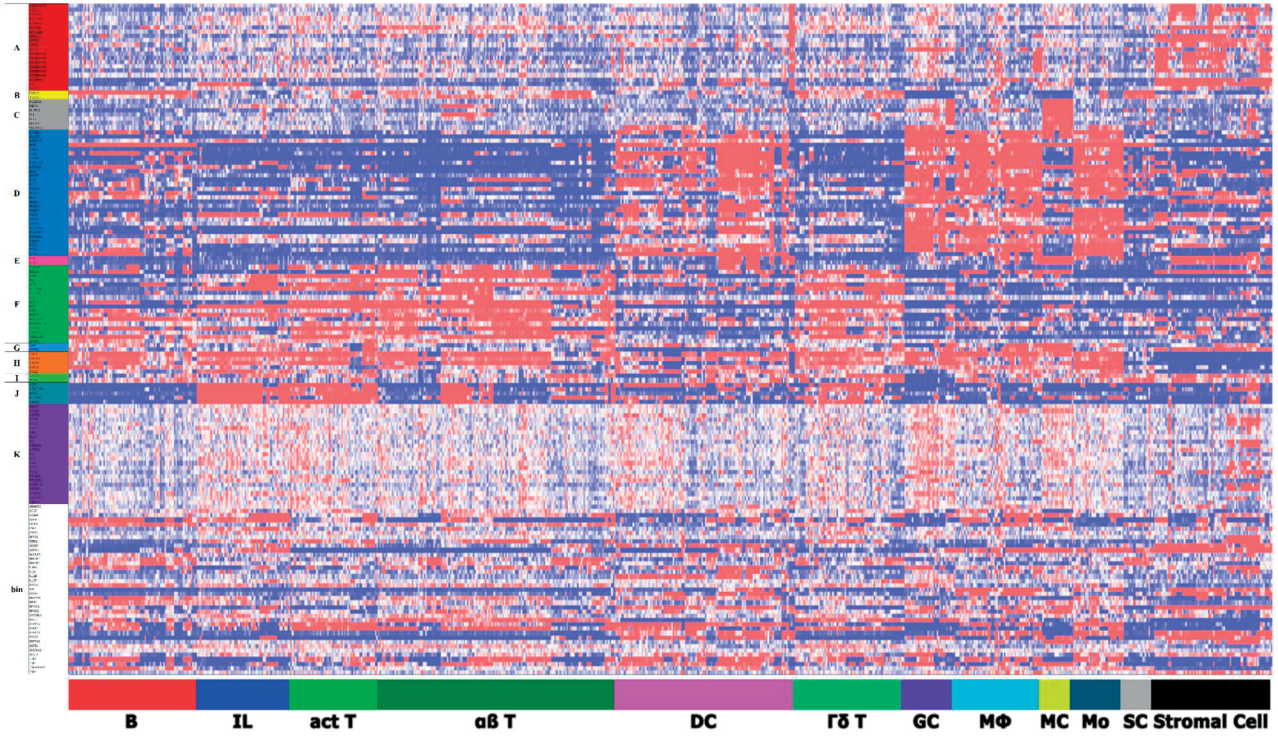

Figure 3. Heat map representing the atopy-related gene expression across the immune cell lineage of healthy mice ordered according to the identified clusters within the atopic syndrome

Data on the expression of atopy-related genes across the immune cell lineages was constructed using the Omniviz software, in which changes in gene expression were visualized by a gradient from red (high expression) to blue (low expression). Genes were alphabetically ordered according to the identified genetic clusters within the atopic syndrome. Abbreviations: B, B lymphocyte; IL, innate lymphocyte; act T, activated T lymphocyte; αβ T, αβ T lymphocyte; DC, dendritic cell; Γδ T, Γδ T lymphocyte, GC, granulocyte; MΦ, macrophage; MC, mast cell; Mo, monocyte; SC, stem cell.

Functional pathway analysis

Functional pathway analysis in IPA of the atopy-related genes both with and without taking unclustered "bin" genes into account resulted in T helper (Th) lymphocyte-mediated pathways. Taking all atopy-related genes (n=160) into account, it resulted in the specific pathways "T helper lymphocyte differentiation", "Th1 and Th2 activation pathway", and "Th2 pathway", in which respectively 22, 28, and 24 atopy-related genes were involved (Table S2a). Additionally, pathway analysis of the clustered atopy-related genes only (n=108) resulted in the specific pathways "Th1 and Th2 activation pathway" (n=22 genes), "T-helper lymphocyte differentiation" (n=16 genes), and "Th2 pathway" (n=19 genes) (Table S2b).

DISCUSSION

This is the first study that describes clusters in the clinically heterogeneous phenotype of the atopic syndrome based on gene expression profiles of immune cell lineages of healthy mice. The overlap between atopy-related genes (n=160) and monogenic PID genes (n=278) was limited to 22 (5.3%) genes. We identified seven distinct clusters within the atopic syndrome based on the expression profiles of atopy-related genes. Functional pathway analysis of all known atopy-related genes resulted in identification of Th lymphocyte-mediated processes underlying the atopic syndrome.

The atopic syndrome is a prevalent comorbidity in a number of PIDs, suggesting that the atopic syndrome can be a symptom of PIDs and that immune dysregulation plays a role in the pathogenesis. Interestingly, the number of overlapping genes in this study was limited (5.3%) and did not belong to one PID category according to the IUIS phenotypic classification or immunologic component.[32] Nonetheless, the overlapping genes were bundled in just two of the seven atopy-related gene clusters (cluster D and F), which suggests that these endotypes of the atopic syndrome are associated with the predisposition to develop a PID. However, atopy-related mutations in these genes might differ from the disease-causing mutations of the PIDs.

Current literature reports nine PIDs to be possibly related to the atopic syndrome, including autosomal dominant HIES (AD-HIES; *STAT3*), autosomal recessive HIES (AR-HIES; *DOCK8*), Comèl Netherton syndrome (*SPINK5*), hypogammaglobulinemia, selective IgA deficiency (SIgAD), IgM deficiency, IPEX (*FOXP3*), chronic granulomatous disease (CGD; *CYBA, CYBB, NCF1*, *NCF2* and *NCF4*), and phospholipase C gamma 2 (PLCG2) gene associated antibody deficiency and immune dysregulation (PLAID; *PLCG2*), and 28 additional genetic PID conditions.[27,37] Only eight genes (*STAT3, DOCK8*, *SPINK5*, *FLG*, *ARPC1B*, *PGM3*, *ERBIN*, and

TYK2) were extracted from HGMD using the atopy phenotype search. Furthermore, only two of the 22 overlapping atopy-related and PID-related genes identified in this study were reported in literature to be involved in PIDs and the atopic syndrome.[27] The discrepancy between literature and HGMD could firstly be explained by the recent expansion of novel mutations derived from next generation sequencing (NGS). Secondly, the atopic manifestations in PIDs, as described in literature, might be an occasional finding and not related to the disease causing genes of PIDs. Thirdly, the heterogeneous course and presentation of the atopic syndrome may make it difficult to associate genetic mutations with atopic manifestations. Moreover, the infectious symptoms in PIDs might be a more prominent clinical feature than the atopic manifestations, which therefore could have resulted in a registration bias.

We found a low number of mutations in most atopy-related genes in human (six or less mutations in 157 of the 160 genes), suggesting that other phenomena contribute to the disease such as post-translational modifications. Alternatively, various genes that interact with environmental factors might be involved in the atopic syndrome, in which each gene contributes only to a small amount of the overall disease risk.[38] Furthermore, the differences between the clusters could indicate that immune regulation plays a role in the atopic syndrome next to underlying genetic mechanisms.

Strikingly, two of the identified clusters (D and F) have a completely opposite expression profile, both in lymphoid and myeloid cell lineages. An explanation for this phenomenon may be that both clusters share the same upstream regulator. Depending on a gain or loss of function mutation in this enhancer, the gene expression profile can be influenced by an agonist or antagonist of this regulator. By performing a functional pathway analysis of the atopy-related genes in only clusters D and F, we would explore the functional significance of these clusters. The analysis resulted in the pathways "T helper cell differentiation", "TREM1 signaling" and "Th1 and Th2 activation pathway", which is completely corresponding with the pathways involved in all atopy-related genes (data not shown). Therefore, we could unfortunately not differentiate between the functional significance of all atopy-related genes and those included in clusters D and F.

The identified Th lymphocyte-mediated pathway supports the hypothesis that changes in the immune system underlie and could be involved in the pathogenesis of atopy. In AD it has been previously described that acute skin lesions are characterized by Th2 lymphocyte infiltration with a shift towards predominantly Th1 lymphocytes in the chronic phase.[39-41] In addition, asthma was reported as a Th2 lymphocyte -mediated diseases driven by allergen exposure.[42] Moreover, patients with FA and AR are characterized by allergen-specific Th2 lymphocyte-mediated responses showing that the obtained Th lymphocyte-pathways

involved in atopy are in agreement with these of the individual atopic diseases.[43-45] In most of our identified clusters (except clusters F, G, H, I and J) the atopy-related genes do not show increased expression in T lymphocytes (Figure 3). Therefore, genes in these clusters might be expressed in immunologic cells that co-interact with T lymphocytes, including Th lymphocytes, or in cells that are progenitors of Th lymphocytes.

This study has some limitations. Firstly, as we could not include terms concerning the skin barrier in the search, we might have missed gene expression profiles of barrier cells. However, by using the terms "atopic dermatitis" and "eczema" we have identified important barrier genes, like *COL6A5*, *FLG* (subtypes), *FLG2*, and *KLK7*. Secondly, some discrepancies were found in the HGMD database. The genes from the atopic phenotype search did not completely overlap with the results from the search on atopy-related mutations per gene. Therefore, we identified atopic phenotypes per gene on the results of both searches. Thirdly, we clustered genes based on their expression profiles in the ImmGen dataset, which uses characterized immune cells of mice. The gene expression profiles of immune cell lineages in healthy mice may not be identical to these in (atopic) humans. This explained why we could not cluster all human atopy-related genes including *FLG*, which is an important atopy gene based on the number of atopy-related mutations (n=62). Furthermore, the data from mice cannot directly be applied for subgrouping of the atopic syndrome in humans. Therefore, large cohorts of patients with the atopic phenotype should be sequenced using NGS to investigate whether atopy clusters could be generated based on the gene expression profiles of immune cell lineages of atopic human. Identification of clusters of atopy-related genes by NGS potentially opens novel ways to select eligible patients for pharmaceutical studies and could predict therapeutic responses.

CONCLUSION

This study shows a model, using data of healthy mice, to define clusters of the atopic syndrome based on gene expression profiles of immune cell lineages. We identified seven distinct clusters within the atopic syndrome in which Th lymphocyte-mediated pathways were most often involved. This supports the hypothesis that both genetic mechanisms and immune dysregulation have a role in the pathogenesis of the atopic syndrome. Our results also opens up the possibility for identification of novel therapeutic targets towards a more tailored approach and personalized medicine.

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SUPPLEMENTARY MATERIAL

Table S1. Atopy-related genes from the Human Gene Mutation Database

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
ADCYAP1R1	Adenylate Cyclase Activating Polypeptide 1 Receptor Type I	Asthma	NA	A
CCL11	C-C Motif Chemokine Ligand 11	Asthma	NA	A
COL6A5	Collagen Type VI Alpha 5 Chain	Dermatitis	4	A
CTNNA3	Catenin Alpha 3	Food allergy	2	A
FRMD6	FERM Domain Containing 6	Asthma	1	A
KCNMB1	Potassium Calcium-Activated Channel Subfamily M Regulatory Beta Subunit 1	Asthma	1	A
LRRC32	Leucine Rich Repeat Containing 32	Dermatitis	6	A
MYLK	Myosin Light Chain Kinase	Asthma	1	A
NGFR	Nerve Growth Factor Receptor	Asthma	NA	A
SELP	Selectin P	Atopy	1	A
SERPINA1	Serpin Family A Member 1	Asthma	1	A
SERPINE1	Serpin Family E Member 1	Asthma	NA	A
ST5	Suppression Of Tumorigenicity 5	Asthma	1	A
TWIST1	Twist Family BHLH Transcription Factor 1	IgE	NA	A
PARP1	Poly(ADP-Ribose) Polymerase 1	Asthma	NA	B
TUFM	Tu Translation Elongation Factor, Mitochondrial	Asthma	1	B
FCER1A	Fc Fragment Of IgE Receptor Ia	Dermatitis	1	C
HRH4	Histamine Receptor H4	Dermatitis	NA	C
IL1RL1	Interleukin 1 Receptor Like 1	Dermatitis	2	C
IL4	Interleukin 4	IgE Asthma	3	C
IL13	Interleukin 13	Atopy IgE Asthma Rhinitis	2	C
MS4A2	Membrane Spanning 4-Domains A2	Atopy IgE Asthma Rhinitis	5	C
SLC6A12	Solute Carrier Family 6 Member 12	Asthma	1	C
ADRB2	Adrenoceptor Beta 2	Dermatitis Asthma	2	D
ALOX5	Arachidonate 5-Lipoxygenase	Asthma	1	D
ARPC1B[†]	Actin Related Protein 2/3 Complex Subunit 1B	Allergy	1	D
BTK[†]	Bruton Tyrosine Kinase	Asthma	1	D
CD14	Cluster of Differentiation 14 Molecule	Asthma Rhinitis	NA	D
CD86	Cluster of Differentiation 86 Molecule	Asthma	1	D

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
CSF1R	Colony Stimulating Factor 1 Receptor	Asthma	1	D
CYSLTR1	Cysteinyl Leukotriene Receptor 1	Atopy Asthma	2	D
FCGR2B	Fc Fragment Of IgE Receptor IIb	Atopy	NA	D
HLX	H2.0 Like Homeobox	Asthma	2	D
HNMT	Histamine N-Methyltransferase	Dermatitis Asthma	NA	D
IL6R	Interleukin 6 Receptor	Dermatitis	NA	D
IL18	Interleukin 18	Asthma	1	D
INPP4A	Inositol Polyphosphate-4-Phosphatase Type I A	Asthma	1	D
IRAK3	Interleukin 1 Receptor Associated Kinase 3	Asthma	2	D
IRF2	Interferon Regulatory Factor 2	Dermatitis	1	D
MMP9	Matrix Metallopeptidase 9	Asthma Allergy	2	D
MMP12	Matrix Metallopeptidase 12	Asthma	NA	D
NLRP3	NLR Family Pyrin Domain Containing 3	Food allergy	2	D
NOD1	Nucleotide Binding Oligomerization Domain Containing 1	IgE Asthma	1	D
NOD2	Nucleotide Binding Oligomerization Domain Containing 2	Atopy Dermatitis Rhinitis	NA	D
ORAI1†	Calcium Release-Activated Calcium Modulator 1	Dermatitis	1	D
PLA2G4A	Phospholipase A2 Group IVA	Asthma	2	D
PLA2G7	Phospholipase A2 Group VII	Atopy Asthma	2	D
PTGER2	Prostaglandin E Receptor 2	Asthma	1	D
STAT6	Signal Transducer And Activator Of Transcription 6	IgE Dermatitis Asthma	3	D
TLR2	Toll Like Receptor 2	Dermatitis Asthma	1	D
TLR6	Toll Like Receptor 6	Asthma	1	D
TLR9	Toll Like Receptor 9	Dermatitis Asthma	1	D
CCL2	C-C Motif Chemokine Ligand 2	Asthma	NA	E
CCL7	C-C Motif Chemokine Ligand 7	Asthma	1	E
CTLA4†	Cytotoxic T-Lymphocyte Associated Protein 4	Asthma	1	F
EPHX1	Epoxide Hydrolase 1	Asthma	NA	F
ICOS†	Inducible T Cell Costimulator	Allergy	1	F
IL2	Interleukin 2	Allergy	2	F
IL7R†	Interleukin 7 Receptor	Dermatitis	NA	F

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
IL12RB1†	Interleukin 12 Receptor Subunit Beta 1	Dermatitis	1	F
IL17RB	Interleukin 17 Receptor B	Asthma	1	F
IL21†	Interleukin 21	Asthma	1	F
IL21R†	Interleukin 21 Receptor	IgE	1	F
ITK†	Interleukin 2 Inducible T Cell Kinase	Asthma	1	F
LTA	Lymphotoxin Alpha	Asthma	1	F
PDCD4	Programmed Cell Death 4	Asthma	1	F
PECAM1	Platelet And Endothelial Cell Adhesion Molecule 1	Asthma	NA	F
PPARGC1B	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Beta	Asthma	2	F
S1PR1	Sphingosine-1-Phosphate Receptor 1	Asthma	2	F
TBXA2R	Thromboxane A2 Receptor	IgE Dermatitis Asthma	2	F
TRAF3IP2†	TNF Receptor-Associated Factor 3 Interacting Protein 2	Dermatitis	2	F
ZPBP2	Zona Pellucida Binding Protein 2	Asthma	2	F
CAT	Catalase	Asthma	1	G
SMPD1	Sphingomyelin Phosphodiesterase 1	Allergy	1	G
CD53	Cluster of Differentiation 53 Molecule	Asthma	1	H
DOCK8†	Dedicator Of Cytokinesis 8	IgE	45	H
STK10	Serine/Threonine Kinase 10	Asthma	1	H
TGFB1	Transforming Growth Factor Beta 1	Asthma	NA	H
TYK2†	Tyrosine Kinase 2	IgE	1	H
GSTP1	Glutathione S-Transferase Pi 1	Dermatitis Asthma	NA	I
PGM3†	Phosphoglucomutase 3	Atopy IgE	6	I
CCL5	C-C Motif Chemokine Ligand 5	Dermatitis Asthma	1	J
CYSLTR2	Cysteinyl Leukotriene Receptor 2	Atopy	1	J
IFNG	Interferon Gamma	Atopy	NA	J
IL12RB2	Interleukin 12 Receptor Subunit Beta 2	Atopy	3	J
TBX21	T-Box 21	Asthma	1	J
BDNF	Brain Derived Neurotrophic Factor	Asthma	NA	K
CCL26	C-C Motif Chemokine Ligand 26	Asthma	1	K
CDHR3	Cadherin Related Family Member 3	Asthma	1	K
CFTR†	Cystic Fibrosis Transmembrane Conductance Regulator	Asthma	6	K
CHIA	Chitinase, Acidic	IgE Asthma	6	K

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
CHIT1	Chitinase 1	Asthma	NA	K
DBH	Dopamine Beta-Hydroxylase	Asthma	NA	K
FLG2	Filaggrin Family Member 2	Dermatitis	1	K
GC	GC, Vitamin D Binding Protein	Asthma	NA	K
GSDMA	Gasdermin A	Asthma	1	K
GSTO2	Glutathione S-Transferase Omega 2	Asthma	NA	K
IL9	Interleukin 9	Dermatitis	1	K
IL31	Interleukin 31	Dermatitis	1	K
KLK7	Kallikrein Related Peptidase 7	Dermatitis	1	K
NOS1	Nitric Oxide Synthase 1	Asthma	1	K
NOS2	Nitric Oxide Synthase 2	Atopy	NA	K
PTGDR	Prostaglandin D2 Receptor	Asthma Allergy	4	K
PTGDR2	Prostaglandin D2 Receptor 2	Asthma	2	K
SCGB1A1	Secretoglobin Family 1A Member 1	Asthma	1	K
SPINK5[†]	Serine Peptidase Inhibitor, Kazal Type 5	Atopy Asthma	3	K
TCHHL1	Trichohyalin Like 1	Dermatitis	1	K
TMEM79	Transmembrane Protein 79	Dermatitis	1	K
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1	Asthma	1	K
ADAM33	ADAM Metallopeptidase Domain 33	Asthma	NA	bin
ATG5	Autophagy Related 5	Asthma	2	bin
CASP8[†]	Caspase 8	Asthma	NA	bin
CD38	Cluster of Differentiation 38 Molecule	Asthma	1	bin
CEP68	Centrosomal Protein 68	Asthma	1	bin
CSF2	Colony Stimulating Factor 2	Dermatitis	1	bin
CSTA	Cystatin A	Dermatitis	1	bin
DEFB1	Defensin Beta 1	Dermatitis	NA	bin
F2RL1	F2R Like Trypsin Receptor 1	Atopy	1	bin
GRASP	General Receptor For Phosphoinositides 1 Associated Scaffold Protein	Asthma	1	bin
GSTM1	Glutathione S-Transferase Mu 1	Dermatitis Asthma	NA	bin
HAVCR1	Hepatitis A Virus Cellular Receptor 1	Asthma	3	bin
HMGB1	High Mobility Group Box 1	Dermatitis	3	bin
IL4R	Interleukin 4 Receptor	Atopy Dermatitis Asthma	6	bin
IL10[†]	Interleukin 10	Asthma	NA	bin

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
IL12B[†]	Interleukin 12B	Dermatitis Asthma	2	bin
IL17F[†]	Interleukin 17F	Asthma	1	bin
KAT6A	Lysine Acetyltransferase 6A	Food allergy	1	bin
LPL	Lipoprotein Lipase	Dermatitis	NA	bin
LTC4S	Leukotriene C4 Synthase	Asthma Allergy	2	bin
MAP3K1	Mitogen-Activated Protein Kinase 1	Asthma	1	bin
NAT2	N-Acetyltransferase 2	Asthma	2	bin
NFKBIA	Nuclear Factor Kappa B Inhibitor Alpha	Asthma	1	bin
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1	Asthma	NA	bin
ORMDL3	Orosomucoid Like 3	Asthma	3	bin
PAG1	Phosphoprotein Membrane Anchor With Glycosphingolipid Microdomains 1	Allergy	1	bin
PHF11	PHD Finger Protein 11	Dermatitis Asthma	2	bin
PTGS2	Prostaglandin-Endoperoxide Synthase 2	Atopy Asthma	NA	bin
RBFOX1	RNA Binding Fox-1 Homolog 1	Food allergy	2	bin
SART1	U4/U6.U5 Tri-SnRNP-Associated Protein 1	Atopy	1	bin
SCGB3A2	Secretoglobin Family 3A Member 2	Asthma	1	bin
STAT3[†]	Signal Transducer And Activator Of Transcription 3	IgE	107	bin
TLR1	Toll Like Receptor 1	Asthma	NA	bin
TNF	Tumor Necrosis Factor	Asthma	NA	bin
TNFRSF13B[†]	Tumor Necrosis Factor Receptor Superfamily Member 13B	Asthma	NA	bin
TSLP	Thymic Stromal Lymphopoietin	Asthma	1	bin
ACE	Angiotensin I Converting Enzyme	Asthma	NA	NA
CCL3L1	C-C Motif Chemokine Ligand 3 Like 1	Asthma	NA	NA
CHI3L1	Chitinase 3 Like 1	Asthma	1	NA
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19	Asthma	NA	NA
CYP2J2	Cytochrome P450 Family 2 Subfamily J Member 2	Asthma	NA	NA
ERBIN	Erb-B2 Interacting Protein	IgE	1	NA
FCGR2A	Fc Fragment Of IgE Receptor IIa	Atopy Allergy	1	NA
FLG	Filaggrin	Dermatitis Food allergy Asthma	62	NA
FLG10.2	Fillagrin Alternative Isoform, Repeat 10.2	Dermatitis	NA	NA
FLG11	Fillagrin Alternative Isoform, Repeat 11	Dermatitis Asthma	1	NA

Table S1. Atopy-related genes from the Human Gene Mutation Database (continued)

Gene symbol	Gene description	Atopic symptom involved	Number of atopy mutations	Cluster[1]
GSDMB	Gasdermin B	Asthma	4	NA
LCE3C	Late Cornified Envelope 3C	Dermatitis	NA	NA
MUC7	Mucin 7, Secreted	Asthma	1	NA
TLR10	Toll Like Receptor 10	Asthma Rhinitis	1	NA
VSTM1	V-Set And Transmembrane Domain Containing 1	Dermatitis	1	NA
ACE	Angiotensin I Converting Enzyme	Asthma	NA	NA
CCL3L1	C-C Motif Chemokine Ligand 3 Like 1	Asthma	NA	NA
CHI3L1	Chitinase 3 Like 1	Asthma	1	NA
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19	Asthma	NA	NA
CYP2J2	Cytochrome P450 Family 2 Subfamily J Member 2	Asthma	NA	NA
ERBIN	Erb-B2 Interacting Protein	IgE	1	NA
FCGR2A	Fc Fragment Of IgE Receptor IIa	Atopy Allergy	1	NA
FLG	Filaggrin	Dermatitis Food allergy Asthma	62	NA
FLG10.2	Fillagrin Alternative Isoform, Repeat 10.2	Dermatitis	NA	NA
FLG11	Fillagrin Alternative Isoform, Repeat 11	Dermatitis Asthma	1	NA
GSDMB	Gasdermin B	Asthma	4	NA
LCE3C	Late Cornified Envelope 3C	Dermatitis	NA	NA
MUC7	Mucin 7, Secreted	Asthma	1	NA
TLR10	Toll Like Receptor 10	Asthma Rhinitis	1	NA
VSTM1	V-Set And Transmembrane Domain Containing 1	Dermatitis	1	NA

Abbreviations: NA, not available. [1]Genes were not available for clustering because they could not be identified in the mouse immune system of ImmGen. [†]Overlapping genes between atopy-related gene list and PID-related gene list (n=22).

Table S2. Ingenuity pathway analysis – top three pathways of all atopy genes (n=160)

Pathways	-log (*p*-value)[1]	Ratio[2]	Molecules
T Helper Cell Differentiation	2,95E01	3,01E-01	*IL21, STAT6, IFNG, IL4R, IL12RB1, IL10, IL21R, IL6R, IL12RB2, STAT3, TBX21, IL13, IL18, IL2, TGFB1, IL12B, NGFR, ICOS, CD86, IL17F, TNF, IL4*
Th1 and Th2 Activation Pathway	2,84E01	1,51E-01	*IL1RL1, IL12RB1, IL31, TBX21, IL2, TGFB1, IL9, IL4, STAT6, IFNG, PTGDR2, IL4R, IL10, HAVCR1, IL6R, TYK2, IL12RB2, STAT3, TLR9, IL13, TSLP, IL18, IL17RB, IL12B, LTA, ICOS, S1PR1, CD86*
Th2 Pathway	2,49E01	1,6E-01	*STAT6, IFNG, IL4R, PTGDR2, IL12RB1, IL10, HAVCR1, IL1RL1, TYK2, IL12RB2, IL31, TLR9, IL13, TBX21, TSLP, IL17RB, IL2, TGFB1, IL12B, ICOS, IL9, S1PR1, CD86, IL4*

[1]The –log (*p*-value) indicates the probability of the association of atopy-related genes with the pathway by random chance alone. [2]The ratio is calculated by the number of atopy-related genes in a given pathway that have a –log (*p*-value) equal to or greater than 1.3 (default cutoff value), divided by the total number of atopy-related genes that make up that pathway.

Table S2b. Ingenuity pathway analysis – top 3 pathways of atopy genes without 'bin' genes (n=108)

Pathways	-log (*p*-value)[1]	Ratio[2]	Molecules
Th1 and Th2 Activation Pathway	2,37E01	1,19E-01	*STAT6, IFNG, PTGDR2, IL12RB1, IL1RL1, TYK2, IL6R, IL12RB2, IL31, TBX21, IL13, TLR9, IL18, IL17RB, IL2, TGFB1, LTA, ICOS, IL9, S1PR1, CD86, IL4*
T Helper Cell Differentiation	2,18E01	2,19E-01	*IL21, IFNG, STAT6, IL12RB1, IL21R, IL6R, IL12RB2, TBX21, IL13, IL18, IL2, TGFB1, NGFR, ICOS, CD86, IL4*
Th2 Pathway	2,1E01	1,27E-01	*STAT6, IFNG, PTGDR2, IL12RB1, IL1RL1, TYK2, IL12RB2, IL31, TBX21, IL13, TLR9, IL17RB, IL2, TGFB1, ICOS, IL9, S1PR1, CD86, IL4*

[1]The –log (*p*-value) indicates the probability of the association of atopy-related genes with the pathway by random chance alone. [2]The ratio is calculated by the number of atopy-related genes in a given pathway that have a –log (*p*-value) equal to or greater than 1.3 (default cutoff value), divided by the total number of atopy-related genes that make up that pathway.

Chapter 5

The prevalence of antibody responses against *Staphylococcus aureus* antigens in patients with atopic dermatitis: a systematic review and meta-analysis

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ABSTRACT

Background

Staphylococcus (*S.*) *aureus* plays a role in the pathogenesis of atopic dermatitis (AD), possibly via the expression of various virulence antigens. An altered antibody response towards these antigens might contribute to inflammation.

Objective

We aimed to provide an overview of the varying prevalence and odds of antibody responses against *S. aureus* antigens in patients with AD.

Methods

Data were systematically obtained from EMBASE, MEDLINE, Web of Science, Scopus, Cochrane, PubMed, and Google Scholar up to 12 February 2016. We selected all original observational and experimental studies assessing antistaphylococcal antibodies in serum of patients with AD. Prevalence and odds ratios (ORs) of immunoglobulin (Ig) E, IgG, IgM, and IgA against *S. aureus* in patients with AD vs. healthy controls were pooled using the random-effects model. We calculated I^2 statistics to assess heterogeneity and rated study quality using the Newcastle-Ottawa Scale.

Results

Twenty-six articles (2369 patients) were included, of which 10 were controlled studies. Study quality was fair to poor. Patients with AD had a higher prevalence of IgE against staphylococcal enterotoxin (SE)A (OR 8.37, 95% confidence interval 2.93–23.92) and SEB (OR 9.34, 95% confidence interval 3.54–24.93) compared with controls. Prevalence of antistaphylococcal IgE was 33% for SEA, 35% for SEB and 16% for toxic shock syndrome toxin-1. However, study heterogeneity and imprecision should be taken into consideration when interpreting the results. Data on IgG, IgM, and IgA, as well as other antigens, are limited.

Conclusion

Patients with AD more often show an IgE antibody response directed against *S. aureus* superantigens compared with healthy controls, supporting a role for *S. aureus* in AD pathogenesis.

INTRODUCTION

Atopic dermatitis (AD) is a multifactorial disorder that arises from interactions between immune dysregulations, genetic predisposition, skin barrier defects and environmental factors.[1,2] Both the lesional and nonlesional skin and the nose of patients with AD are more likely to be colonized with *Staphylococcus* (*S.*) *aureus* compared with healthy controls.[3] Recent studies have shown that abundance of *S. aureus* is associated with AD severity, suggesting a causal role for *S. aureus* in the pathogenesis of AD.[2,4-9] However, the exact mechanisms by which *S. aureus* aggravates inflammation in AD are not fully understood.[10]

S. aureus expresses a variety of virulence factors that could contribute to AD inflammation. Based on their biological function, these antigens can be divided in four groups: (i) Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) such as Clumping factor A (ClfA), which helps *S. aureus* adhere to the host cells; (ii) cell-membrane damaging molecules such as alpha toxin, which can induce keratinocyte cell death; (iii) household enzymes such as lipase, which provides cell nutrition; and (iv) immune modulating proteins (superantigenic and nonsuperantigenic).[10-13] The latter include the group of staphylococcal superantigens, which have the ability to activate mast cells and T lymphocytes directly, resulting in the release of proinflammatory cytokines.[5,14-16] Expression of these *S. aureus* antigens varies between the different *S. aureus* isolates. However, it has been proven difficult to identify associations between the genetic composition of *S. aureus* strains and AD.[17-22]

Evaluation of the antibody response to these *S. aureus* antigens gives an indication of the antigens that are expressed by the bacterium *in vivo* and will give insight into how the immune system of patients with AD counteracts these antigens. This might help us to understand the role of *S. aureus* in AD pathogenesis, as well as the mechanisms by which *S. aureus* causes inflammation. Since 1982, several studies have reported serum antibodies against *S. aureus* in patients with AD.[23-35] However, the prevalence of antistaphylococcal antibodies in these studies vary widely. This is probably due to low sample sizes and different methods used to detect antibodies (e.g. enzyme-linked immunosorbent assay (ELISA) or AlaSTAT). Moreover, studies often focus on few antigens and/or antibody classes.

The aim of this systematic review was to provide an overview of the pooled prevalence and odds of antibodies (immunoglobulin (Ig) E, IgG, IgM, and IgA) against *S. aureus* antigens in serum of patients with AD compared with healthy controls. Additionally, we reviewed the relationship between AD severity and anti-*S. aureus* antibodies.

MATERIALS AND METHODS

Study participants and outcomes

All original observational and experimental human studies were included. No restrictions were made on publication date or language. Case reports were excluded.

Patients of all ages with AD, irrespective of disease severity, and in which anti-*S. aureus* antibodies were measured were included. Healthy controls were defined as persons who had neither AD nor an atopic constitution (food allergy, asthma, allergic rhinitis) nor another skin disease.

The primary outcome was the proportion of patients with AD with antibodies (IgE, IgG, IgM, and IgA) in serum against *S. aureus* antigens compared with healthy controls. The secondary out-come was the relationship between AD severity and antistaphylococcal antibodies.

Search strategy

The systematically electronic search was conducted in EMBASE, MEDLINE, Web of Science, Scopus, Cochrane, PubMed, and Google Scholar up to 12 February 2016. A cross-reference check was performed to identify other relevant studies.

Study selection and data extraction

Initially, all studies identified in the systematic search were screened for relevance by title and abstract. Duplicates and studies that did not meet our inclusion criteria were excluded (Appendix 1). The remaining articles were assessed for eligibility by full-text review. Translation of non-English studies was conducted officially. Study selection and data extraction were performed independently by two researchers (FvB and either JT or JdW). Disagreements were resolved and consensus was reached. If one population was described in different articles, we included the study with the most detailed description of the results.

The methodological quality of the articles was scored based on an adapted version of the Newcastle-Ottawa Scale (NOS).[3] Studies could reach a maximum score of nine points for case-control studies and five points for uncontrolled studies. Using a scoring algorithm, the controlled studies were classified as being of poor, fair or good methodological quality, based on their NOS scores (Appendix 2).[36] The overall quality of evidence was discussed according to the principles of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (i.e. limitations in study design or execution, inconsistency of results, indirectness of evidence, imprecision, publication bias).[37]

Statistical analysis

A meta-analysis was performed using a random-effects model in case of at least two available studies. We extracted the prevalence of antistaphylococcal antibodies in patients with AD and controls from the included studies. If required, we calculated the prevalence with the available raw data. The prevalence of antistaphylococcal antibodies were pooled. Furthermore, in controlled studies the prevalence in patients and controls were compared, expressed as ORs with a 95% confidence interval (CI). The antibody prevalence was descriptively presented for single studies. When the antibody prevalence in the control group was 0%, an OR could not be calculated and a continuity correction factor using the Mantel-Haenszel method was added to both the patient and control group (based on the unbalanced group ratio).[38,39]

Heterogeneity was assessed using the Higgins I^2 test.[40,41] However, I^2-values should be interpreted cautiously in small meta-analyses.[42] In case of substantial (I^2 50-90%) or considerable (I^2 75-100%) heterogeneity, sources were explored using subgroup analyses for the variables age, method of antibody identification and geographical region of the study centres (≥10 available studies). Possible publication bias was assessed in case of ≥10 studies using funnel plots and Egger's test (p-value <0.05).[43,44] Analyses were performed using Comprehensive Meta-Analysis version 2.2 (Biostat, Englewood, NJ, U.S.A.). This systematic review was conducted and reported according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines.[45]

RESULTS

Study characteristics and quality

The literature search identified 2789 studies. After removal of duplicates, 1,323 studies remained. Screening on title and abstract yielded 113 full-text articles. Finally, 26 articles with a total of 2369 patients were included for further analysis (Figure 1).[24,25,27-35,46-60] Twenty-one articles reported the sex of the patients, with a mean percentage of male participants of 53.4% (range 28.1-81.8).[25,28-30,33-35,46-49,51-60] The mean age was 24.1 years (range 4.4-68.9), reported in 15 articles.[25,28-30,33,34,46-49,51,53-55,58] Thirteen articles scored the AD severity using the Scoring Atopic Dermatitis (SCORAD), with three articles reporting a mean of 33.6 (range 32.2-36.0), corresponding to moderate AD.[30,32-35,46,47,49-52,57,59,61] Nine articles used other scoring criteria for the AD severity.[25,28,29,31,48,54-56,58] Most studies were conducted in Europe and Asia.

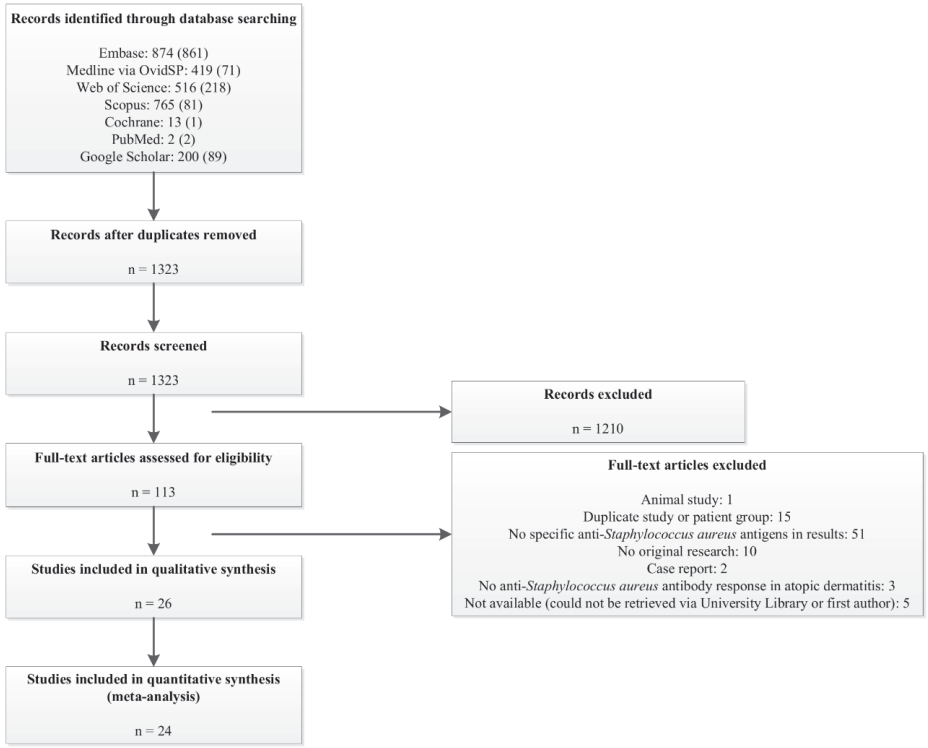

Figure 1. Flow chart of search strategy and study selection

Methods for identification of antistaphylococcal antibodies consist mainly of radioimmunoassay (RIA) tests, ELISA, and AlaSTAT, an enzyme immunoassay method for the measurement of allergen-specific IgE (Table S1). One study measuring multiple antibodies used both a RIA test for IgE and an ELISA for IgG.[31] NOS scores of the 11 controlled studies were rated as good (n=1), fair (n=5) or poor (n=5).[24,25,27-33,36,53,60] The main reason for downgrading the quality of these studies was incomparability of the patient and control groups. The quality scores of the 15 studies without a control group varied between two and four points out of five (Table S1 and Appendix 2).[34,35,46-52,54-59]

Prevalence of antibodies against *Staphylococcus aureus*

IgE

Twenty-four studies including 2206 patients reported the prevalence of antistaphylococcal IgE. [24,25,27-31,33-35,46-59] These studies predominantly determine the antibody response against staphylococcal enterotoxin (SE) A, SEB, SEC, SED, and toxic shock syndrome toxin (TSST)-1 (19, 23, seven, three and 10 studies, respectively) (Table S2).[24,25,27-31,34,35,46-59] Pooled prevalence of antistaphylococcal IgE in patients was 33% for SEA (95% CI 23-45; I^2 94.23)[24,25,27-30,34,35,46-48,50,51,53,54,56-59], 35% for SEB (95% CI 27-43; I^2 91.36)[24,25,27-31,34,35,46-59], 14%

for SEC (95% CI 8-22; I^2 78.26)[24,34,46,47,52,56,58], 5% for SED (95% CI 1-16; I^2 70.49)[24,34,47] and 16% for TSST-1 (95% CI 10-25; I^2 85.28)[24,27,34,35,46-48,52,56,58] (Table 1). There was a great variation in prevalence between studies (0.8-78.8% for SEA, 1.4-72.9% for SEB, 5.4-40.0% for SEC, 0.0-10.7% for SED and 1.4-53.3% for TSST-1), probably resulting in the substantial to considerable heterogeneity. One study showed a prevalence of 35.8% of fibronectin-binding protein (FBP)-specific IgE, another study found a prevalence of 48.1% of IgE against lipoteichoic acid (LTA).[27,33] Undetectable to very low prevalences of IgE against the staphylococcal antigens SEE, SEI, SEH, SEK, SEJ, exfoliative toxin (ET)-1 and ETA were found in several single studies (Table 2).[24,31,47]

IgG, IgM and IgA

Prevalence of IgG against *S. aureus* antigens was determined in four studies.[31,32,55,60] The pooled prevalence of IgG against SEB, reported in two studies (114 patients), was 64% (95% CI 42-81; I^2 78.84).[55,60] Besides, in single studies the IgG prevalence was 77.0% for SEA, 77.0% for TSST-1, and 34.6% for ETA.[31,60] IgG subclass 2 (IgG_2) was found in 87.0% of the patients with AD against SEB and in 61.5% against SEC_1.[32] Only one study determined antistaphylococcal IgM, and detect antibodies against SEB in 62.5% of the patients with AD (Table 2).[55] None of the selected articles studied antistaphylococcal IgA.

Odds of antibodies against *Staphylococcus aureus*

Of the 26 articles, 11 studies compared patients with AD to healthy controls (759 patients vs. 328 controls).[24,25,27-33,53,60] Nine studies reported the prevalence of antistaphylococcal IgE (596 patients vs. 189 controls).[24,25,27-31,33,53] These studies mainly described antibody responses against SEA and SEB (seven and eight articles, respectively).[24,25,27-31,53] Pooled analyses showed that antistaphylococcal IgE was found significantly more often in the serum of patients compared with controls, with ORs of 8.37 for SEA (95% CI 2.93-23.92; $p<0.001$; I^2 0.00)[24,25,27-30,53] and 9.34 for SEB (95% CI 3.54-24.93; $p<0.001$; I^2 0.00)[24,25,27-31,53] (Table 3). The pooled OR of IgE against TSST-1, reported in two studies (83 patients vs. 20 controls), was 23.33 (95% CI 0.47-1153.93, $p=0.114$, I^2 0.00) (Table 3).[24,27] Prevalences of other antigens, including SEC, SED, ETA, ET-1, FBP, and LTA, were described in single controlled studies and pooled estimates could not be provided.[24,27,31,33] The prevalence of all these *S. aureus* antigens were equal or increased in patients vs. controls (Table 4). As most antibody prevalences in control groups were 0%, the ORs could not be calculated. Prevalences of IgG in patients and controls were compared in three studies.[31,32,60] Compared with controls, patients were found to have higher IgG prevalences to ETA and SEB and lower prevalences of IgG to SEA and TSST-1.[31,60] In patients, the IgG_2 prevalence to SEC_1 was lower and to SEB higher than in controls.[32] However, most differences in prevalence between patients and controls were small. No studies compared the antistaphylococcal IgM or IgA responses between patients and controls.

Table 1. IgE against staphylococcal enterotoxin (SE) A, B, C and D; and toxic shock syndrome toxin (TSST)-1 in patients with atopic dermatitis

Antigen (subgroup)	Number of studies	Number of patients	Pooled proportion of patients with detectable antigens (95% CI)	Heterogeneity (I^2)
SEA				
All studies	19	1852	0.33 (0.23-0.45)	94.23
Studies including age <18	6	507	0.31 (0.11-0.63)	96.56
Studies including age ≥18	7	859	0.27 (0.17-0.42)	88.22
Studies including RIA methods*	8	1139	0.19 (0.12-0.29)	86.36
Studies including ELISA method	3	169	0.61 (0.34-0.82)	86.83
Studies including AlaSTAT method	6	461	0.42 (0.28-0.57)	89.04
Studies including Immunoblot method	1	27	0.48 (0.30-0.66)	-
Studies performed in Europe	11	1220	0.24 (0.16-0.34)	87.87
Studies performed in Asia	7	576	0.51 (0.33-0.70)	93.75
Studies performed in USA	1	56	0.32 (0.21-0.45)	-
SEB				
All studies	23	2111	0.35 (0.27-0.43)	91.36
Studies including age <18	8	631	0.25 (0.13-0.43)	92.73
Studies including age ≥18	8	968	0.38 (0.29-0.48)	84.70
Studies including RIA methods*	11	1418	0.25 (0.18-0.34)	86.77
Studies including ELISA method	4	209	0.47 (0.24-0.72)	89.39
Studies including AlaSTAT method	6	461	0.48 (0.33-0.64)	90.42
Studies including Immunoblot method	1	27	0.63 (0.44-0.79)	-
Studies performed in Europe	12	1304	0.28 (0.21-0.36)	84.70
Studies performed in Asia	10	751	0.48 (0.36-0.61)	90.06
Studies performed in USA	1	56	0.18 (0.10-0.30)	-
SEC				
All studies	7	540	0.14 (0.08-0.22)	78.26
SED				
All studies	3	317	0.05 (0.01-0.16)	70.49
TSST-1				
All studies	10	1110	0.16 (0.10-0.25)	85.28
Studies including age <18	5	631	0.13 (0.05-0.28)	85.00
Studies including age ≥18	3	1039	0.12 (0.05-0.27)	84.65
Studies including RIA methods*	6	918	0.12 (0.05-0.25)	90.38
Studies including ELISA method	2	109	0.18 (0.11-0.26)	0.00
Studies including Immunoblot method	1	27	0.41 (0.24-0.60)	-
Studies performed in Europe	7	945	0.15 (0.07-0.28)	90.09
Studies performed in Asia	2	109	0.18 (0.11-0.26)	0.00
Studies performed in USA	1	56	0.21 (0.13-0.34)	-

Abbreviations: SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin; RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; CI, confidence interval. *CAP fluorescent enzyme immunoassay (FEIA), ImmunoCAP, and UniCAP.

Table 2. IgE, IgG and IgM against *Staphylococcus aureus* antigens in patients with atopic dermatitis

Antibody	*Staphylococcus aureus* antigen	Number of studies	Number of patients	(Pooled) proportion of patients with detectable antigens (95% CI)	Heterogeneity (I^2)
IgE	SEE	1	140	0.01	
	SEI	1	140	0.01	
	SEH	1	140	0.00	
	SEK	1	140	0.00	
	SEJ	1	140	0.00	
	ETA	1	26	0.00	
	FBP	1	95	0.36	
	LTA	1	27	0.48	
IgG	SEA	1	74	0.77	
	SEB	2	114	0.64 (0.42-0.81)	78.84
	TSST-1	1	74	0.77	
	ETA	1	26	0.35	
IgG_2	SEB	1	77	0.87	
	SEC_1	1	78	0.62	
IgM	SEB	1	40	0.63	

Abbreviations: Ig, immunoglobulin; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein; LTA, lipoteichoic acid; CI, confidence interval.

Table 3. IgE against staphylococcal enterotoxin (SE) A and SEB and toxic shock syndrome toxin (TSST)-1 in patients with atopic dermatitis vs. healthy controls

Staphylococcus aureus antigen	Number of studies	Number of patients	Number of controls	Pooled OR in patients vs controls (95% CI)	Heterogeneity (I^2)
SEA	7	475	139	8.37 (2.93-23.92)*	0.00
SEB	8	501	172	9.34 (3.54-24.93)*	0.00
TSST-1	2	83	20	23.33 (0.47-1153.93)	0.00

Abbreviations: SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; OR, odds ratio; CI, confidence interval. *Significant result.

Subgroup analysis

Subgroup analyses of the variables age, method of antibody identification and geographical region of the study centres were performed to detect possible sources of heterogeneity. The prevalence of IgE against SEA, SEB, and TSST-1 did not differ significantly between children and adults (31% vs. 27%, 25% vs. 38%, and 13% vs. 12%, respectively). Studies using the ELISA method showed higher pooled prevalences of IgE against SEA, SEB, and TSST-1 compared with studies using RIA tests (61% vs. 19%, 47% vs. 25%, and 18% vs. 12%, respectively). Lastly, studies conducted in Asia showed higher pooled prevalences of IgE to SEA, SEB, and TSST-1 than studies conducted in Europe (51% vs. 24%, 48% vs. 28%, and 18% vs. 15%, respectively) (Table 1).

Table 4. IgE and IgG against *Staphylococcus aureus* antigens in patients with atopic dermatitis vs. healthy controls

Antigen	*Staphylococcus aureus* antibody	Number of studies	Number of patients	Number of controls	Mean proportion of patients with detectable antigens	Mean proportion of controls with detectable antigens	*p*-value
IgE	SEC	1	56	15	0.05	0.0	-
	SED	1	56	15	0.05	0.0	-
	ETA	1	26	33	0.00	0.0	-
	ET	1	56	15	0.02	0.0	-
	FBP	1	95	17	0.36	0.0	-
IgG	SEA	1	74	111	0.77	0.88	-
	SEB	1	74	111	0.73	0.69	-
	TSST-1	1	74	111	0.77	0.85	-
	ETA	1	26	14	0.35	0.14	-
IgG_2	SEB	1	77	27	0.87	0.78	-
	SEC_1	1	78	28	0.62	0.86	-

Abbreviations: Ig, immunoglobulin; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein.

Relationship between atopic dermatitis severity and antibodies against *Staphylococcus aureus*

Considering the low number of studies reporting a mean SCORAD, we could not calculate an overall association between AD severity and antistaphylococcal antibodies. However, several individual studies reported a significant association between superantigen-specific (e.g. SEA, SEB) IgE and AD severity, measured by SCORAD, the criteria of Rajka or the modified Leicester system.[29,32,55,58] This association could not be confirmed in four comparable studies.[25,28,46,58] Sohn *et al*.[55] looked at IgG against SEB and did not find a relationship with AD severity. However, Mrabet-Dahbi *et al*.[32] found that patients with a deficiency of antistaphylococcal IgG_2 to SEC_1 had a more severe AD phenotype. Based on these contradictory studies, no conclusions can be drawn about the association between the antistaphylococcal antibody response and severity of AD.

Publication bias

Funnel plots of the pooled prevalence of IgE against SEA, SEB, and TSST-1 showed no asymmetry. Egger's tests had intercepts of 0.52 for SEA (95% CI -4.40-5.44, p=0.826), -0.44 for SEB (95% CI -3.78-2.91, p=0.789), and -0.82 for TSST-1 (95% CI -4.40-2.76, p=0.611) confirming no publication bias.

DISCUSSION

This systematic review includes 26 studies and 2352 patients with AD. IgE responses against SEA and SEB in serum were found more often in patients with AD than in healthy controls. IgE, IgG, and IgM against a very limited panel of other antigens were reported in single studies. No data are available on antistaphylococcal IgA. Pooled prevalences of antistaphylococcal IgE in patients with AD are 33% for SEA, 35% for SEB, and 16% for TSST-1. Substantial to considerable heterogeneity and imprecision (small studies) limit the quality of evidence and should be taken into consideration when interpreting the results. Subgroup analyses were performed to account for differences in outcome measures (indirectness). Quality of evidence was probably not influenced by publication bias.

Subgroup analyses suggest that the antibody prevalence is dependent on the method of antibody identification (ELISA vs. RIA) and the geographical region of the study centres (Asia vs. Europe). This is in accordance with the study of Taylor *et al*.[62] that found ELISA more sensitive than RIA to detect IgG_1 in mice. It might also explain the higher prevalence of antibodies in Asia than in Europe, as Asian studies use ELISA techniques more often. Furthermore, ethnicity-dependent antibody response has been suggested, at least for TSST-1.[63] Because heterogeneity in subgroup analyses remains high, pooled prevalences and odds were probably also influenced by other variables, such as AD severity. Unfortunately, we were not able to explore this as only a few studies reported a mean SCORAD. These individual studies showed contradictory results about the association between AD severity and IgE against predominantly superantigens.

The *S. aureus* antigens SEA and SEB belong to the group of immune modulators and act as superantigens. This indicates that they have the ability to stimulate T lymphocytes directly, resulting in T lymphocyte proliferation and cytokine release, causing epithelial damage.[14-16] The increased anti-SEA and anti-SEB IgE responses could be the result of increased expression of these antigens by the *S. aureus* bacteria in patients with AD, indicating SEA and SEB as possible bacterial mechanisms to aggravate or even initiate inflammation in AD. However, the studies included in this systematic review predominantly examined the prevalence of antibodies against the superantigens SEA, SEB, and TSST-1, and other common antigens, such as ClfA and lipase, were not tested. In addition, SEA, SEB, and TSST-1 are present in only 14%, 24%, and 14%, respectively, of the *S. aureus* isolates.[64] These data suggest a bias in the assessment of staphylococcal antigens and also indicate a large genetic diversity amongst the colonized *S. aureus* strains. Furthermore, the increased IgE responses against these antigens may be the result of immunological cross-reactivity, where the corresponding antigen-coding genes of SEA, SEB and/or TSST-1 are not present in the isolate.[65-67]

This is the first systematic review summarizing the available data on the prevalence of antistaphylococcal antibodies in patients with AD and the involved antigens. The broad selection criteria (e.g. all languages, only exclusion of case reports, and nonoriginal studies) resulted in collecting the majority of articles about this subject and limiting selection bias. However, there are still some limitations in this study. Firstly, most articles did not report the AD treatment at time of antibody measurement. The use of antimicrobial therapy might decrease the *S. aureus* load and *S. aureus* antibody titres.[4] In addition, the anti-inflammatory effect of systemic glucocorticosteroids could cause a decrease in serum antibody concentrations and might also reduce *S. aureus.*[6,68,69] Even emollient monotherapy showed a decrease of *S. aureus* on the skin.[70,71] In the studies that did report the treatment at baseline, the therapies consist mainly of topical corticosteroids or no treatment at all (n=9). Secondly, cut-off values of antibody identification methods were highly variable, not mentioned or unclear in and between several methods. Through subgroup analysis, we tried to correct for this variability partly. Lastly, mainly antistaphylococcal IgE was assessed, of which the choice for determination was often unsubstantiated or based on results of previous studies. Patients with AD have frequently high IgE responses to environmental antigens, for example *S. aureus*. In addition, IgG is the most common antibody in the extravascular fluid and, among others, plays a role in the neutralization of toxins.[72,73]

To investigate further the role of (the immune response against) *S. aureus* in AD pathogenesis, future studies should focus on other antibody subtypes and other *S. aureus* antigens. IgG subclasses should be measured to detect possible biomarkers for AD severity, such as a selective deficiency in IgG_2 against SEC_1 in the study of Mrabet-Dahbi *et al*.[32] Furthermore, assessment of the antibody response against other *S. aureus* antigens, like MSCRAMMs, membrane-damaging molecules, housekeeping antigens, and other types of immune modulating proteins, might give more insight whether an increased IgE response is a secondary phenomenon of increased *S. aureus* colonization of AD skin.

In conclusion, this systematic review with meta-analysis shows that patients with AD have higher prevalences of IgE against the *S. aureus* antigens SEA and SEB than healthy controls, taking the large heterogeneity into consideration. These antigens, belonging to the group of immune modulators, are known as superantigens and have the ability to cause inflammation and epithelial damage. This supports a role for *S. aureus* in the pathogenesis of AD. IgE, IgG, and IgM against a very limited panel of other antigens were studied in single studies. No data are available on antistaphylococcal IgA.

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Table S1. Study characteristics per study

	Country	Patients					Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score	N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
Rojo 2014[46]	Spain	32/ 30[b]	65.6	23	-	SCORAD -				IgE	UniCAP 10 kU/L	2	SEA: 0.47 SEB: 0.27 SEC: 0.40 TSST-1: 0.53	
Ott 2014[47]	Germany	140	60.7	6.2	-	SCORAD 36				IgE	FEIA -	3	SEA: 0.06 SEB: 0.01 SEC: 0.12 SED: 0.00 SEE: 0.01 SEI: 0.01 SEH: 0.00 SEK: 0.00 SEJ: 0.00 TSST-1: 0.01	
Jinnestål 2014[35]	Sweden	30	30.0	32.5 (med)	Topical treatment, no UV treatment	SCORAD -	10[c]	30.0	41 (med)	IgE	ImmunoCAP 0.35 kU/L	4	SEA: 0.30 SEB: 0.20 TSST-1: 0.20	
Sonesson 2013[48]	Sweden	513	32.6	26.6	-	Rajka & Langeland 5.7				IgE	ImmunoCAP 0.35 kU/L	3	SEA: 0.18 SEB: 0.25 TSST-1: 0.15	
Kou 2012[49]	Japan	121/109[b]	57.0	35.7	Topical corticosteroid, 24 patients used cyclosporine	SCORAD 42 (med)	50[c]	54.0	31.0	IgE	UniCAP 0.70 UA/mL	3	SEB: 0.49	
Golysheva[a] **2012**[50]	Russia	133	-	Range 1-55	-	SCORAD -				IgE	ImmunoCAP -	2	SEA: 0.16 SEB: 0.31	

Table S1. Study characteristics per study (continued)

	Country	Patients					Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score	N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
Bozek 2012[34]	Poland	121	63.6	68.9	-	SCORAD 32.2	106[c]	-	68.1	IgE	CAP assay 0.35 kU/L	2	SEA: 0.01 SEB: 0.40 SEC: 0.07 SED: 0.11 TSST-1: 0.03	
Reginald 2011[33]	Austria & Germany	95	47.4	34.4	-	SCORAD -	17	29.4	36.2	IgE	ELISA -	3	FBP: 0.36	FBP: 0.00
Langer 2007[51]	Germany	32	28.1	31.5	-	SCORAD 33.4	9	-	-	IgE	CAP FEIA 0.35 kU/L	2	SEA: 0.44 SEB: 0.47	
Gutová 2006[52]	Czech Republic	84	50.0	Range 4 mo – 9 y	-	SCORAD -	10[c]	-	-	IgE	CAP (SEC, TSST-1) - Immulite 2000 (SEB) -	2	SEB: 0.06 SEC: 0.11 TSST-1: 0.10	
Mrabet-Dahbi 2005[32]	Germany	89	-	31 (med)	No corticosteroid and systemic or topical AB 4 weeks prior to the study	SCORAD 45 (med)	28	-	27 (med)	IgG_2	CAP (IgE) 0.35 kU/L ELISA (IgG_2) -	6	SEB: 0.87 (IgG_2) SEC_1: 0.62 (IgG_2)	SEB: 0.78 (IgG_2) SEC_1: 0.86 (IgG_2)
Yagi 2004[31]	Japan	105/26[b]	-	-	-	Modified Leicester system -	33	-	-	IgE, IgG	UniCAP (IgE) 0.35 kU/L ELISA (IgG) -	3	SEB: 0.19 (IgE) ETA: 0.00 (IgE) ETA: 0.35 (IgG)	SEB: 0.06 (IgE) ETA: 0.00 (IgE) ETA: 0.15 (IgG)

Table S1. Study characteristics per study (continued)

	Country	Patients					Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score	N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
Tabuchi 2004[53]	Japan	22	81.8	27.5	-	-	8	50.0	31.9	IgE	AlaSTAT 0.10 IU/mL	3	SEA: 0.50 SEB: 0.50	SEA: 0.00 SEB: 0.00
Ide 2004[54]	Japan	140	65.0	4.4	According to guidelines	1999 Japanese Therapeutic Guidelines for Atopic Dermatitis -				IgE	ImmunoCAP 0.7 U/mL	2	SEA: 0.18 SEB: 0.29	
Sohn 2003[55]	South-Korea	40	45.0	5.2	-	Criteria of Rajka -	40[c]	65.5	6.6	IgE, IgG, IgM	ELISA 12.11 U/mL (IgE) 26.11 U/mL (IgG) 19.83 U/mL (IgM)	3	SEB: 0.68 (IgE) SEB: 0.53 (IgG) SEB: 0.63 (IgM)	
Nomura 2002[56]	Japan	15	73.3	6 mo (med)	Systematic washing of skin using soap and topical corticosteroid, antihistamines and some patients used systemtic AB	Modified Leicester system -				IgE	ELISA 1.07 U (SEA) 12.6 U (SEB) 7.4 U (SEC) 8.3 U (TSST-1)	2	SEA: 0.20 SEB: 0.07 SEC: 0.13 TSST-1: 0.13	
Zollner 2000[30]	Germany	65	41.5	41	No AB or systemic immunosuppressives 4 weeks prior to the study	SCORAD -	65	-	-	IgE	AlaSTAT 0.7 U/mL	5	SEA: 0.23 SEB: 0.29	SEA: 0.00 SEB: 0.00

Table S1. Study characteristics per study (continued)

	Country	Patients					Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score	N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
Lin 2000[29]	Taiwan	60	66.7	7.2	-	Criteria of Rajka -	24	41.7	8.4	IgE	ELISA 0.16 kU/L (SEA) 0.7 kU/L (SEB)	7	SEA: 0.70 SEB: 0.70	SEA: 0.25 SEB: 0.17
Breuer 2000[57]	Germany	71	40.8	32 (med)	No treatment	SCORAD -				IgE	AlaSTAT 0.35 kU/L	4	SEA: 0.44 SEB: 0.39	
Nomura 1999[58]	Japan	94	59.6	7.8	-	Modified Leicester system 35.4				IgE	ELISA 1.07 U (SEA) 12.4 U (SEB) 7.8 U (SEC) 8.3 U (TSST-1)	3	SEA: 0.79 SEB: 0.35 SEC: 0.21 TSST-1: 0.18	
Morishita 1999[28]	Japan	149	44.3	21.4	-	Criteria of Rajka -	11	27.3	26.9	IgE	AlaSTAT 0.35 IU/mL	5	SEA: 0.54 SEB: 0.65	SEA: 0.00 SEB: 0.00
Bunikowski 1999[59]	Germany	58	65.5	30 mo (med)	No topical or systemic antimicrobial drugs 2 weeks prior to the study	SCORAD -	22[c]	-	73 mo (med)	IgE	AlaSTAT 0.7 kU/L	3	SEA: 0.19 SEB: 0.31	
Campbell 1998[60]	Australia	74	59.5	-	-	-	111	-	-	IgG	ELISA 2.1 LU (SEA) 2.4 LU (SEB) 8.7 LU (TSST-1)	3	SEA: 0.77 SEB: 0.73 TSST-1: 0.77	SEA: 0.88 SEB: 0.69 TSST-1: 0.85

Table S1. Study characteristics per study (continued)

	Country	Patients					Controls			Antibody	Cut-off detection method	NOS	Patients	Controls
		N	% Male	Mean age (y)	Treatment at baseline	Mean AD severity score	N	% Male	Mean age (y)				Prevalence positive anti-*S. aureus* antibodies	Prevalence positive anti-*S. aureus* antibodies
Nissen 1997[27]	Denmark	34/ 27[b]	-	31 (med)	-	-	5	-	-	IgE	Immunoblot -	2	SEA: 0.48 SEB: 0.63 TSST-1: 0.41 LTA: 0.48	SEA: 0.00 SEB: 0.00 TSST-1: 0.00 LTA: 0.00
Tada 1996[25]	Japan	96	42.7	20.2	-	Criteria of Rajka -	11	27.7	29.4	IgE	AlaSTAT 0.35 IU/L	5	SEA: 0.66 SEB: 0.73	SEA: 0.00 SEB: 0.00
Leung 1993[24]	USA	56	-	-	-	-	15	-	-	IgE	ELISA -	9	SEA: 0.32 SEB: 0.18 SEC: 0.05 SED: 0.05 TSST-1: 0.21 ET: 0.02	SEA: 0.00 SEB: 0.00 SEC: 0.00 SED: 0.00 TSST-1: 0.00 ET: 0.00

Abbreviations: N, number of patients or controls; y, year; mo, months; AD, atopic dermatitis; NOS, Newcastle-Ottawa Scale; *S. aureus*, *Staphylococcus aureus*, med, median; AB, antibiotics; UV, ultraviolet, SCORAD, SCORing Atopic Dermatitis; Ig, immunoglobulin; FEIA, fluorescent enzyme immunoassay; SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; FBP, fibronectin-binding protein; ET, exfoliative toxin; LTA, lipoteichoic acid. [a]Only abstract available. [b]Number of patients included in study (characteristics refer to this number) / number of patients included in the outcome. [c]Control group included in the study but the outcome was not reported.

Table S2. Studies reporting IgE antibodies against *Staphylococcus aureus* antigens in patients with atopic dermatitis

***Staphylococcus aureus* antigen**	**Number of studies**	**Number of controlled studies**
SEA	19	7
SEB	23	8
SEC	7	1
SED	3	1
SEE	1	
SEI	1	
SEH	1	
SEK	1	
SEJ	1	
TSST-1	10	2
ETA	1	
FBP	1	
LTA	1	
ET1	1	1

Abbreviations: SE, staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1; ET, exfoliative toxin; FBP, fibronectin-binding protein; LTA, lipoteichoic acid.

Appendix 1. Inclusion criteria for selecting studies for this systematic review

Types of studies

- All original observational and experimental human studies which assess the presence of anti-staphylococcal antibodies in the serum of patients with atopic dermatitis, reported per *Staphylococcus aureus* antigen.
- All study designs, except for case reports.

Participants

- Patients of all ages with atopic dermatitis irrespective of disease severity, and presence of antistaphylococcal antibodies. Atopic dermatitis diagnosed by a medical doctor.

Controls

- Persons who do not have atopic dermatitis neither an atopic constitution (asthma, allergic rhinitis, food allergy) or another skin disease.

Outcome measures

- Primary: Specific antibodies (IgE, IgG, IgM, IgA) against *Staphylococcus aureus* antigens.
- Secondary: The relationship between atopic dermatitis disease severity and specific antibodies (IgM, IgG, IgA, IgE) antibodies against *Staphylococcus aureus* antigens.

Appendix 2. Quality assessment score

Modified Newcastle-Ottawa quality assessment scale for cohort or cross sectional studies

Stars indicate the points allocated if the item criterion is met. A maximum score of 9 can be allocated to each article. Uncontrolled studies can reach a maximum score of 5.

Selection

1. Representativeness of the exposed cohort

a) Truly representative of the general atopic dermatitis population ★
b) Somewhat representative of the general atopic dermatitis population ★
c) Selected group of atopic dermatitis patients: hospital based, tertiary center, inpatients, outpatients)
d) No description of the derivation of the cohort

2. Selection of the non-exposed cohort

a) Representative of the average community (healthy control, community control) ★
b) Selected group of controls (hospital controls, other dermatological condition)
c) No description of the derivation of the cohort

3. Ascertainment of atopic dermatitis
a) Diagnosed by dermatologist ★
b) Diagnosed by physician other than dermatologist ★
c) Diagnosed by clinical assessment ★
d) Based on self-report
e) No description of atopic dermatitis case definition

4. Definition of the non-exposed cohort
a) No history of disease (endpoint) ★
b) No description of source

Comparability
1. Comparability of exposed and non-exposed cohorts on the basis of design or analysis
a) Atopic dermatitis patients and healthy controls are matched for age ★
b) Atopic dermatitis patients and healthy controls are matched for any additional factor ★
c) No controlling for confounding or matching

Outcome
1. Assessment of outcome: measurement method of antibody response against *Staphylococcus aureus* antigens
a) Determined by ELISA, CAP, RAST, micro-array, AlaSTAT ★
b) Not mentioned

2. Treatment during sampling
a) No treatment ★
b) Systemic treatment
c) Topical treatment
d) Not mentioned

3. Missing data
a) Same rate for both atopic dermatitis patients and healthy controls / no missing data ★
b) Different rate for both atopic dermatitis patients and healthy controls, but well described / missing data, but well described ★
c) Different rate for both atopic dermatitis patients and healthy controls and not explained
d) Not mentioned

Modified Scoring algorithm controlled studies

Quality rating	Points in Selection Domain	Points in Comparability Domain	Points in Outcome Domain
Good	≥ 3	≥ 1	≥ 2
Fair	2	0	≥ 2
Poor	0-1	0	0-1

Chapter 6.1

Targeted antistaphylococcal therapy with endolysins in atopic dermatitis and the effect on steroid use, disease severity and the microbiome: study protocol for a randomized controlled trial (MAAS trial)

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ABSTRACT

Background

Atopic dermatitis (AD) is associated with a reduced skin microbial diversity and overgrowth of *Staphylococcus* (*S.*) *aureus*. However, the importance of *S. aureus* colonisation in the complex pathogenesis remains unclear and studies on the effect of antistaphylococcal therapy in noninfected AD show contradictory results. Long-term interventions against *S. aureus* might be needed to restore the microbial balance, but carry the risk of bacterial resistance induction. Staphefekt, an engineered bacteriophage endolysin, specifically kills *S. aureus* leaving other skin commensals unharmed. Bacterial resistance towards endolysins has not been reported, nor is it expected, which allows us to study its effect as long-term anti-staphylococcal treatment in non-infected AD.

Methods

This is a multi-centre, placebo-controlled, double-blinded and randomized superiority trial with a parallel group design. A total of 100 participants, aged 18 years or older, diagnosed with moderate-to-severe atopic dermatitis and using a topical corticosteroid in the weeks before enrolment are included in the study. The study is executed in the Erasmus MC University Medical Center Rotterdam in collaboration with the Havenziekenhuis Rotterdam. After a two-week run-in period to standardize the corticosteroid use with triamcinolone acetonide 0.1% cream, participants will be randomized to either treatment with Staphefekt in a cetomacrogol-based cream or a placebo for 12 weeks, followed by an eight-week follow-up period. The primary objective is to assess the difference in the need for corticosteroid co-therapy between the Staphefekt and the placebo group, measuring the number of days per week of corticosteroid cream (triamcinolone) use. Secondary objectives include the difference in use of corticosteroid cream measured in grams, differences in clinical efficacy, quality of life (QoL), microbial composition (including *S. aureus*) between the Staphefekt and the placebo group, and the safety and tolerability.

Discussion

The results of this trial will provide data about the effect of long term antistaphylococcal therapy with Staphefekt on corticosteroid use, clinical symptoms and QoL in patients with moderate-to-severe AD. Additional data about growth characteristics of the skin microbiome, including *S. aureus*, will give insight in the role of the microbiome as a factor in the pathophysiology of AD.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is associated with a reduced quality of life (QoL), primarily due to an itchy skin.[1-3] The disease is characterised by a reduced skin microbial diversity and overgrowth of *Staphylococcus (S.) aureus,* a bacterium that can aggravate skin inflammation via the production of staphylococcal enterotoxins that stimulate the release of pro-inflammatory cytokines.[4-7] However, the importance of *S. aureus* colonisation in the complex pathogenesis, compared to the other genetic and immunologic factors involved, remains unclear.

Current treatment approaches for AD include topical treatment with emollients and anti-inflammatory therapy with (topical) immunosuppressive agents (corticosteroids and calcineurin inhibitors), according to the international guidelines.[8,9] Antistaphylococcal therapy is only recommended in cases of fever or clinically infected skin.[8,9] Clinical studies that evaluated the added value of antistaphylococcal therapy in noninfected AD have shown contradictory results. Bath Hextall *et al.*[10] performed a systematic review of 26 studies and showed that antistaphylococcal agents reduced the amount of *S. aureus* on the skin in AD. However, the bacteriological reduction did not translate into a decrease of clinical symptoms. These studies mainly investigated short-term therapies of less than one month duration and comprised small and poor-quality studies. As discontinuation of therapy after a short treatment period can result in quick regrowth of *S. aureus*, the results of this systematic review do not necessarily mean that anti-staphylococcal agents do not work.[11] A more recent review of Brüssow *et al.*[12] summarizes two intervention trials that reported significant improvement of disease severity in noninfected AD after two and three months of therapy with antistaphylococcal therapy (bleach baths).[13,14] We hypothesize that long-term therapy may be needed to reduce *S. aureus* overgrowth and maintain a stable and balanced skin microbial composition. Ultimately, this could result in disease improvement, prevention of AD flares, and less need for (topical) immune suppression. However, long-term use of antibiotics can induce bacterial resistance, and both the use of antibiotics and dilute bleach baths can cause unnecessary harm to the commensal flora, that is hypothesized to have anti-staphylococcal properties.[15,16]

In the context of the increasing incidence of bacterial resistance, the interest in bacteriophages and their endolysins as antibacterial therapy has been renewed.[17] Staphefekt SA.100 is an engineered chimeric endolysin that specifically lyses the cell membrane of *S. aureus* via endopeptidase and putative amidase activities.[18-20] Long-term application of Staphefekt on the skin, targeting only *S. aureus* and leaving skin commensals unharmed, may improve long-term AD outcomes, such as the number of disease flares, and may reduce the use of topical corticosteroids. Bacterial resistance to Staphefekt or other endoly-

sins has not been observed and could not be induced, which enables us to study the effect of long-term antistaphylococcal treatment in noninfected AD using this endolysin-based agent.[19,21,22]

The aim of this randomized controlled trial, the MAAS trial, is to evaluate the effect of a three-month antistaphylococcal therapy with Staphefekt on the frequency and quantity of topical corticosteroid use, clinical symptoms and QoL in patients with moderate-to-severe AD. In addition, data on the growth characteristics of the skin microbiome, including *S. aureus*, will be collected, which will gain insight into the role of the microbiome as a factor in the pathophysiology of AD.

MATERIALS AND METHODS

Design and setting

The MAAS trial (microbiome in atopic dermatitis during antistaphylococcal therapy and the effect on steroid use), is a multi-centre, randomized, double-blinded, placebo-controlled superiority trial with a parallel group design. The study aims to evaluate the effect of Staphefekt on the use of corticosteroids, disease severity, QoL, and composition of the microbiome in patients with AD (Figure 1). The study was designed by the department of Dermatology of the Erasmus MC University Medical Center Rotterdam and will be executed in collaboration with the Havenziekenhuis Rotterdam. Enrolment and follow-up visits take place at these two locations. Participants who comply with the criteria for inclusion and exclusion will start with a two-week run-in period to standardize the corticosteroid use with triamcinolone acetonide 0.1% cream. After completion of the run-in phase, participants will be randomized to either treatment with Staphefekt or a placebo for 12 weeks, followed by an eight-week follow-up period. An Eczema Area Severity Index (EASI) above 50.0 after the run-in phase is a contraindication for further participation**.** During the course of the study, participants visit the outpatient clinic six times (visit 1 through 6) and data will be collected on corticosteroid use, disease severity, QoL, skin microbiome, and adverse events.

Ethical considerations

This study follows the Dutch Medical Research Involving Human Subjects Act 1998 (WMO) and the principles of the Helsinki Declaration 2008. All study procedures have been reviewed and approved by the Medical Ethics Committee of the Erasmus MC University Medical Center Rotterdam, the Netherlands (MEC-2016-233). Protocol amendments will be submitted for review at the Medical Ethics Committee.

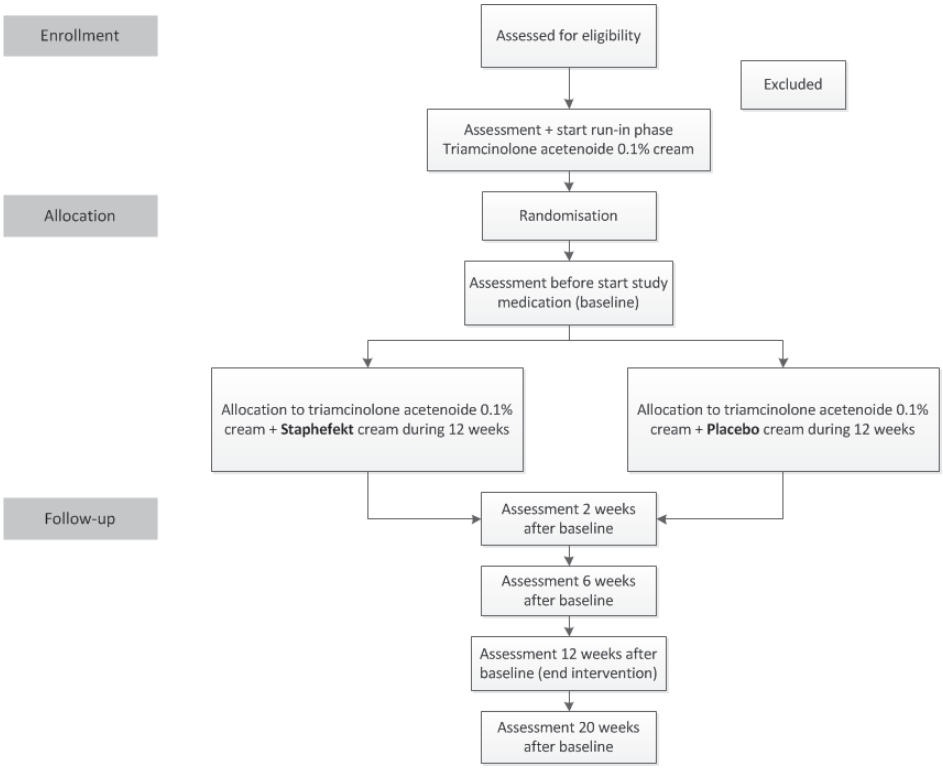

Figure 1. Flowchart of the study design

Participants

This study will enroll adults (18 years or older) diagnosed with atopic dermatitis according to the UK working party diagnostic criteria for AD.[23-25] Participants are eligible for enrolment if they have a score between 7.1 and 50.0 on the EASI for disease severity. Topical corticosteroids must have been prescribed before enrolment. All patients must be able to read and understand the patient information and provide written informed consent. Patients are not eligible for enrolment if they used (1) systemic antibiotics or corticosteroid in the two months prior to enrolment; (2) oral immunosuppressive agents or UV therapy in the three months before enrolment; or (3) local antibiotics or Staphefekt (from commercial sources) one week before enrolment. Other criteria for exclusion are a known contact allergy to any of the components of the study drug (e.g. propylene glycol), clinically infected AD, or the existence of other skin condition(s) that could interfere with the assessment of the AD severity.

Recruitment, inclusion and consent

Participants with AD will be recruited from the dermatology outpatient clinic of the Erasmus MC and the Havenziekenhuis Rotterdam. Furthermore, Dutch dermatologists are informed about the study via the Dutch Trial Network and via scientific conferences. Patients with AD are informed via the patient support group and via online media, such as DermHome (https://www.huidhuis.nl/). In addition, recruiting advertisements will be placed on student fora and in local newspapers. Patients who are interested in participation in the trial can contact the researcher directly via email or phone. After a first screening with regard to the inclusion and exclusion criteria via email or phone, potentially eligible participants receive an information letter and will be invited at the dermatology outpatient clinic to further assess eligibility. Patients that fulfil the inclusion criteria and are willing to participate, will be included in the study after providing written informed consent.

Sample size

The sample size for this study was calculated based on the primary outcome, namely the difference in mean days per week corticosteroid use over 12 weeks between the Staphefekt arm and the placebo arm, in patients that are positive for *S. aureus* on the skin at baseline. This is the first study measuring clinical outcomes of Staphefekt in patients with AD. We expect to find a mean topical corticosteroid use of 5 days/week in the placebo group. This was based on a study of Hon *et al.*[26] that showed decreased use of topical corticosteroids when taking bleach baths, an antistaphylococcal therapy. Based on the results of this study, we anticipate an effect size of 1.25 day/week reduction of topical corticosteroid use in the Staphefekt arm. A sample size was calculated using an unpaired t-test to compare means in a superiority trial design. With a power of 0.80, alpha of 0.05, and standard deviation (SD) of 2.0, 40 patients are needed per treatment arm. Assuming 10% drop out and 90% of the patients being positive for *S. aureus* on the skin lesions, 50 patients will be assigned to each of the two treatment arms.

Randomization and blinding

The participants are randomly assigned in a 1:1 fashion to either treatment with Staphefekt or placebo. Stratified block randomization for AD severity is performed to ensure equal distribution of patients with moderate and severe AD over the treatment arms (EASI 7.1-21 and EASI 21.1-50). Randomization is done by an independent biostatistician of the Erasmus MC, using the statistical software package R version 3.2.2. The participants, the researchers and laboratory analyst are blinded for the intervention. The pharmacy manages the randomization list and provides blinded study medication.

Intervention

After enrolment, all participants start a run-in phase of two weeks, in which they receive a standardized dosing regimen of topically applied triamcinolone acetonide 0.1% cream (Table 1). After the run-in period, the patient and the researcher evaluate further participation, with very severe AD (EASI >50.0) as a contraindication for continuation. The run-in period and randomization is followed by a 12-week treatment period and an eight-week follow-up period. During the treatment period, Staphefekt or placebo cream will be applied on the total skin surface twice daily to reach optimal reduction of *S. aureus,* as both lesional and non-lesional skin are often colonised.[4] The Staphefekt endolysin is made available in a cetomacrogol-based cream. The placebo is composed of the same cetomacrogol-based cream, without Staphefekt. During the treatment and follow-up period triamcinolone will be used according to the corticosteroid dosing regimen (Table 1). Measurements and assessments will be performed at enrolment (start of the run-in phase, visit 1), baseline (start treatment with Staphefekt/placebo, visit 2a), 0.5 hours after baseline and 2, 6, 12, and 20 weeks after baseline (visits 2b to 6). Table 2 provides an overview of the measurements per visit. Unless it is in the best interest of the patients, for example in case of an eczema flare, patients are not allowed to use systemic or topical immunosuppressive medication (including calcineurin inhibitors), antibiotics or antiseptics during the study. Escape medication will be prescribed according to current treatment guidelines and its use will be registered. At start of the study, patients receive an emollient according to patient's preference for use during the course of the study. The use of this emollient will be registered by weighing the tubes at each visit.

Table 1. Corticosteroid dosing regimen

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Saturday	x x	x	x	x					
Sunday	x x	x			x	x	x	x	
Monday	x x	x	x	x					
Tuesday	x x	x			x	x			
Wednesday	x x	x	x	x					
Thursday	x x	x			x	x	x	x	
Friday	x x	x	x	x					

Start in week 1 or 2 depending on severity of the atopic dermatitis. If the symptoms allow, reduce the use of corticosteroid cream weekly according to the scheme. Return to week 1 or 2 in case of an exacerbation. Based on patient' assessment.

Detailed sample and laboratory procedures

Sampling procedures are based on the 'Manual of Procedures' for microbiome sampling of the Human Microbiome Project.[32] All samples are obtained by one of the researchers wearing gloves (sterile for the skin scrub). Sterile Copan 490CE.A swabs are used to sample

the skin, nasal cavity and pharynx. Skin samples are taken from lesional skin, preferably located at the antecubital folds or the popliteal fold. The skin surface is swabbed during 30 seconds. The mucosal surfaces of both the anterior nares are gently rubbed going round the area for 10 seconds. The rear of the oropharynx is swabbed for 5 seconds, using a tongue depressor. For the skin scrub sample, a ring with an internal diameter of 4 cm will be placed on the same skin lesion where the swab was collected, but on a non-overlapping area: 1 ml of swab-solution (0,85% NaCl, 0.1% bacteriological peptone, 0.1% Tween 80) is pipetted in the ring. After rubbing over the skin with a Copan 480CE swab during 1 minute, the swab solution will be pipetted out the ring into an Eppendorf tube. The swabs will be sent to the laboratory by mail at the day of collection. A semi-quantitative culture technique and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOFF) for identification of *S. aureus* will be performed. The scrub samples will be stored at -80 ° at the Erasmus MC Rotterdam until 16S rRNA-sequencing and quantitative *S. aureus* analysis.

Primary and secondary outcomes

The primary outcome of this study is the days per week of corticosteroid use, compared between the Staphefekt and the placebo group over 12 weeks. Patients report their triamcinolone use daily in a secured digital platform, 'DermHome'.* Additionally, the use of triamcinolone cream will be measured in grams by weighing the study medication at time of issue and return (each visit). Secondary outcomes include clinical efficacy and QoL from baseline through week 12 and week 20, change of the microbial composition (including *S. aureus*), and safety. Clinical efficacy is measured using the EASI, the Investigators Global Assessment (IGA), and registration of the number of flares.[27] A flare is defined as an exacerbation that requires the need to intensify treatment, from a doctor or patient's perspective. This implies any stronger topical therapy or the need for systemic treatment. A 50% increase of the EASI score compared to baseline is used as an indication to intensify treatment. The Pruritus Numerical Rating Scale (NRS) and the Patient-Oriented Eczema Measure (POEM) are included as patient-reported efficacy outcomes.[28,29] QoL is measured using the Skindex-29.[30,31] Changes in the microbiome are evaluated by comparing the changes in bacterial composition between the treatment groups, determined by 16S rRNA sequencing of the skin scrub samples. Reduction of *S. aureus* is determined by quantitative polymerase chain reaction (qPCR), and culture for the comparison between visit 2a and visit 2b. Safety and tolerability is assessed by monitoring the incidence of (serious) adverse device events through the end of the study, evaluated by medical check-ups that include evaluation of vital signs. Reportable adverse events will be reported within the set timelines to the competent authorities. Table 2 gives a detailed overview of the measurements per visit.

Table 2. Study assessments

		Run-in	Baseline/ Allocation	Intervention				Follow-up
		Visit 1	**Visit 2a**	**Visit 2b**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
ENROLMENT								
Eligibility screen		x						
Informed consent		x						
Baseline questionnaire		x						
Allocation			x					
INTERVENTION								
ASSESSMENTS								
Efficacy	Questionnaire triamcinolone use (primary outcome)							
	Weight triamcinolone tube at issue and return	x	x		x	x	x	x
	EASI[27]	x	x		x	x	x	x
	IGA		x		x	x	x	x
	Pruritis NRS[28]							
	POEM[29]		x		x	x	x	x
Quality of life	Skindex-29[30,31]		x		x		x	x
Microbiome	Swab skin	x	x	x	x		x	x
	Scrub skin		x		x		x	x
	Swab nose	x	x				x	x
	Swab throat	x	x				x	x
Safety	Medical check-up	x	x		x	x	x	x
Other	Photograph (overview + close-up sampled lesion)		x		x	x	x	x
	Questionnaire use of emollients and escape medication		x		x	x	x	x

Abbreviations: EASI, eczema area and severity index; IGA, investigators global assessment; NRS, numerical rating scale; POEM, patient-orientated eczema measure. Visit 1, enrolment in the trial and start of a two weeks run-in phase; Visit 2a, start of the intervention (baseline); Visit 2b, 0.5 hours after baseline; Visit 3, 2 weeks after baseline; Visit 4, 6 weeks after baseline; Visit 5, 12 weeks after baseline and end of the intervention; Visit 6, follow-up visit 20 weeks after baseline. All visits take place plus or minus two days from the indicated timeframe.

Data collection, monitoring and data analysis

Data collected during the visits are entered in Open Clinica. This data management system allows direct data entry. Data entry is monitored by an independent researcher according to a predefined monitoring plan. Triamcinolone use and itch scores filled in daily by the patients in 'DermHome' will be extracted in a SPSS format and combined with the Open Clinica database. Patients confidentiality will be ensured by using identification numbers. Data will be analysed on an intention-to-treat basis. A mixed linear regression model will be used to examine if there is a significant difference in corticosteroid use over 12 weeks between the intervention and the placebo group.[33] This model accounts for repeated measurements in each patient and is valid in the case of missing data. Covariates that could influence the outcome variable will be included in the model. Subgroup analysis will be performed to analyse patients who are positive for *S. aureus* on the skin versus patients that are negative for *S. aureus* before the start of the intervention. Positive patients are defined as having positive cultures both at visit 1 and 2a. Negative patients must have two negative cultures. Patients that have one positive and one negative culture will not be included in the subgroup analysis. Secondary outcomes will also be analysed using a mixed model analysis (linear or logistic according to the type of data). The findings of this study will be published in national and international journals and will be communicated to the relevant patient associations.

*'DermHome' is a secured digital treatment and research platform, developed with Patient 1 BV, Almere.[34] The platform provides an user-friendly individual account that allows patients to report their pruritus score and triamcinolone use daily. Thereby, the platform provides digital information about the study, including the use of the study medication, and an option to contact the researcher and to upload photos in case of questions. After every visit the researcher can make notes in the digital file about findings, agreements, and future appointments.

DISCUSSION

The MAAS trial is a randomized, placebo-controlled trial that investigates the effect of three-month antistaphylococcal therapy with Staphefekt on topical corticosteroid use, clinical symptoms, and QoL in adults with moderate-to-severe AD. Additionally, data will be collected about the growth characteristics of the skin microbiome, including *S. aureus*. Taking into consideration the current literature on antistaphylococcal therapy, a study design using a long-term antistaphylococcal intervention, measuring long-term outcomes, was chosen.

Evidence for the clinical efficacy of Staphefekt, registered as a class 1 medical device in Europe, is based on *in vitro* studies and a case series.[18-20] These *in vitro* studies showed that Staphefekt kills different strains of *S. aureus* (also methicillin-resistant strains), without harming the commensal flora or inducing bacterial resistance.[19] A case series describes clinical improvement of *S. aureus* related symptoms, such as folliculitis and superinfected dermatitis, and no development of resistance during long-term daily treatment with Staphefekt based on the minimal inhibitory concentrations of the cultured *S. aureus* strains over time.[35] The lack of resistance induction can be expected, as bacterial killing by an endolysin is independent of the involvement of the bacterial metabolism. The co-evolution of bacteriophages and their host bacteria over millions of years ensures that phage endolysins attack essential bonds in the bacterial cell wall that cannot be adapted by the host.[22] Thereby, the lytic activity of exogenously applied endolysins results in lysis of the target cells within seconds, restricting the possibility to adapt and develop resistance. Furthermore, attacking several bonds of the bacterial wall simultaneously by the use of more than one enzymatically active domain in the Staphefekt molecule makes resistance even less likely to develop.[18]

Because of the proteinaceous nature of endolysins, immunogenicity can be of concern. The literature shows the possibility of the formation of non-neutralizing antibodies against lysins other than Staphefekt.[22] In a study in which the presence of anti-Staphefekt IgG was evaluated in serum from 21 Staphefekt-naive healthy human donors, pre-existent IgG antibodies recognizing Staphefekt epitopes could be detected in all the donors (unpublished data). This can be explained as humans are exposed daily to *S. aureus* and, therefore, to bacteriophages and their lysins. However, Staphefekt is a large protein molecule (>50 kDa), making penetration through the skin and mucosa and subsequent antibody reactions unlikely.[36]

Calculation of the sample size for this study was hampered as no information was available about the effect of Staphefekt on corticosteroid use and clinical efficacy in AD. Therefore, the study should be considered as hypothesis generating, giving insight into effect sizes and distributions of clinical outcomes. Our expected effect size was based on a study of Hon *et al*.[26] that studies the effect of bleach on corticosteroid use in AD. We chose a slightly higher effect size, because we expect the effect of Staphefekt that specifically targets *S. aureus* to be more efficacious than bleach. We consider this effect size, a reduction in corticosteroid use of more than one day a week over 12 weeks, as clinically relevant because of the (low) risk of side effects and a general reluctance of patients to use corticosteroids, resulting in poor compliance and a lack of treatment efficacy.[37,38]

No consensus has been reached yet on a standardized outcome for long-term AD control, the primary goal of our study. The Harmonising Outcome Measures for Eczema (HOME) initiative reached consensus on the use of EASI and POEM as doctor-based and patient-based measures of AD severity, of which both are included as secondary outcomes in this trial.[39] According to the authors of HOME, measures of long-term control could include time to flare and the use of rescue medicine.[39] Next to corticosteroid use, both these study outcomes were included in this study as secondary parameters.

In conclusion, this study will evaluate the effects of a three-month targeted antistaphylococcal therapy with Staphefekt in moderate-to-severe AD. The lack of resistance induction allows long-term treatment with this antistaphylococcal agent. This study will provide the first data on the use of antistaphylococcal therapy with Staphefekt in AD and may provide new insights into the role of *S. aureus* in the pathophysiology of AD.

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Chapter 6.2

Endolysin treatment against Staphylococcus aureus in adults with atopic dermatitis: a randomized controlled trial

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To the Editor:

Staphylococcus (*S.) aureus* density is increased in many patients with atopic dermatitis (AD) and is thought to contribute to the disease pathogenesis, interacting with an altered skin barrier and immunological changes.[1] *S. aureus* might induce or aggravate inflammation via different mechanisms, for example through excretion of virulence factors, even if the *S. aureus* overgrowth is primarily caused by other factors.[2] Current guidelines only recommend antimicrobial therapy directed against *S. aureus* in clinically infected AD based on a Cochrane review where no clinical benefit of short-term antimicrobial treatment in noninfected AD was found.[3]

Arguably, long-term anti-staphylococcal treatment, such as antibiotics, might reduce symptoms in AD.[4] However, this is undesired because antibiotics can affect the commensal microbiota and could induce bacterial resistance.[5] In contrast, long-term treatment of AD with an endolysin that targets only *S. aureus* is feasible. It might improve AD symptoms and reduce the use of corticosteroids consecutively.[2,6] Therefore, we aimed to determine the topical corticosteroid (TCS)-sparing effect and safety of 12 weeks of endolysin treatment against *S. aureus* in patients with AD.

We performed a double-blind, vehicle-controlled superiority trial (MAAS trial, ClinicalTrials.gov NCT02840955) in 100 adult patients with TCS-treated, not clinically infected, moderate-to-severe AD, which was defined by an Eczema Area and Severity Index (EASI) of 7.1 to 50.0. After a two-week run-in period to standardize the TCS treatment with triamcinolone acetonide 0.1% cream, patients were randomly assigned 1:1 to a 12-week intervention with either a topical endolysin against *S. aureus* or a vehicle twice daily, followed by an eight-week follow-up period. The vehicle and recombinant chimeric endolysin, Staphefekt™ SA.100, were provided by Micreos Human Health (Bilthoven, The Netherlands) and topically applied in a cetomacrogol cream. Details on patient inclusion, randomization, and study procedures at six assessments are described in a previously published study protocol (Figure S1).[2]

The primary outcome, the TCS-sparing effect of endolysin treatment against *S. aureus*, was evaluated by the patients, who registered daily use of a TCS (yes/no) over 12 weeks. Secondary outcomes included differences in TCS use measured in grams, clinical efficacy, quality of life using the Skindex-29, *S. aureus* load on the skin, and safety and tolerability of endolysin treatment. Clinical efficacy was measured using the EASI, Investigators Global Assessment, Patient-Oriented Eczema Measure, pruritus Numeric Rating Scale, and by registration of the number of flares. The *S. aureus* load on the skin was assessed by a semi-quantitative culture and quantitative polymerase chain reaction (qPCR, Appendix 1).

Generalized linear mixed-effect models for repeated measurements were used to analyze the primary and dichotomous secondary outcomes, and linear mixed-effect models were used for continuous secondary outcomes. Data were analyzed as intention-to-treat and per-protocol. Furthermore, a subgroup analysis was performed in patients with a positive *S. aureus* skin culture at two time points before start of the intervention.

Eighty-eight (88.0%) patients completed the intervention, and 87 (87.0%) completed follow-up (13% dropout rate, Figure S2). Patients' characteristics were comparable between the endolysin and vehicle groups (Table 1). Over the 12-week intervention period (corresponding to 8400 days for 100 patients), patients in the endolysin group used a TCS for 1889 (45.0%) days compared with 1566 (37.3%) days in the vehicle group. There was no statistically significant difference in the probability of TCS use per day between the groups in the intention-to-treat analysis, per-protocol analysis, and in the subgroup of *S. aureus*-positive patients (p=0.97, p=0.40 and p=0.08, respectively, Table 2). Sensitivity analyses showed no differences in the odds ratio of TCS use per assessment day. Except for the number of doctor-reported AD flares during the intervention period (per-protocol, n=2 in endolysin group vs. n=10 in vehicle group, p=0.03), no statistically significant differences were found in the secondary outcomes after both intention-to-treat and per-protocol analyses (Table S1 and S2). At baseline, 62 (64.6%) patients had positive results for *S. aureus* based on skin culture and 24 (24.7%) by qPCR. Both methods showed no significant difference in *S. aureus* reduction (Table S3 and S4). During the study, one serious adverse event occurred in the endolysin group eight weeks after the last application of endolysin cream (pleural effusion with hospitalization), which was considered unlikely to be related to the study intervention (Table S5).

Our results are in accordance with data from a Cochrane review showing no significant effect of short-term anti-*S. aureus* therapy in patients with noninfected AD.[3] We cannot confirm the positive results of other longer-term studies. However, these studies used broad-spectrum antimicrobials and mainly included patients with signs of bacterial infection.[4] Patients with clinically infected AD were excluded from our study, and a possible effect of anti-*S. aureus* endolysins in this patient group should be determined in future studies.

Several hypotheses could explain our results. First, use of triamcinolone in the run-in phase resulted in a decrease in AD severity (Table 1), which might have masked a possible benefit of endolysin treatment.

Second, daily use of an emollient and good compliance with the treatment could have resulted in a reduction of triamcinolone use in both the endolysin and vehicle groups.[7]

Table 1. Baseline characteristics

	Total (n=100)	Staphefekt (n=50)	Vehicle (n=50)
Age			
years; median (IQR)	33.5 (25.5-47.5)	36.5 (25.0-51.0)	32.5 (24.0-44.0)
Sex (male)			
n (%)	55 (55.0)	24 (48.0)	31 (62.0)
Race, n (%)			
American Indian or Alaska Native	5 (5.0)	2 (4.0)	3 (6.0)
Asian	10 (10.0)	2 (4.0)	8 (16.0)
Black or African American	8 (8.0)	5 (10.0)	3 (6.0)
White	77 (77.0)	41 (82.0)	36 (72.0)
Atopic disease, n (%)			
Food allergy	43 (43.0)	18 (36.0)	25 (50.0)
Rhinoconjunctivitis	63 (63.0)	28 (56.0)	35 (70.0)
Asthma	47 (47.0)	25 (50.0)	22 (44.0)
EASI, median (IQR)			
Screening (V1)	12.9 (9.2-19.0)	13.7 (8.9-19.1)	12.5 (9.2-19.0)
Baseline (V2)	8.0 (5.0-13.5)[4]	8.3 (5.0-14.7)[5]	8.0 (4.9-12.9)[6]
IGA, median (IQR)			
Baseline (V2)	2.0 (2.0-3.0)[4]	2.0 (2.0-3.0)[5]	2.0 (2.0-3.0)[6]
POEM, mean (SD)			
Baseline (V2)	12.9 (6.2)[4]	14.5 (8.3-17.0)[5]	13.0 (8.0-15.0)[6]
Pruritus NRS, median (IQR)			
Baseline (V2)	3.0 (2.0-4.0)[4]	3.0 (2.0-4.0)[5]	3.0 (2.0-4.0)[6]
Skindex-29, mean (SD)			
Baseline (V2)	35.1 (17.1)[4]	37.3 (15.3)[5]	32.9 (18.7)[6]
Use of topical corticosteroids at screening, n (%)			
Class 1	3 (3.0)	1 (2.0)	2 (4.0)
Class 2	13 (13.0)	6 (12.0)	7 (14.0)
Class 2-3	11 (11.0)	3 (6.0)	8 (16.0)
Class 3	44 (44.0)	22 (44.0)	22 (44.0)
Class 3-4	3 (3.0)	2 (4.0)	1 (2.0)
Class 4	18 (18.0)	11 (22.0)	7 (14.0
Unknown	8 (8.0)	6 (12.0)	4 (8.0)
***Staphylococcus aureus* skin culture*, n (%)**			
Positive	56 (56.0)	32 (64.0)	24 (48.0)
Intermediate	20 (20.0)	7 (14.0)	13 (26.0)
Negative	20 (20.0)	9 (18.0)	11 (22.0)
Missing	4 (4.0)	2 (4.0)	2 (4.0)

Abbreviations: EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; SD, standard deviation. Missings: [1]n=15 (15.0%), [2]n=8 (16.0%), [3]n=7 (14.0%), [4]n=3 (3.0%), [5]n=2 (4.0%), [6]n=1 (2.0%). *Positive is defined as having a positive culture at visit 1 and visit 2a; intermediate is defined as having one positive culture and one negative culture at visit 1 and visit 2a; negative is defined as having two negative cultures at visit 1 and visit 2a.

Table 2. Generalized Linear Mixed-Effect model results for the difference in topical corticosteroid use per day during the 12-week intervention period

Analysis	Time period or time point in days from baseline	Patients included in analysis		Topical corticosteroid use 'yes', n (%)		OR (95% CI)	*P*-value[1]
		Staphefekt, n	**Vehicle**, n	**Staphefekt**, n (%)	**Vehicle**, n (%)		
Intention-to-treat	Intervention						0.97
	14	40	37	26 (65.00)	20 (54.05)	0.99 (0.95 – 1.03)	0.49
	42	37	37	26 (70.27)	22 (59.46)	0.99 (0.78 – 1.24)	0.91
	84	30	34	18 (60.00)	21 (61.76)	1.04 (0.87 – 1.23)	0.68
Per-protocol	Intervention						0.40
	14	22	21	14 (63.64)	10 (47.62)	1.09 (0.98 – 1.21)	0.10
	42	17	18	12 (70.59)	11 (61.11)	1.36 (0.84 – 2.20)	0.21
	84	13	13	7 (53.85)	5 (38.46)	1.69 (1.14 – 2.51)	**0.01**
***Staphylococcus aureus* positive**[2]	Intervention						0.08
	14	26	16	17 (65.38)	7 (43.75)	0.87 (0.78 – 0.96)	**0.01**
	42	22	17	16 (72.73)	8 (47.06)	0.85 (0.49 – 1.48)	0.56
	84	18	15	11 (61.11)	11 (73.33)	1.29 (0.84 – 1.96)	0.24

NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use. [1]Overall effect of endolysin treatment during intervention period was calculated with a Likelihood-Ratio test, the effect per time point using a Wald test with t-distribution. [2]Defined as having a positive culture both at visit 1 and visit 2a (endolysin n=32, vehicle n=24). Sensitivity analyses, performed by adding/subtracting 0.25 times the standard deviation to/of the odds, showed no differences in the odds ratio of topical corticosteroid use per assessment day.

Because AD is a heterogeneous disease, anti-*S. aureus* treatment might not be suitable for all patients with AD, indicating the need for subphenotyping. Because only 56% of our study population had two consecutive positive *S. aureus* skin cultures (indicating persistent colonization) before start of the intervention, the target population that would probably benefit the most from endolysin treatment was small.

Our data suggest that endolysin treatment has no effect on *S. aureus in vivo.* However, patients might have been recolonized with *S. aureus* from the nose because 73% of them were nasal carriers (data not shown). Alternatively, cetomacrogol as the basis of the endolysin cream might have created a barrier on the skin that prevented the endolysin to reach and subsequently kill *S. aureus*. However, some reduction in *S. aureus* load would have been expected in both treatment groups because of the use of TCSs and emollients in this study, which both have been shown to reduce the *S. aureus* load on the skin.[8,9] Nonetheless, it is unclear whether complete eradication of *S. aureus* is required for clinical improvement because a case series showed a clear clinical improvement without *S. aureus* reduction using a qualitative culture in *S. aureus*-related dermatoses.[6] In addition, the discrepant results between culture and qPCR indicate the complexity of the interpretation

of *S. aureus* testing. Despite the limitations and outcome, this study provides estimates of AD symptoms, use of TCSs, and the percentage of persistent *S. aureus* carriers that can be used for future clinical studies.

In conclusion, long-term targeted endolysin treatment against *S. aureus* in this study was well tolerated but had no TCS-sparing effect in patients with AD. However, an effect cannot be excluded because good compliance with the treatment and concurrent application of TCSs, emollients, or both might have masked a clinical benefit.

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Table S1. Results for the difference in secondary clinical outcomes – intention-to-treat analysis

Outcome	Time period or visit	Staphefekt Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Mean grams/week topical corticosteroid use, median (IQR)	Intervention[4]	4.5 (2.2-8.7)	3.6 (1.9-7.4)			0.66
	Intervention + follow-up[5]	5.1 (1.9-8.9)	4.1 (2.3-10.4)			0.91
Proportion of patients who indicated to have used less corticosteroids compared with baseline, n (%)	Intervention + follow-up					0.19
	3	36 (76.60)	34 (72.34)		0.77 (0.45 – 1.29)	0.32
	4	30 (65.22)	27 (60.00)		0.79 (0.51 – 1.22)	0.29
	5	22 (50.00)	20 (45.45)		0.91 (0.61 – 1.38)	0.67
	6	23 (53.49)	13 (29.55)		0.32 (0.21 – 0.51)	<0.001†
Change in EASI from baseline, mean (SD)	Intervention + follow-up					0.57
	3	-0.61 (4.88)	-1.63 (5.09)	1.03 (-1.30 – 3.36)		0.38
	4	0.11 (6.40)	-0.88 (6.22)	1.08 (-1.41 – 3.57)		0.39
	5	0.29 (7.40)	-1.08 (6.16)	1.28 (-1.48 – 4.04)		0.36
	6	0.43 (7.83)	-1.71 (7.26)	2.61 (-0.53 – 5.75)		0.10
IGA corrected for baseline	Intervention + follow-up					0.78
Proportion of patients with a reduction of ≥2 points in IGA from baseline, n (%)	3	0 (0.00)	2 (4.26)			0.46[6]
	4	1 (2.17)	2 (4.44)			0.62[6]
	5	1 (2.27)	3 (6.82)			0.62[6]
	6	1 (2.33)	5 (11.36)			0.20[6]
Change in POEM from baseline, mean (SD)	Intervention + follow-up					0.98
	3	-3.15 (4.64)	-2.94 (5.52)	-0.23 (-2.55 – 2.09)		0.84
	4	-2.93 (5.80)	-2.09 (5.43)	-0.54 (-2.87 – 1.78)		0.64
	5	-2.43 (6.13)	-2.02 (6.76)	-0.57 (-3.01 – 1.88)		0.65
	6	-1.79 (6.32)	-1.09 (6.68)	-0.79 (-3.57 – 1.99)		0.57

Table S1. Results for the difference in secondary clinical outcomes – intention-to-treat analysis (continued)

Outcome	Time period or visit	Staphefekt Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Change in pruritus NRS from baseline, mean (SD)	Intervention + follow-up					0.56
	3	0.26 (1.57)	0.21 (1.64)	0.03 (-0.63 – 0.69)		0.92
	4	0.20 (1.77)	-0.09 (1.43)	0.37 (-0.31 – 1.05)		0.28
	5	0.23 (1.85)	-0.05 (1.70)	0.32 (-0.43 – 1.07)		0.39
	6	-0.05 (2.23)	0.14 (1.98)	-0.09 (-0.97 – 0.79)		0.84
Proportion of patients with a reduction of ≥2 points in pruritus NRS from baseline, n (%)	Intervention + follow-up					0.65
	3	14 (29.79)	10 (21.28)		0.56 (0.24 – 1.28)	0.17
	4	16 (34.78)	11 (24.44)		0.57 (0.26 – 1.23)	0.15
	5	14 (31.82)	17 (38.64)		1.44 (0.71 – 2.94)	0.31
	6	16 (37.21)	15 (34.09)		0.84 (0.42 – 1.68)	0.62
Proportion of patients with a reduction of ≥3 points in pruritus NRS from baseline, n (%)	Intervention + follow-up					0.71
	3	7 (14.89)	3 (6.38)		0.41 (0.11 – 0.53)	0.18
	4	5 (10.87)	4 (8.89)		0.69 (0.17 – 2.77)	0.60
	5	7 (15.91)	6 (13.64)		0.83 (0.32 – 2.18)	0.71
	6	11 (25.58)	8 (18.18)		0.50 (0.22 – 1.13)	0.09
Change in Skindex-29 from baseline, mean (SD)	Intervention + follow-up					0.71
	3	-7.15 (9.83)	-4.86 (9.32)	-2.63 (-6.81 – 1.54)		0.21
	5	-7.35 (10.54)	-4.82 (12.96)	-2.35 (-7.22 – 2.51)		0.34
	6	-8.38 (12.98)	-4.66 (13.87)	-3.35 (-8.97 – 2.26)		0.24
Number of doctor reported flares from baseline, n (%)	Intervention[7]	14 (10.22)	17 (12.50)			0.55
Number of patient reported flares from baseline, n (%)	Intervention[7]	14 (10.22)	17 (12.50)			0.58

Table S1. Results for the difference in secondary clinical outcomes – intention-to-treat analysis (continued)

Outcome	Time period or visit	Staphefekt Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Mean time (days) to doctor reported flare from baseline, median (IQR)	Intervention[8]	44.50 (35.25-84.00)	43.00 (30.50-84.50)			0.95
Mean time (days) to patient reported flare from baseline, median (IQR)	Intervention[8]	40.50 (20.00-72.25)	30.00 (15.00-64.00)			0.71
	Follow-up[9]	30.50 (16.75-50.50)	22.00 (8.50-43.50)			0.52
Number of patients with at least one (serious) AE, n	Intervention + follow-up	40	40			1.00
Number of (serious) AEs, n	Intervention + follow-up	82	74		1.14 (0.83 – 1.56)[10]	0.42

Abbreviations: CI, confidence interval; EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; OR, odds ratio; SD, standard deviation. Patients included in analyses per visit in endolysin and vehicle group: visit 3, n=47 and n=47; visit 4, n=46 and n=45; visit 5, n=44 and n=44; visit 6, n=43 and n=44. NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use in the analysis of the mean grams/week topical corticosteroid use. [1]Differences in change presented for analyses using a Linear Mixed-Effect model. [2]OR presented for analysis using a Generalized Linear Mixed-Effect model. [3]*P*-values were calculated using a Chi-Square test or Fisher's Exact test for categorical data, where appropriate. A non-parametric Mann-Whitney U Test for independent samples was used for continuous variables. The overall effect of endolysin treatment during the intervention and follow-up period ((Generalized) Linear Mixed-Effect models) was analyzed with a Likelihood-Ratio test and per visit using a Wald test with t-distribution. [4]Endolysin group n=32, vehicle group n=34. [5]Endolysin group n=32, vehicle group n=32. [6] Since the number of patients per cell are ≤5 a Fisher's Exact test per visit was used instead of a Generalized Linear Mixed-Effect model. A *P*-value of 0.0125 will be considered significant after Bonferroni correction. [7]For every visit from baseline through week 12, 137 visits in endolysin group and 136 visits in vehicle group, it was registered if a flare occurred yes/no. [8]Endolysin group n=14, vehicle group n=17. [9]Endolysin group n=8, vehicle group n=5. [10]Rate ratio with 95% CI. [†]Significant result.

Table S2. Results for the difference in secondary clinical outcomes – per-protocol analysis

Outcome	Time period or visit	Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Mean grams/week topical corticosteroid use, median (IQR)	Intervention[4]	6.0 (3.3-10.9)	4.1 (2.6-7.9)			0.44
	Intervention + follow-up[5]	6.1 (4.2-7.3)	5.0 (2.6-14.7)			1.00
Proportion of patients who indicate to have used less corticosteroids compared with baseline, n (%)	Intervention + follow-up					0.40
	3	19 (76.00)	21 (72.41)		0.91 (0.41 – 2.00)	0.81
	4	8 (42.11)	11 (52.38)		1.52 (0.70 – 3.34)	0.30
	5	7 (43.75)	11 (73.33)		3.17 (1.06 – 9.48)	0.04
	6	8 (50.00)	5 (33.33)		0.50 (0.18 – 1.39)	0.18
Change in EASI from baseline, mean (SD)	Intervention + follow-up					0.81
	3	-1.17 (5.70)	-0.64 (4.99)	-0.45 (-3.64 – 2.73)		0.78
	4	-0.58 (7.19)	1.73 (6.14)	-1.95 (-5.55 – 1.65)		0.28
	5	-0.59 (7.79)	-0.71 (4.16)	-0.77 (-4.83 – 3.30)		0.71
	6	-0.94 (6.44)	-0.20 (5.61)	-1.45 (-5.92 – 3.03)		0.52
Change in IGA from baseline	Intervention + follow-up					0.86
Proportion of patients with a reduction of ≥2 points in IGA from baseline, n (%)	3	0 (0.00)	1 (3.45)			1.00[6]
	4	0 (0.00)	1 (4.76)			1.00[6]
	5	1 (6.25)	1 (6.67)			1.00[6]
	6	1 (6.25)	2 (13.33)			0.60[6]
Change in POEM from baseline, mean (SD)	Intervention + follow-up					0.77
	3	-4.04 (4.72)	-3.14 (5.78)	-0.91 (-4.11 – 2.30)		0.57
	4	-3.63 (5.56)	-2.76 (6.31)	-0.78 (-4.25 – 2.70)		0.66
	5	-3.06 (5.95)	-2.93 (7.44)	-0.14 (-3.83 – 3.54)		0.94
	6	-3.00 (3.93)	-0.87 (6.55)	-2.13 (-5.82 – 1.55)		0.25

Table S2. Results for the difference in secondary clinical outcomes – per-protocol analysis (continued)

Outcome	Time period or visit	Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Change in pruritus NRS from baseline, mean (SD)	Intervention + follow-up					0.50
	3	0.08 (1.53)	0.34 (1.88)	-0.24 (-1.13 – 0.65)		0.59
	4	-0.16 (1.34)	-0.10 (1.38)	-0.29 (-1.29 – 0.71)		0.56
	5	0.19 (1.56)	0.00 (1.69)	0.11 (-1.09 – 1.31)		0.85
	6	-0.06 (2.41)	0.80 (1.86)	-0.89 (-2.38 – 0.60)		0.24
Proportion of patients with a reduction of ≥2 points in pruritus NRS from baseline, n (%)	Intervention + follow-up					0.58
	3	8 (32.00)	7 (24.14)		0.63 (0.22 – 1.78)	0.39
	4	7 (36.84)	3 (14.29)		0.32 (0.07 – 1.38)	0.13
	5	5 (31.25)	5 (33.33)		1.06 (0.23 – 5.01)	0.94
	6	8 (50.00)	5 (33.33)		0.42 (0.10 – 1.81)	0.24
Proportion of patients with a reduction of ≥3 points in pruritus NRS from baseline, n (%)	3	3 (12.00)	3 (10.34)		0.41 (0.11 – 0.53)	1.00[6]
	4	0 (0.00)	2 (9.52)		0.69 (0.17 – 2.77)	0.49[6]
	5	2 (12.50)	3 (20.00)		0.83 (0.32 – 2.18)	0.65[6]
	6	5 (31.25)	4 (26.67)		0.50 (0.22 – 1.13)	1.00[6]
Change in Skindex-29 from baseline, mean (SD)	Intervention + follow-up					0.66
	3	-6.62 (9.39)	-5.41 (10.26)	-1.53 (-7.33 – 4.27)		0.60
	5	-6.14 (9.67)	-5.00 (15.72)	-2.21 (-10.58 – 6.16)		0.60
	6	-8.41 (14.56)	-2.99 (14.05)	-6.60 (-16.48 – 3.27)		0.19
Number of doctor reported flares from baseline, n (%)	Intervention[7]	2 (3.33)	10 (15.38)			0.03†
Number of patient reported flares from baseline, n (%)	Intervention[7]	5 (8.33)	6 (9.23)			0.86
Mean time (days) to doctor reported flare from baseline, median (IQR)	Intervention[8]	22.50[11]	42.00 (11.75-53.25)			0.76

Table S2. Results for the difference in secondary clinical outcomes – per-protocol analysis (continued)

Outcome	Time period or visit	Endolysin	Vehicle	Difference in change (95% CI)[1]	OR (95% CI)[2]	*P*-value[3]
Mean time (days) to patient reported flare from baseline, median (IQR)	Intervention[9]	39.00 (15.00-53.50)	29.00 (5.00-47.00)			0.66
	Follow-up[9]	14.00[11]	32.00[11]			NA
Number of patients with at least one (serious) AE, n[12]	Intervention + follow-up	33	33			1.00
Number of (serious) AEs, n[12]	Intervention + follow-up	61	53		1.20 (0.84 – 1.72)[13]	0.31

Abbreviations: CI, confidence interval; EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NA, not available; NRS, Numeric Rating Scale; POEM, Patient Oriented Eczema Measure; OR, odds ratio; SD, standard deviation. Patients included in analyses per visit in Staphefekt and placebo group: visit 3, n=25 and n=29; visit 4, n=19 and n=21; visit 5, n=16 and n=15; visit 6, n=16 and n=15. NOTE: Given the low number of patients using escape medication (n=5), we did not correct for its use in the analysis of the mean grams/week topical corticosteroid use. [1]Differences in change presented for analyses using a Linear Mixed-Effect model. [2]OR presented for analysis using a Generalized Linear Mixed-Effect model. [3]*P*-values were calculated using a Chi-Square test or Fisher's Exact test for categorical data, where appropriate. A non-parametric Mann-Whitney U Test for independent samples was used for continuous variables. The overall effect of endolysin treatment during the intervention and follow-up period ((Generalized) Linear Mixed-Effect models) was analyzed with a Likelihood-Ratio test and per visit using a Wald test with t-distribution. [4]Endolysin group n=14, vehicle group n=10. [5]Endolysin group n=15, vehicle group n=10. [6]Since the number of patients per cell are ≤5 a Fisher's Exact test per visit was used instead of a Generalized Linear Mixed-Effect model. A *P*-value of 0.0125 will be considered significant after Bonferroni correction. [7]For every visit from baseline through week 12, 60 in endolysin group and 65 in vehicle group, it was registered if a flare occurred yes/no. [8]Endolysin group n=2, vehicle group n=10. [9]Endolysin group n=5, vehicle group n=6. [10]Endolysin group n=1, vehicle group n=2. [11]No IQR because n≤2. [12]AEs that occurred until 8 weeks (follow-up period in intention-to-treat analysis) after the first protocol deviation, i.e. no use of endolysin or vehicle on total skin surface twice daily, were analyzed. [13]Rate ratio with 95% CI. [†]Significant result.

Table S3. Results for the difference in reduction of *Staphylococcus aureus* 0.5 hour after first application determined by semi-quantitative culture

Analysis	Decrease in semi-quantitative culture of *S. aureus*[1]	Endolysin, n (%)[2]	Vehicle, n (%)[3]	*P*-value[4]
Intention-to-treat	Yes	6 (12.5)	4 (8.3)	0.74
	No	42 (87.5)	44 (91.7)	

Abbreviations: *S. aureus*, *Staphylococcus aureus*. [1]Decrease is defined as a decrease of at least 1 point on semi-quantitative scale (scale ranges from 0-4). [2]Patients included in analysis in endolysin group: n=49. [3]Patients included in in analysis in vehicle group: n=48. [3]*P*-values were calculated using a Fisher's Exact test for categorical data.

Table S4. Results for the difference in reduction of *Staphylococcus aureus* from baseline determined by qPCR

Analysis	Time period	Log10 reduction in qPCR for *S. aureus*		*P*-value[1]
		Endolysin, n (%)	**Vehicle**, n (%)	
Intention-to-treat[2]	Visit 2a to visit 3	3 (6.5)[6]	4 (8.5)[6]	1.00
	Visit 2a to visit 5	3 (6.8)[7]	8 (18.2)[7]	0.20
Per-protocol[3]	Visit 2a to visit 3	2 (7.1)	3 (9.4)	1.00
	Visit 2a to visit 5	1 (6.3)	5 (35.6)[8]	0.07
***S. aureus* positive**[4-6]	Visit 2a to visit 3	3 (33.3)	4 (30.8)	1.00
	Visit 2a to visit 5	3 (33.3)	9 (66.7)	0.20

Abbreviations: *S. aureus*, *Staphylococcus aureus*; qPCR, quantitative polymerase chain reaction. [1]*P*-values were calculated using a Fisher's Exact test for categorical data. [2]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=47 and n=47; visit 2a to visit 5, n=44 and n=44. [3]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=25 and n=29; visit 2a to visit 5, n=16 and n=15. [4]Patients included in endolysin and vehicle group: visit 2a to visit 3, n=9 and n=13; visit 2a to visit 5, n=9 and n=12. [5]Analysis additionally to analyses described in the study protocol. [6]*Staphylococcus aureus* positive is defined as having a positive qPCR at visit 2a (endolysin group n=10, vehicle group n=14). Missings: [6]n=1 (2.1%), [7]n=1 (2.3%), [8]n=1 (6.7%).

Table S5. Incidence of (non-) Treatment Emergent Adverse Events – Overall and per study phase

	Total (n=100)		**Endolysin (n=50)**		**Vehicle (n=50)**	
	Patients, n (%)	**Events**, n	**Patients**, n (%)	**Events**, n	**Patients**, n (%)	**Events**, n
Overall (V1-V6)	83 (83.0)	183	43 (86.0)	96	40 (80.0)	87
Run-in (V1-V2)						
At least 1 non-TEAE	21 (21.0)	27	11 (22.0)	14	10 (20.0)	13
At least 1 serious non-TEAE	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 non-TEAE leading to study discontinuation	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 non-TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Intervention (V2-V5)						
At least 1 TEAE	73 (73.0)	125	36 (72.0)	67	37 (74.0)	58
At least 1 serious TEAE	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 TEAE leading to study discontinuation	3 (3.0)	3	1 (2.0)	1	2 (4.0)	2
At least 1 TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Follow-up (V5-V6)						
At least 1 TEAE	25 (25.0)	31	15 (30.0)	15	10 (20.0)	16
At least 1 serious TEAE	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
At least 1 TEAE leading to study discontinuation	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
At least 1 TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0

Abbreviations: TEAE, Treatment Emergent Adverse Event (adverse events after the first endolysin or vehicle administration); V, visit.

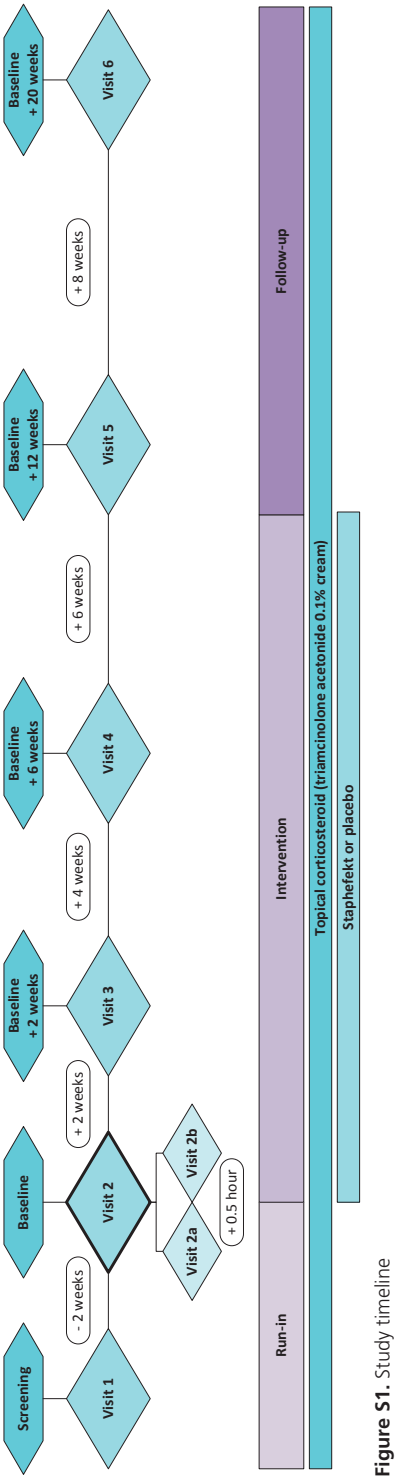

Figure S1. Study timeline

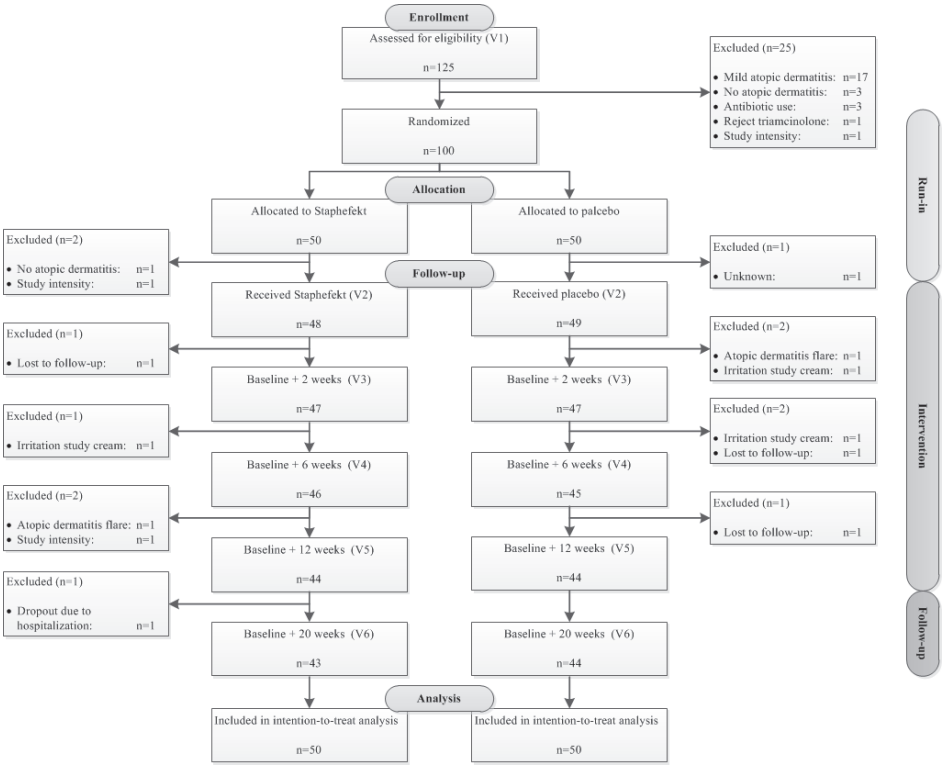

Figure S2. Flowchart of the study design

Appendix 1. Microbial methods

a. Microbial sampling methods

Samples of the skin were collected using sterile Copan 490CE.A swabs for culture analysis and skin scrubs for qPCR analysis. Skin swabs were collected from a skin lesion at the first visit, preferably located at the antecubital fold or the popliteal fold. For all consecutive visits the swab was collected from the location chosen at the first visit. The scrubs were collected from the lower arm, adjacent to the antecubital fold according to methods described previously.[23] Scrub samples were stored at -80 °C.

b. Semi-quantitative culture and qPCR for Staphylococcus aureus

Bacterial cultures were performed using routine diagnostic culture procedures, using blood agar plates and specific S. aureus culture plates (ChromID S. aureus Elite agar (SAIDE), Biomérieux, France) for overnight incubation and subsequent species determination by MALDI-TOF (Bruker Daltonics, Bremen, Germany). For DNA isolation 150 uL from the sample was added to 350 uL lysis buffer, 500 uL Phenol (Tris pH 8) and 500 uL 0.1 mm zirconium beads. This mixture was mechanically disrupted by bead beating twice for 2 minutes, followed by centrifuging for 10 minutes at 1690 RCF to separate the aqueous and phenolic phases. The aqueous phase was purified using AGOWA mag Mini DNA isolation kit. After elution, we used qPCR to determine the total load of S. aureus with the following primers and probes: 16S-S.aur-F1 (5'-GCG AAG AAC CTT ACC AAA TCT TG-3'), 16S-S.aur-R1 (5'-TGC ACC ACC TGT CAC TTT GTC-3'), 16S-S.aur MGB Taqman® probe (5'-CAT CCT TTG ACA ACT CT-3') with NED™ label.

Chapter 7

General discussion

Primary immunodeficiency diseases (PIDs) are generally characterized by an increased risk of infectious complications due to specific inborn errors in immune cell function. However, patients with PIDs also present with features of significant immune dysregulation, often leading to autoimmunity, autoinflammation, (hematological) malignancies and allergic disorders.[1-3] Previously, immunodeficiency and autoimmunity were considered to be mutually exclusive conditions. Increased understanding of the complex immune regulatory and signaling mechanisms involved, coupled with the application of genetic analysis, reveals more and more the relation between primary immunodeficiency syndromes and autoimmune diseases.[4] Monogenic defects can cause rare diseases that predominantly present with autoimmune symptoms or recurrent infections (immunodeficiency). However, it has been increasingly recognized that various genetic variant give rise to a clinical phenotype of both immunodeficiency and autoimmunity. Also other features of immune dysregulation, like (hematological) malignancy, may be attributed to genetic variants found in PIDs. As an example, a major complication of activated phosphatidylinositol 3-kinase-δ (PI3K-δ) syndrome (APDS) is malignancy (especially B-cell lymphoma), which is the result of uncontrolled PI3K-δ activity in lymphocytes.[5]

The first aim of this thesis was to gain more insight in the nature and prevalence of infectious and noninfectious skin disorders and manifestations within the atopic syndrome, including atopic dermatitis (AD), food allergy (FA), asthma and allergic rhinitis (AR), in patients with PIDs. Recognition of these symptoms could facilitate earlier diagnosis of PIDs. As skin disorders, including AD, and atopic manifestations were demonstrated as frequently occurring symptoms in PIDs, it suggests that immune dysregulation also plays a role in the multifactorial pathogenesis of AD and the atopic syndrome. Moreover, common pathways appear to be involved in the pathogenesis of these disorders and PIDs. Therefore, this thesis aimed to identify these common pathways of immune dysregulation in PIDs, atopy and AD. Knowledge on immune dysregulation processes in PIDs was used to gain more insight in the pathogenesis of AD and the atopic syndrome, in which endotypes were identified. Moreover, the antibody response and microbiome in AD were studied to better understand the interaction between various pathogenic factors in AD and immune dysregulation. Based on the predominance of *S. aureus* in the skin microbiome of AD patients, a clinical trial was performed using an endolysin selectively targeting *S. aureus.* In the following paragraphs the main findings of this thesis are summarized and discussed.

MAIN FINDINGS OF THE THESIS

Skin disorders are prominent clinical features in primary immunodeficiency diseases

PIDs are characterized by an increased risk of infections, autoimmune disease, autoinflammatory complications, malignancy and allergic disorders. In Chapter 2 of the thesis it was demonstrated that skin disorders are also frequently occurring symptoms in patients with PIDs, based on a systematic literature analysis of 67 mainly cross-sectional studies. A complete spectrum of skin disorders was composed, categorized in 15 main groups of cutaneous manifestations, across 30 PID phenotypes. Presence of skin manifestations characteristic for ataxia-telangiectasia (AT), hyper immunoglobulin (Ig) E syndrome (HIES), selective IgA deficiency (SIgAD), autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) and chronic granulomatous disease (CGD) have been identified. For example, skin abscesses were found to be a well-defined characteristic for HIES and CGD, and alopecia and skin pigmentation disorders for SIgAD and APECED. In various PIDs skin disorders were already known to be among the clinical characteristics and frequently occurring manifestations, such as telangiectasia in AT and granuloma and skin abscesses in CGD. However, this thesis also reveals infectious and noninfectious skin disorders of which an association with a PID was not generally recognized. Examples include alopecia in SIgAD and hypopigmented macules in AT. Although various skin conditions were described in patients with a PID, the prevalence of these skin conditions in a matched control group remains largely unknown. Interpretation of the data should, therefore, be done with caution. Nonetheless, the high prevalence of skin conditions in PIDs suggests that these could be used as an additional warning sign to raise suspicion for underlying PID. Linking skin disorders to PIDs provides a valuable tool that could raise PID awareness in clinical practice.

Skin infections and nail disorders are warning signs for primary immunodeficiency diseases

In Chapter 3.1 of the thesis, a Dutch cohort of 45 pediatric patients and 207 adult patients with a PID and 56 unaffected partner-controls was studied using a questionnaire. A history of skin disorders, in particular skin infections and nail disorders, was more frequently reported in adult PID patients (80.2%) when compared with adult partner-controls (41.1%, $p<0.001$). As far as we know this was the first study evaluating the nature and prevalence of skin disorders in a mainly Western population of PID patients. Data retrieved from this cohort of PID patients were mostly consistent with the data summarized in our systematic review (Chapter 2), which included mainly data derived from Middle-Eastern countries. However, in the Dutch patient cohort viral and bacterial skin infections, erythematous skin lesions and skin rashes were more frequently reported than in current literature.

Skin disorders were found to develop earlier in life in adult patients with PIDs (mean age 20.9 (SD 22.0) years) when compared with unaffected partner-controls (mean age 33.7 (SD 26.6) years, p=0.02) suggesting an association between the skin condition and immune dysfunction in PID. Moreover, skin disorders preceded a diagnosis of PID on average 20.3 (SD 20.5) years in adult patients, whereas classical PID symptoms, such as respiratory tract infections, developed on average 15.9 (SD17.7) years before the PID diagnosis in this patient cohort. Based on these results, skin disorders frequently appeared to be presenting symptoms, while they are not included in the warning signs for PIDs.

Current literature appointed eczematous dermatitis as common finding and presenting clinical manifestation among PIDs, especially in rare PIDs which are not well represented in our cohort.[6] In our study, (atopic) dermatitis also was the most commonly reported presenting skin disorder in PID patients. However the prevalence did not significantly differ from partner-controls (29.5% vs. 25.0%, respectively) and even less adult patients reported (atopic) dermatitis as first developed skin disorder compared with partner-controls (15.0% vs. 52.2%, respectively). Moreover, the prevalence of a history of (atopic) dermatitis in both adult patients and partner-controls included in this study corresponds to the lifetime prevalence of atopic dermatitis in the Dutch population, which is up to 25% in children and 1-7% in adults.[7,8] Therefore, we feel that (atopic) dermatitis is not a distinctive presenting manifestation in the development of PIDs in general. By contrast, we hypothesize that (a combination of) other skin manifestations, like skin infections and nail disorders (prevalence in adult patients vs. adult controls 61.4% vs. 17.9%, p<0.001, and 38.6% vs. 7.1%, p=0.005, respectively), are more useful in raising suspicion of an underlying PID in addition to the presence of the currently used warning signs for PIDs.[9]

Staphylococcus aureus-targeting treatment in patients with primary immunodeficiency diseases could be beneficial in reducing severity of skin disease

Staphylococcus (*S.*) *aureus* is involved in the pathogenesis of many common infectious skin disorders (i.e. *S. aureus*-induced skin infections) and the inflammatory skin disorder AD. In patients with PIDs, only a few studies reported culture-proven *S. aureus*-associated skin disorders (Chapter 2).[6,10-12] These skin disorders included a papulopustular eruption, eczematous dermatitis, (cold) abscesses and wounds in HIES[13-16], skin infections in Comèl Netherton syndrome[17] and suppurative dermatitis in patients with CGD.[18] Furthermore, colonization with *S. aureus* was found to be significantly correlated with skin disease severity in PID patients.[19]

In our Dutch population of adult PID patients, including mostly predominantly antibody deficiencies (PADs), a positive *S. aureus* culture at a single time point was found in 40.0%

(12/30) of the skin disorders with suspected *S. aureus*-related etiology (Chapter 3.1). These positive cultures originated from dermatitis lesions, ecthyma and folliculitis. The prevalence of lesional *S. aureus* in our PID population was lower than the prevalence of lesional *S. aureus* in patients with AD (70%).[20] *S. aureus* colonization in less than half of the clinical suspected *S. aureus*-related skin lesions in our study could reflect intermittent carriage of *S. aureus*.[21] We suppose that repetitive culturing during clinical examination to identify skin lesions colonized with *S. aureus* can contribute in selection of PID patients, which might have benefit from *S. aureus*-targeting treatment to reduce skin disease severity.

Atopic manifestations are underestimated clinical features in primary immunodeficiency diseases

The systematic review presented in Chapter 2 of the thesis shows that original data on atopic manifestations in PIDs are limited, as studies comprise small patient samples and the diagnosis of atopic manifestations is generally not based on diagnostic tests. Although atopic manifestations are described in various PID phenotypes, presence of these manifestations is mainly reported in immunodeficiencies affecting both cellular and humoral immunity (combined immunodeficiencies; CIDs), like DOCK8 deficiency, and CIDs with associated or syndromic features, such as Comèl Netherton syndrome.[6,22] In addition, atopic manifestations have also been reported in PADs, like SIgAD, with a prevalence comparable to the general population.[23-28]

In Chapter 3.2 we showed that all atopic manifestations, including AD, FA, asthma and AR, were highly prevalent in 47 children and 206 adults with a PID based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and diagnostic criteria or tests.[29] Using the questionnaire, we found in adult patients a self-reported prevalence of 49.5%, 10.7%, 55.7% and 49.8% for AD, FA asthma and AR, respectively. Remarkably, a discrepancy was shown between the prevalence of AD based on patient-reported skin disorders (Chapter 3.1) and the ISAAC questionnaire (Chapter 3.2). Self-reported ever experienced atopic manifestations were significantly more common in adult PID patients when compared with 56 adult partner-controls. Although our study population mainly involved patients with PADs and CIDs, atopic manifestations were reported in a large spectrum of PIDs across the various phenotypes, which is in contrast with previous literature (Chapter 2). The prevalence of FA, asthma and AR based on diagnostic test results was significantly lower than the prevalence reported by adult patients (FA 4.8%, asthma 16.4% and AR 19.2%). This discrepancy could be due to overreporting of clinical symptoms related to atopy, the relapsing-remitting course of atopic manifestations over time, or because PID patients are known to commonly have asthma-like airway complaints without of a positive diagnostic test.[30] Unfortunately, data on diagnostic tests from the control group or another

reference population were not available. Nonetheless, estimates of atopic manifestation prevalence in PID patients were provided in this study that can be used for future studies.

Compared with our review (Chapter 2), in which diagnosis of atopic manifestations was generally based on medical records, we found a significantly higher prevalence of atopic manifestations in patients with a PAD and comparable numbers of patients with atopic manifestations in CIDs. The exact mechanism underlying the development of atopic manifestations in PIDs remains to be elucidated. Nonetheless, the pathogenic pathway involved in the atopic syndrome, in which T lymphocytes play a central role, could suggest that patients with a PID affecting cellular immunity are more prone to develop atopic manifestations.[31] However, it is known that also in PADs T lymphocyte abnormalities are found, which could contribute to development of the atopic syndrome in these patients.[32,33] According to our data, we propose early evaluation of atopic manifestations in patients with CIDs and PADs to prevent clinical deterioration. Future studies should focus on identification of specific atopic characteristics of PIDs to evaluate whether they could serve as a potential warning sign for an underlying PID.

New clustering algorithm shows endotypes within the atopic syndrome based on expression profiles of immune cell lineages

As mentioned in the introduction of the thesis, the atopic syndrome has a heterogeneous clinical presentation, which probably is the result of an interaction between physiological, biological, immunological and genetic mechanisms.[34] Atopic manifestations are prevalent comorbidities in PIDs, suggesting that genetic defects in pathways that are involved in monogenetic PIDs could also play a role in the pathogenesis of the atopic syndrome.[6,35] In Chapter 4 we defined subclasses within the atopic syndrome via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages. We identified 160 atopy-related genes, of which 22 genes were overlapping with disease-causing genes of monogenic PIDs.[36] Seven distinct clusters within the atopy-related genes were identified. The overlapping genes involved in both the atopic syndrome and PIDs were bundled in two of the atopy-related gene clusters suggesting that these endotypes of the atopic syndrome could be associated with the predisposition to develop a PID. However, these data should be confirmed *in vivo* as atopy-related variants in the overlapping genes might differ from disease-causing variants resulting in of PID.

Based on the known atopy-related genes and corresponding expression profiles of immune cell lineages, T helper (Th) lymphocytes seem to play a crucial role in the pathogenesis of the atopic syndrome, which is in accordance with previous findings. Disturbed T lymphocyte function has been described in AD, FA, asthma and AR.[37-43] This finding supports the hypothesis that changes in the immune system underlie and could be involved in the

pathogenesis of the atopic syndrome next to genetic mechanisms, which have previously been reported in literature.[44-53]

Patients with atopic dermatitis have an increased IgE response against *Staphylococcus aureus*

In Chapter 5 of the thesis a systematic review on the antibody response against *S. aureus* was performed to gain more insight in how the immune system of AD patients counteracts the bacterium. This might help us to better understand the role of *S. aureus* in AD pathogenesis, as well as the mechanisms by which *S. aureus* causes inflammation. We significantly more often found an IgE response against *S. aureus* immune modulating superantigens (33% for staphylococcal enterotoxin (SE) A and 35% for SEB) in patients with AD as compared with healthy controls (pooled odds ratio with 95% confidence interval 8.37 (2.93-23.92) for SEA and 9.34 (3.54-24.93) for SEB). Subgroup analyses to explain the high heterogeneity in the pooled analyses suggest that the antibody response is dependent on the method of antibody identification (ELISA vs. RIA) and the geographical region of the study centre (Asia vs. Europe) in accordance with the study of Taylor *et al*.[54] The increased IgE response against immune modulating antigens suggests that *S. aureus* encourages epithelial damage via direct T lymphocyte stimulation and subsequent T lymphocyte proliferation and cytokine release.[55-57] Nonetheless, it is unclear whether the increased IgE response is specific for patients with AD as comparable studies in other *S. aureus*-related diseases (i.e. *S. aureus* bacteraemia, folliculitis) are not available. Furthermore, it is unknown whether the elevated IgE response in AD is a consequence of the increased skin permeability, abundance of SEA and SEB carrying *S. aureus* strains or an altered immune response against *S. aureus*. For example, the anti-SEA and anti-SEB IgE responses could be the result of increased expression of these antigens by AD-specific *S. aureus* strains, indicating SEA and SEB expression as possible bacterial mechanisms to aggravate or even initiate inflammation in AD. However, previous studies describe absence of a prevailed *S. aureus* genotype, suggesting that the IgE response is the result of immune dysregulation in AD in contrast to colonization with an AD-specific *S. aureus* strain.[58-61]

Targeted intervention against *Staphylococcus aureus* had no effect on symptoms of atopic dermatitis

The effect of long-term treatment of AD with an endolysin that selectively targets *S. aureus* was studied using a double-blind, vehicle-controlled design and described in Chapters 6.1 and 6.2 of this thesis. Over the 12-week intervention period (corresponding to 8400 days for 100 patients), patients in the endolysin group used a topical corticosteroid (TCS) for 1889 (45.0%) days compared with 1566 (37.3%) days in the vehicle group. There was no statistically significant difference in the probability of TCS use per day between the groups nor a difference in reduction of clinical disease severity scores. These data

are in accordance with data from a Cochrane review showing no significant effect of short-term nonspecific anti-*S. aureus* therapy (e.g. antibiotics and antibacterial therapeutic clothes) in patients with noninfected AD.[62] We could not confirm the positive results of other longer-term studies. However, these studies used broad-spectrum antimicrobials and mainly included patients with signs of bacterial infection.[63] Patients with clinically infected AD were excluded from our study. A possible effect of anti-*S. aureus* endolysins in patients with clinically infected AD should be determined in future studies.

Several other hypotheses could explain our results, which has implications for future studies. First, use of triamcinolone in the run-in phase resulted in a decrease in AD severity, which could have masked a possible benefit of endolysin treatment. Second, anti-*S. aureus* treatment might not be suitable for all patients with AD because it is a heterogeneous disease, indicating the need for subphenotyping. Since only 56% of our study population had two consecutive positive *S. aureus* skin cultures (indicating persistent colonization) before start of the intervention, the target population that would probably benefit the most from endolysin treatment was small.

However, the most likely reason for the absence of a significant reduction in TCS use in this study might be that there was no difference in *S. aureus* load reduction between the endolysin and vehicle treated groups determined by semi-quantitative culture and qPCR. Possibly, patients were recolonized with *S. aureus* from the nose since 73% of them were nasal *S. aureus* carriers.[64,65] Furthermore, cetomacrogol used as basis of the cream could have formed a barrier on the skin that prevents the endolysin to reach and subsequently kill *S. aureus*. Some reduction in *S. aureus* load could, however, have been expected in both treatment groups due to the use of corticosteroids and emollients in this study, which both have shown to reduce the *S. aureus* load on the skin.[66-70] Nonetheless, it is unclear whether complete eradication of *S. aureus* is required for clinical improvement as a case series showed a clear clinical improvement without significant *S. aureus* reduction using a qualitative culture in clinically infected AD.[71]

In conclusion, endolysin treatment as studied in this randomized controlled trial did not contribute to unraveling the contribution of the microbiome in the pathogenesis of AD due to absence of a clinical effect on AD and reduction of *S. aureus* load. Nonetheless, endolysin treatment was well tolerated and this study provides estimates of AD symptoms, use of TCSs and the percentage of persistent *S. aureus* carriers that can be used for future clinical studies.

IMMUNE DYSREGULATION: A MODEL FOR RESEARCH ON PRIMARY IMMUNODEFICIENCY DISEASES, SKIN DISORDERS AND ATOPY

Primary immunodeficiency disease as immune dysregulation disorder

PIDs are characterized by a compromised or entirely absent function of a part of the immune system, which makes people vulnerable for infections. Therefore, PIDs are generally considered to be immunodeficiency diseases, as implied by its name. However, there is increasing evidence that non-infectious complications, including autoimmune and autoinflammatory complications, (hematological) malignancies and allergic disorders, are involved in PIDs as well.[1-3] Autoimmune disorders, such as type 1 diabetes mellitus, rheumatoid arthritis and psoriasis, are the result of an immune response directed against normal parts of the body, termed auto-antigens. In autoinflammatory disorders, like familiar mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), the innate immune system is abnormally activated, leading to recurrent episodes of fever and inflammation.[72] Both autoimmune and autoinflammatory conditions are characterized by disruption of the normal function of the immune system, also called immune dysregulation. Interestingly, different forms of immune dysregulation, both as primary or as accompanying problem, next to the immunodeficiency seem to occur in one and the same PID. Therefore, PIDs should be considered as immune dysregulation disorders instead of immunodeficiency diseases.[73]

Immune dysregulation in skin disorders and atopy

Immune dysregulation also plays a role in the multifactorial pathogenesis of skin disorders and atopy.[74] For example, chronic inflammation caused by different triggers, such as biological agents (e.g. bacteria, viruses), physical agents (e.g. UV radiation) and immunologic disorders (e.g. PIDs), is suggested as one of the hallmarks in skin carcinogenesis.[75] In AD, a number of immunological abnormalities have been described, such as increased serum IgE levels, elevated Th2-type cytokines in acute lesions and Th1-type cytokines in chronic lesions, and decreased expression of antimicrobial peptides.[76] The immunological dysregulation might precede barrier changes (inside-out hypothesis) or could be the effect of barrier dysfunction and subsequent penetration of environmental stimuli into the skin (outside-in hypothesis).[77] It could be suggested that immune dysregulation is primary affected in AD as not all patients with AD have a genetic polymorphism in one of the barrier genes (e.g. *FLG*, *KIF3A*, *OVOL1* and *ADAMTS*).[78,79] The fact that a significant amount of genes involved in AD is associated with immune dysregulation further supports a genetic predisposition of immunological alterations in AD.[44] More than half of the patients with AD will develop asthma and other allergic diseases (i.e. FA and AR) involved in the atopic syndrome, which indicates that both local and systemic immunity are involved.[80,81] In a Dutch study group

of children with moderate-to-severe AD more than 60% of the patients had a diagnosis of FA and even more than 80% of the patients were diagnosed with asthma and AR.[82] The pathway involved in the atopic syndrome could be characterized by auto-allergy, in which atopy seems to stand at the boundary between allergy and autoimmunity. Herein, the presence of IgE antibodies against self-proteins is an important pathogenic feature, emphasizing the role of immune dysregulation in the atopic syndrome.[83-85]

Spectrum of immune dysregulation

The spectrum of clinical features associated with PIDs has broadened due to the recent identification of many novel causative genes.[86] Patients with a PID and noninfectious complications are increasingly recognized with features of immune dysregulation, including autoimmunity, inflammation, lymphoproliferation and malignancy.[87] Depending on the pathways involved and the corresponding number and functionality of immune cells involved, the clinical manifestations of diseases involving immune dysregulation are characterized by a higher susceptibility to infections or, on the other hand, an increase in manifestations of autoimmunity.[4] Moreover, it has been increasingly recognized that various genetic variants give rise to a clinical phenotype of both immunodeficiency and autoimmunity. One could visualize immune dysregulation as a spectrum with infections on one end and autoimmunity on the other end. PIDs can present with a variety of symptoms covering the entire spectrum (Figure 1). Within PIDs, for example, patients with severe combined immunodeficiency (SCID) are particularly susceptible to opportunistic infections, whereas patients with APECED usually present with autoimmune manifestations in the endocrine glands.

INFECTIONS **AUTOIMMUNITY**

Figure 1. Spectrum of immune dysregulation

Patients with (mild) AD and other atopic manifestations are characterized by an infectious phenotype as the immune system in these diseases is directed against external factors. On the other hand, patients with increased severity of atopic manifestations may be characterized by autoimmunity as levels of IgE autoantibodies have been shown to be associated with disease severity.[84]

Overlap in immune dysregulation: a model for future studies

Taking the hypothesis of a spectrum one step further, different variants of immune dysregulation result in a diversity of clinical manifestations within the same patient. We found that 80% percent of the patients with a PID has a history of skin disorders and around

one third suffered from AD during life, which was higher as compared with controls. Furthermore, atopic manifestations were found significantly more prevalent in PID patients than in a matched control group. These results show a clinical overlap in presence of skin disorders, atopic manifestations within the atopic syndrome and PIDs. Expression profiles of immune cell lineages substantiated a genetic overlap between (monogenic) PIDs and endotypes within the atopic syndrome as well. Therefore, we propose a model that assumes overlap between immune dysregulation disorders (PID, skin disorders and atopy) (Figure 2). AD is a clinical example of the overlap between skin disorders and the atopic syndrome, in CGD both (non-atopy related) skin disorders and PID are involved and HIES is an example of disease in which all three components are affected.

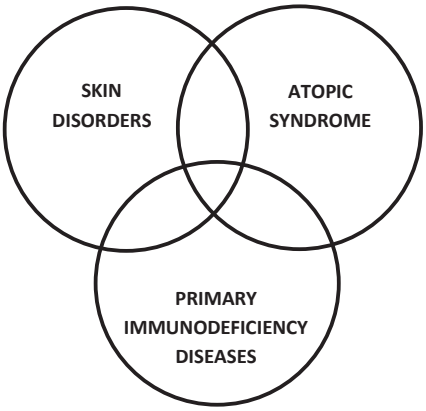

Figure 2. Overlap in immune dysregulation

Presence of manifestations of immune dysregulation could fluctuate throughout life, suggesting immune dysregulation has a dynamic spectrum of diseases involved. Our assumption emphasizes that more attention should be paid to simultaneous presence of multiple immune dysregulation disorders in clinical care. The model as shown in Figure 2 should be used both in clinical care and for research purposes. Caretakers should consider other immune dysregulation disorders in patients who present with one of the disorders. In this context, detailed registration of these clinical manifestations of each of the components within the model could give further insight in the clinical overlap of immune dysregulation manifestations within a patient (group). Additionally, the model can help design future studies getting more insight in the pathogenesis and role of immune dysregulation within the associated diseases.

CLINICAL IMPLICATIONS OF RESULTS OF THE THESIS

The diagnostic delay of primary immunodeficiency diseases might be reduced by using skin symptoms as warning signs

As mentioned in the introduction of the thesis, the delay between onset of the first symptoms and diagnosis of PIDs in the Netherlands is up to 14.5 years and might result in a reduced quality of life.[88-93] In our questionnaire-based studies (Chapters 3.1 and 3.2), patients reported a diagnostic delay up to 15.9 years from the first classical PID symptom,

such as respiratory tract infections. Skin disorders were shown to precede the diagnosis of PIDs for many years. Moreover, patients with a PID appear to develop skin disorders at a younger age compared with people without a PID.

Within the spectrum of PIDs, skin infections and nail disorders, were significantly more prevalent in adult patients compared with partner controls. In addition, a history of asthma and AR was reported significantly more often by adult patients than controls. Although (atopic) dermatitis was the most frequently noted skin disorder in patients, is was not found to be a specific skin condition related to PIDs.

Although current literature appointed eczematous dermatitis as common finding and presenting clinical manifestation among PIDs, we feel that reduction of the diagnostic delay of PIDs in general could be achieved by recognition of non-dermatitis-like skin disorders, like skin infections and/or nail disorders in addition to the presence of the currently used warning signs for PIDs. Moreover, data shown in this thesis indicate that atopic manifestations, of which at least asthma and AR, might serve as a potential warning sign for an underlying PID as well. To further discriminate between the different types of PIDs based on patients' clinical symptoms, we composed an overview of PIDs per skin disorders that could serve as a valuable support tool for PID awareness in clinical practice and for registries (Chapter 2). However, skin disorders as well as atopic manifestations are also frequently described in the general population.[6] Therefore, it is of importance to realize that presence of specific skin symptoms alone does not necessarily point towards a (specific) PID. Nonetheless, recognition of specific cutaneous manifestations in combination with other clinical features suggestive of an immunodeficiency, like the warning signs for PIDs, should raise awareness to an underlying PID and may facilitate earlier diagnosis of PIDs.[9] Moreover, identification of specific atopic characteristics of PIDs in future studies could further increase the suspicion of an underlying PID. In this context, the diagnosis of suspected PIDs or PID-classes based on clinical symptoms, for example multiple skin disorders and recurrent upper airway infections, could be confirmed with laboratory tests. Using the multi-stage diagnostic protocol of de Vries[3] or the phenotypic approach for PID classification and diagnosis by Bousfiha *et al.*[94], a first diagnostic step in case of a supposed antibody deficiency or neutropenia could be blood count and differentiation, IgG, IgA, IgM and IgE. In case of a possible combined immunodeficiency disease, these tests should be supplemented by lymphocyte subpopulations.

Extended registration of clinical features will improve the diagnostic and therapeutic processes in patients with primary immunodeficiency diseases

Data from our review and questionnaire-based studies (Chapters 2, 3.1 and 3.2) demonstrate a high prevalence of skin disorders and atopic manifestations in patients with

a PID. However, most PIDs are rare and a reliable prevalence of skin disorders and atopic manifestations in PIDs can only be obtained by reporting these clinical features in larger cohorts. The international PID database of The European Society for Immunodeficiencies (ESID) registers, among others, data on warning signs of PIDs. These currently applied warning signs focus on the presence of infectious skin disorders. Noninfectious cutaneous symptoms and atopic manifestations are not included. Based on data of studies included in this thesis, showing skin disorders and the atopic syndrome as highly prevalent and potentially distinctive symptoms of PIDs, we suggest to collect more detailed data on all skin disorders and atopic manifestations in the ESID registry in order to improve the diagnostic and therapeutic processes in patients with PIDs. In this context, higher number of data on these manifestations reported per PID might further improve our composed support tool for PID awareness in clinical practice, creating the possibility to better discriminate between PID phenotypes based on cutaneous and/or atopic symptoms. Furthermore, extensive registration of these symptoms in patients with a PID might provide reliable data on the prevalence of cutaneous and atopic manifestations of rare PID phenotypes. Second, collection of data on cutaneous *S. aureus* carriage on skin lesions in PIDs might identify patients that could have benefit from *S. aureus*-targeting treatment to reduce skin disease severity.[19] In this thesis, a discrepancy between the self-reported prevalence of atopic manifestations and the prevalence based on diagnostic criteria or tests was demonstrated indicating the need for registration of test results as well. For example, the standardized use of skin biopsies to confirm the diagnosis of cutaneous manifestations histopathologically should be considered, as clinically comparable skin lesions could demonstrate different histopathological images in patients with PIDs due to an altered immune system.[95] In addition, we recommend having skin conditions diagnosed by a dermatologist within the diagnostic process to increase its reliability.

RECOMMENDATIONS FOR FUTURE STUDIES

Genome sequencing should be further implemented in the diagnostics of primary immunodeficiency diseases

The last two decades there has been an exponential growth in genome sequencing with immense increase in speed and efficiency concomitantly with reduction in cost. Genome sequencing has proved its usefulness in diagnostics for PIDs and is the gold standard in most of the (monogenic) PID diagnoses, but is currently not used as first-line diagnostic. As genome sequencing becomes cheaper and more convenient, both supply and demand will rise. Subsequently, sequencing will be used as standard in care for patients with a PID, which will shorten the diagnostic delay and reduce chronic deterioration due to PID-associated symptoms. By identification of genetic variants in patients with PIDs, we would

be able to correlate clinical features, including cutaneous and atopic manifestations, to specific genetic variants. Moreover, sequencing could identify patients based on detected genetic variants, that require additional specialist care or screening because of an increased risk of associated comorbidities (e.g. dermatologist because of high probability of development of skin disorders, pulmonologist because of association with asthma or other airway complaints). Routine diagnostic genetic testing of patients with a CVID phenotype was recently suggested to improve diagnostics in these patients.[96]

Clustering of atopy-related genes might unravel the heterogeneous presentation of the atopic syndrome

Currently, atopic patients have already been stratified based on clinical and immunological characteristics, including the type of immune response involved.[97] In this thesis, subclasses within the atopic syndrome were for the first time defined via molecular clustering of atopy-related genes based on their expression profiles of immune cell lineages of healthy mice, as reported in Chapter 4. These gene expression profiles may not be identical to those in (atopic) humans and may explain why we could not cluster all human atopy-related genes including *FLG*, which is an important atopy gene based on the number of atopy-related variants (n=62). Furthermore, data from mice cannot directly be applied for subgrouping of the atopic syndrome in humans. Therefore, the genetic material of large cohorts of patients with the atopic phenotype should be sequenced to investigate whether atopy clusters could be generated based on the gene expression profiles of immune cell lineages of atopic human. If clusters could be identified, it would be of interest to correlate these genetic endotypes to atopic phenotypes and, secondly, to study the overlap with disease causing genes of monogenic PIDs. Subsequently, in atopic patients with an endotypic profile associated with PID (based on overlapping genes) referral to an immunologist may be considered in case of warnings signs of a PID.

Overlap in genes involved in atopy and primary immunodeficiency diseases identifies (new) therapeutic targets for the atopic syndrome

In PIDs and the atopic syndrome common pathogenic pathways seem to be involved. Therefore, several gene-targeted and/or pathway-targeted treatment strategies for PIDs could be of clinical benefit in the atopic syndrome as well. The clustering model as shown in Chapter 4 creates possibilities for identification of novel therapeutic targets for subgroups of the atopic syndrome, leading to more personalized and targeted treatment. For example, *STAT3* and *CTLA4* polymorphisms were found to be both involved in atopy and PID (HIES, STAT3 gain-of-function disease and large granular lymphocytosis, and CTLA4-deficiency, respectively). Jakinibs, such a tofacitinib and ruxolitinib, were described as safe and effective treatment modalities in patients with a *STAT3* gain-of-function variant to treat the autoimmune and autoinflammatory manifestations.[98] In patients with a CTLA4-

deficiency, treatment with a CTLA4 mimetic (e.g. abatacept) was shown to improve patient's autoimmune symptoms.[99] Furthermore, abatacept is registered and used as therapy for autoimmune diseases, like rheumatoid artritis.[100] Based on their mode of action, these therapies could also be effective in patients with (severe) atopic manifestations and an autoimmunity phenotype, based on genetic variants in these genes.

The Th lymphocyte mediated pathway was found to be most often involved in the atopic syndrome. Acute AD lesions and FA, asthma and AR are predominantly characterized by a Th2 response with production of, among others, interleukin (IL)-4 and IL-13.[37-43] Dupilumab is a human monoclonal antibody that blocks IL-4 and IL-13 signaling by binding to the IL-4 receptor alpha chain, modulating Th2-mediated inflammation.[101] Blockade by dupilumab of these key drivers of Th2-mediated inflammation has already proved effective in AD, but could potentially reduce symptoms of other atopic manifestations (i.e. FA, asthma and AR) as well.

Using the antibody response against *Staphylococcus aureus* to gain insight in the pathogenesis of atopic dermatitis

In Chapter 5 of the thesis we studied the humoral antibody response against *S. aureus* antigens and found an increased IgE response against immune modulating antigens suggesting *S. aureus* encourages epithelial damage via direct T lymphocyte stimulation.[55-57] To investigate further the role of *S. aureus* and the immune response against this bacterium in the AD pathogenesis, future studies should focus on other antibody subtypes than the IgE mediated response and other *S. aureus* antigens, like microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), membrane-damaging molecules, housekeeping antigens and other types of immune modulating proteins. Totté *et al.* showed that the IgG mediated immune response against immune modulating (non-superantigen) *S. aureus* antigens was associated with the disease severity in children with AD.[102] It can be argued that children with more severe AD have an altered immune response against *S. aureus* antigens or the association might be the result of a higher *S. aureus* load in children with more severe AD. However, in the latter hypothesis the IgG response against all *S. aureus* antigens would be increased instead of a subset. Following the inside-out hypothesis, in which immunologic dysregulation precedes barrier changes, alterations in the immunologic function of AD patients may allow or stimulate cutaneous colonization of *S. aureus*, which expresses several antigens resulting in persistence or exacerbation of AD symptoms.[77] On the other hand (outside-in hypothesis), *S. aureus* strains involved in AD may express more immune modulating antigens, which leads to a more severe AD phenotype and immunologic activation. In this context, it would be of interest to study whether AD patients are colonized by specific *S. aureus* strains. However, previous studies describe absence of a prevailed *S. aureus* genotype, which suggests that a primary immune

dysregulation precedes and stimulates microbial alterations, including abundance of *S. aureus*, within the pathogenesis of AD.[58-61]

Pitfalls in the design of a randomized controlled trial to study new antimicrobial treatment options in patients with atopic dermatitis

In Chapter 6.1 and 6.2 of the thesis we studied a targeted intervention directed against *S. aureus* using an endolysin. However, endolysin treatment as studied in this randomized controlled trial did not contribute to unraveling the contribution of the microbiome within the pathogenesis of AD due to absence of a clinical effect on AD and reduction of *S. aureus* load. According to the limitations in the design of our study, it might be relevant to investigate the effects of a targeted anti-*S. aureus* treatment using a wash-out period, in which topical corticosteroid and other (systemic) AD medications are not allowed, as their (long-term) effect on AD severity may mask an additional effect of the *S. aureus* targeting therapy. Furthermore, inclusion of patients based on two consecutive positive *S. aureus* cultures or clinically infected AD seems to be important to select a more appropriate population for *S. aureus* targeted therapy.

It can be doubted whether endolysins are the most convenient treatment strategy to target the microbiome based on results of our study. However, antibiotics are no suitable alternative as they induce bacterial resistance and have influence of the commensal flora. On the other hand, different promising (non-antibiotic) treatment strategies for modulation of the microbiome, including monoclonal antibodies and probiotics, are under development at the moment, but are not yet available for clinical use.

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Chapter 8

Summary / Samenvatting

Chapter 1 gives a general introduction and provides the aims of the thesis. Primary immunodeficiency diseases (PIDs) encompass a heterogeneous group of more than 430 inheritable defects of immunity. The prevalence of symptomatic PIDs is estimated at 1 in 10,000-12,000 in the general population. PIDs are clinically typically characterized by an increased risk of recurrent and/or severe infections due to a compromised or entirely absent function of a part of the immune system. In addition, patients may suffer from autoimmune and autoinflammatory complications and have an increased risk of development of (hematological) malignancies and allergic disorders. To raise suspicion of a PID, ten general warning signs of PID have been composed, mainly focusing on the presence of infectious complications. Despite the use of these warning signs to improve earlier recognition of an underlying PID, diagnosis of PIDs is still delayed.

It has been well recognized that a wide spectrum of both infectious and noninfectious skin manifestations are common in PIDs and may be among the presenting clinical symptoms. Overall, *Staphylococcus* (*S.*) *aureus*-induced skin infections are the most common infectious skin manifestations reported in PIDs. On the other hand, autoimmune, autoinflammatory, malignant and allergic manifestations are reported, that can be attributed to immune dysregulation. Dermatitis is considered as one of the most prominent noninfectious skin manifestations in PIDs. Although skin manifestations are frequently occurring in PIDs and may even precede the diagnosis of a PID, they are currently not considered as one of the warning signs for PIDs.

In **Chapter 2** of the thesis a systematically obtained overview of literature of the nature and prevalence of skin and atopic manifestations in PIDs is provided. The usefulness of these manifestations as (early) warning signs for suspicion of PIDs was evaluated in order to improve earlier diagnosis of PIDs. The relation between PIDs and *S. aureus*-related skin manifestations was reviewed in more detail. Based on the collected data, it was demonstrated that both infectious and noninfectious skin manifestations frequently occur in patients with PIDs. These included also PIDs in which an association with skin disorders was not generally recognized. The high prevalence of skin manifestations in PIDs suggests that these manifestations can be used as an additional warning sign to raise suspicion for an underlying PID. Through linking skin disorders to specific PIDs, a valuable tool was provided that could raise PID awareness in clinical practice. Data on culture proven *S. aureus*-associated skin disorders in PIDs was limited.

In **Chapter 3.1** the nature and prevalence of (presenting) skin disorders were evaluated in a Dutch population of patients with a PID. A history of skin disorders, in particular skin infections and nail disorders, was found to be more prevalent in patients with a PID compared with partner-controls. Data retrieved from this cohort of PID patients were mostly

consistent with the data summarized in our systematic review, which includes mainly data derived from Middle-Eastern countries (Chapter 2). However, in the Dutch patient cohort viral and bacterial skin infections, erythematous skin lesions and skin rashes were more frequently reported than in current literature. In addition, skin disorders preceded a diagnosis of PIDS for many years and developed earlier in life in patients compared with unaffected partner-controls. As skin disorders were frequently reported as presenting symptoms, we suggest to consider skin disorders as a potential warning sign for an underlying PID. In our cohort, a positive *S. aureus* culture at a single time point was found in 40% of the skin disorders with suspected *S. aureus*-related etiology, which could reflect intermittent carriage of *S. aureus*. We suppose that repetitive culturing during clinical examination to identify skin lesions colonized with *S. aureus* can contribute in selection of PID patients, that could benefit from *S. aureus*-targeting treatment to reduce skin disease severity.

In particular patients with severe (atopic) dermatitis (AD) have an atopic constitution and show tendency towards development of other atopic manifestations, including food allergy (FA), asthma and allergic rhinitis (AR). The atopic manifestations encompass allergic disorders, which are already known as prevalent comorbidities in various PIDs. However, original data on atopic manifestations in PIDs are limited, mainly based on small numbers of patients with PIDs and the diagnosis of atopic manifestations is generally not confirmed by diagnostic tests (Chapter 2). **Chapter 3.2** focused on the nature and prevalence of atopic manifestations in in a cohort of adult and pediatric patients with a PID to identify specific PIDs with a higher chance of developing the atopic syndrome. All atopic manifestations were found to be highly prevalent in patients with PIDs and more common when compared with partner-controls. Moreover, atopic manifestations were reported by patients in a large spectrum of PIDs across the various phenotypes, which is in contrast with previous literature (Chapter 2). Compared with our review (Chapter 2), we found a significantly higher prevalence of atopic manifestations in patients with a predominantly antibody deficiencies (PADs) and comparable numbers of patients with atopic manifestations in combined immunodeficiencies (CIDs). Based on our data, we propose early evaluation of atopic manifestations in patients with PADs and CIDs to prevent clinical deterioration. Future studies should focus on identification of specific atopic characteristics of PIDs to evaluate whether they could serve as a potential warning sign for an underlying PID.

Development of atopic manifestations not always follows the classic sequence and not all atopic patients will develop the complete spectrum of atopic manifestations. Subgroups of the atopic phenotype, termed endotypes, are possibly responsible for the heterogeneous presentation of the atopic syndrome. Atopic manifestations are prevalent comorbidities in various (monogenic) PIDs, which may be due to overlapping pathogenic pathways. Therefore, current insights in the pathways involved in PIDs could be used to define the

endotypic profile of atopic patients in more detail, contributing to determination of more homogeneous subclasses of these patients and additionally better stratification of patients for future pathway-targeted or gene-targeted treatment strategies. **Chapter 4** shows a new clustering algorithm to define endotypes within the atopic syndrome that were obtained via molecular clustering of atopy-related genes based on their expression profiles in immune cell lineages. Seven distinct clusters within the atopy-related genes were identified. Genes that are involved in the atopic syndrome as well as in PIDs were mainly located in two of the seven atopy-related gene clusters. This suggests that these subgroups of the atopic syndrome could be associated with the predisposition to develop PID. Furthermore, T helper lymphocytes were found to play a crucial role in the pathogenesis of the atopic syndrome based on the known atopy-related genes and corresponding expression profiles in immune cell lineages. These findings support the hypothesis that changes in the immune system are involved in the pathogenesis of the atopic syndrome next to genetic mechanisms.

AD is an important skin manifestation within the atopic syndrome and one of the most common chronic inflammatory skin disorders. AD has a multifactorial pathogenesis characterized by three major pathophysiological changes, including (i) abnormalities of the skin barrier; (ii) changes in the immune response; and (iii) alterations in the skin microbiome including abundance of *S. aureus*. However, studies on the interaction between the immune system and *S. aureus* are still scarce. Further evaluation of the antibody response against antimicrobial antigens could provide insights in the antigens that are expressed by the skin microbiome *in vivo* and will reveal how the immune system in AD patients counteracts these antigens. In **Chapter 5** the prevalence of human antibody responses against *S. aureus* antigens was evaluated in patients with AD and compared to healthy controls. Therefore, a systematic literature search and meta-analysis was performed. Patients with AD significantly more often showed an IgE antibody response directed against *S. aureus* immune modulating superantigens (SEA, SEB and TSST-1) when compared to healthy controls. Data on other antibodies as well as other *S. aureus* antigens were limited. This suggests that *S. aureus* encourages epithelial damage via direct T lymphocyte stimulation and subsequent T lymphocyte proliferation and cytokine release.

The specific contribution of each of the three factors to the AD phenotype is still unknown. However, it is suggested that *S. aureus* plays a pivotal role in AD. Therefore, it would be of interest to study a treatment targeting *S. aureus*. **Chapter 6.1** describes the protocol of a randomized vehicle controlled trial that studied the effect of topical treatment with an endolysin that targets *S. aureus* in patients with AD. Subsequently, the results of this trial are reported in **Chapter 6.2** of the thesis. In this study, long-term targeted endolysin treatment against *S. aureus* was found to be well tolerated. However, there was no statistically

significant difference in the probability of topical corticosteroid (TCS) use per day between the groups nor a difference in reduction of clinical disease severity scores, which might be the result of a decrease in AD severity in the run-in phase due to use of triamcinolone cream combined with emolliens. Moreover, there was no difference observed in the reduction of *S. aureus* load between the endolysin and vehicle treated groups determined by semi-quantitative culture and qPCR, corresponding with the results of the clinical outcome. Therefore, endolysin treatment as studied in this randomized controlled trial, in patients with relatively mild and noninfected AD at start of the treatment, did not contribute to unravel the role of *S. aureus* in the pathogenesis of AD. Nonetheless, endolysin treatment was well tolerated and this study provides estimates of AD symptoms, use of TCSs and the percentage of persistent *S. aureus* carriers that can be used for future clinical studies.

Finally, a general overview of the main findings and suggestions for clinical implication and future research is provided in **Chapter 7**. Recent identification of many novel causative genes has broadened the spectrum of clinical features associated with PIDs. Patients with a PID and noninfectious complications are increasingly recognized with features of immune dysregulation, including autoimmunity, autoinflammation, lymphoproliferation and malignancy. Therefore, we suggest to consider PIDs as immune dysregulation syndromes rather than immune deficiency syndromes. Depending on the pathways, genetic variants and number and functionality of immune cells involved, the clinical picture could be dominated by a higher susceptibility to infections or an increased risk of autoimmune or autoinflammatory manifestations. In this thesis immune dysregulation is therefore visualized as a spectrum with infections on one end and autoimmunity on the other end. Patients with PIDs typically show various symptoms within this spectrum, but predominance of specific manifestations can be PID specific. Moreover, results of our studies support the hypothesis that immune dysregulation also plays a role in the multifactorial pathogenesis of skin disorders and atopy. In both AD and other atopic diseases, patients can be characterized by having either a predominant infectious phenotype or an autoimmune phenotype. As immune dysregulation may result in a diversity of clinical manifestations within the same patient, a model that assumes overlap between immune dysregulation disorders (PID, skin disorders and atopy) is proposed in this thesis. Our assumption emphasizes that more attention should be paid to the concurrent presence of various symptoms related to immune dysregulation in clinical care. For example, in order to further consider (specific) skin disorders as a potential warning sign for an underlying PID. In addition, gene-targeted and/or pathway-targeted treatment strategies registered for specific immune dysregulation disorders could be effective in other diseases with clinically overlapping symptoms in which immune dysregulation is involved.

Hoofdstuk 1 geeft een algemene inleiding en tevens de doelstellingen van het proefschrift weer. Primaire immuundeficiënties (PID's) omvatten een heterogene groep van meer dan 430 erfelijke aandoeningen van het immuunsysteem. De prevalentie van symptomatische PID's kan worden geschat op 1 op 10.000-12.000 in de algemene bevolking. PID's worden klinisch typisch gekenmerkt door een verhoogd risico op recidiverende en/of ernstige infecties als gevolg van een verminderde of volledig afwezige functie van een deel van het immuunsysteem. Daarnaast kunnen patiënten klachten hebben van auto-immuun- en auto-inflammatoire complicaties en hebben zij daarnaast een verhoogd risico op het ontwikkelen van (hematologische) maligniteiten en allergische aandoeningen. Om het vermoeden op een PID te wekken, zijn er tien alarmsignalen van PID's beschreven, welke voornamelijk zijn gericht op de aanwezigheid van infectieuze complicaties. Ondanks het gebruik van deze alarmsignalen ter bespoediging van het bemerken van een onderliggende PID, is de diagnose van PID's nog steeds vertraagd.

Het is algemeen erkend dat een breed spectrum van zowel infectieuze als niet-infectieuze huidmanifestaties frequent voorkomt bij patiënten met een PID en mogelijk zelfs tot de presenterende klinische symptomen behoort. Over het algemeen zijn door *Staphylococcus* (*S.*) *aureus* geïnduceerde huidinfecties de meest voorkomende infectieuze huidmanifestaties van PID's. Anderzijds zijn auto-immuun, auto-inflammatoire, maligne en allergische manifestaties beschreven, welke kunnen worden toegeschreven aan immuundysregulatie. Dermatitis wordt beschouwd als een van de meest prominente niet-infectieuze huidmanifestaties van PID's. Hoewel huidmanifestaties frequent voorkomen bij PID's en zelfs vooraf kunnen gaan aan de diagnose van een PID, worden ze momenteel niet beschouwd als een van de alarmsignalen voor PID's.

In **Hoofdstuk 2** van het proefschrift wordt een systematisch verkregen literatuuroverzicht met betrekking tot de aard en prevalentie van huid- en atopische manifestaties van PID's gegeven. Het gebruik van deze manifestaties als (vroege) alarmsignalen voor de verdenking op een PID werd geevalueerd ter bevordering van vroegdiagnostiek van PID's. De relatie tussen PID's en *S. aureus*-gerelateerde huidmanifestaties werd hierbij nader bekeken. Op basis van de verzamelde gegevens werd aangetoond dat zowel infectieuze als niet-infectieuze huidmanifestaties freuent voorkomen bij patiënten met PID's. Deze omvatten ook PID's waarvan een verband met huidaandoeningen nog niet algemeen bekend is. De hoge prevalentie van huidmanifestaties van PID's suggereert dat deze manifestaties kunnen worden gebruikt als additioneel alarmsignaal om de verdenking op een onderliggende PID te wekken. Door huidaandoeningen aan specifieke PIDS te koppelen, werd een waardevol hulpmiddel verschaft dat het bewustzijn van een PID in de klinische praktijk zou kunnen vergroten. Gegevens van door middel van kweek bewezen *S. aureus*-geassocieerde huidaandoeningen in patiënten met PID's bleken beperkt gepubliceerd.

In **Hoofdstuk 3.1** zijn de aard en prevalentie van (presenterende) huidaandoeningen geëvalueerd in een Nederlandse populatie van patiënten met een PID. Een belaste voorgeschiedenis met huidaandoeningen, met name huidinfecties en nagelaandoeningen, werd vaker gerapporteerd bij patiënten met een PID in vergelijking met partner controles. Gegevens uit het cohort van PID-patiënten kwamen grotendeels overeen met de resultaten van ons systematische literatuuronderzoek, wat voornamelijk gegevens bevat afkomstig uit landen in het Midden-Oosten (Hoofdstuk 2). In het Nederlandse patiënten cohort werden echter virale en bacteriële huidinfecties, erythemateuze huidlaesies en huiduitslag vaker gemeld dan in de reeds gepubliceerde literatuur. Daarnaast gingen huidaandoeningen jaren vooraf aan de diagnose van PID's en ontwikkelden ze zich eerder in het leven van patiënten met PID's in vergelijking met onaangedane partner controles. Aangezien huidaandoeningen frequent worden gerapporteerd als presenterende symptomen, suggereren wij om huidaandoeningen te overwegen als potentiaal allarmsignaal voor een onderliggende PID. In ons cohort werd een positieve *S. aureus*-kweek op één tijdstip gevonden bij enkel 40% van de huidaandoeningen met vermoedelijke *S. aureus*-gerelateerde etiologie. Dit zou het intermitterende dragerschap van *S. aureus* kunnen weerspiegelen. We veronderstellen dat herhaaldelijk kweken tijdens klinisch onderzoek noodzakelijk is om huidlaesies te identificeren die zijn gekoloniseerd met *S. aureus*. Dit kan bijdragen aan het selecteren van PID-patiënten, die baat zouden kunnen hebben bij een op *S. aureus* gerichte behandeling om de ernst van huidziekten te verminderen.

Voornamelijk patiënten met ernstig (constitutioneel) eczeem (CE) hebben een atopische constitutie en tonen de neiging om andere atopische manifestaties, bestaande uit voedselallergie (VA), astma en allergische rhinitis (AR), te ontwikkelen. De atopische manifestaties omvatten allergische aandoeningen, die reeds bekend zijn als veelvoorkomende comorbiditeit van verschillende PID's. Originele gegevens over atopische manifestaties van PID's zijn echter beperkt, voornamelijk gebaseerd op kleine aantallen patiënten met een PID en de diagnose van atopische manifestaties is over het algemeen niet bevestigd door middel van diagnostische tests (Hoofdstuk 2). **Hoofdstuk 3.2** richtte zich op de aard en prevalentie van atopische manifestaties in een cohort van volwassen en pediatrische patiënten met een PID, zodat specifieke PID's kunnen worden geïdentificeerd die een grotere kans hebben op het ontwikkelen van het atopisch syndroom. Alle atopische manifestaties bleken zeer vaak voor te komen bij patiënten met PID's en waren prevalenter in vergelijking met partner controles. Bovendien werden atopische manifestaties door patiënten gerapporteerd binnen een groot spectrum van PID's verspreid over de verschillende fenotypen. Dit is in tegenstelling met eerdere literatuur (Hoofdstuk 2). Vergeleken met ons literatuuronderzoek (Hoofdstuk 2), vonden wij een significant hogere prevalentie van atopische manifestaties bij patiënten met overwegend antilichaamdeficiënties (PAD's) en vergelijkbare aantallen patiënten met atopische manifestaties bij gecombineerde immunodeficiënties (CID's). Op

basis van deze gegevens stellen wij voor om bij patiënten met PAD's en CID's vroegtijdige evaluatie van atopische manifestaties uit te voeren om klinische achteruitgang te voorkomen. Toekomstige studies zouden zich moeten concentreren op de identificatie van specifieke atopische kenmerken van PID's met als doel te evalueren of deze manifestaties zouden kunnen dienen als mogelijk waarschuwingssignaal van een onderliggende PID.

Atopische manifestaties presenteren zich niet altijd volgens de klassieke volgorde en niet alle atopische patiënten zullen het volledige spectrum van atopische manifestaties ontwikkelen. Subgroepen van het atopische fenotype, endotypes genoemd, zijn mogelijk verantwoordelijk voor de heterogene presentatie van het atopische syndroom. Atopische manifestaties zijn veel voorkomende comorbiditeiten van verschillende (monogene) PID's, wat mogelijk te wijten is aan overlappende processen. Daarom zouden huidige inzichten in de processen die betrokken zijn bij PID's kunnen worden gebruikt om het endotypische profiel van atopische patiënten in meer detail te definiëren. Dit zou vervolgens kunnen bijdragen aan de identificatie van meer homogene subgroepen van deze patiënten en bovendien tot een betere stratificatie van patiënten voor toekomstige procesgerichte of gen-gerichte behandelstrategieën. **Hoofdstuk 4** toont een nieuw clusteringalgoritme om endotypen binnen het atopische syndroom te definiëren, welke werden verkregen via moleculaire clustering van atopie-gerelateerde genen op basis van hun expressieprofielen in immuun cellijnen. Zeven onderscheidende clusters binnen de atopie-gerelateerde genen werden geïdentificeerd. Genen die betrokken zijn bij zowel het atopische syndroom als PID's waren met name gelokaliseerd in twee van de zeven atopie-gerelateerde gen clusters. Dit suggereert dat deze subgroepen van het atopische syndroom geassocieerd zouden kunnen zijn met de aanleg om een PID te ontwikkelen. Bovendien bleken T-helper lymfocyten een cruciale rol te spelen in de pathogenese van het atopische syndroom op basis van de bekende atopie-gerelateerde genen en bijbehorende expressieprofielen in immuun cellijnen. Deze bevindingen ondersteunen de hypothese dat veranderingen in het immuunsysteem betrokken zijn bij de pathogenese van het atopische syndroom additioneel aan genetische mechanismen.

CE is een belangrijke cutane manifestatie binnen het atopisch syndroom en een van de meest voorkomende chronische inflammatoire huidaandoeningen. CE heeft een multifactoriële pathogenese die wordt gekenmerkt door drie belangrijke pathofysiologische veranderingen, bestaande uit (i) afwijkingen van de huidbarrière; (ii) veranderingen in de immuunrespons; en (iii) veranderingen in het huidmicrobioom, waaronder een overvloed aan *S. aureus*. Studies naar de interactie tussen het immuunsysteem en *S. aureus* zijn echter schaars. Evaluatie van de antilichaamrespons tegen antimicrobiële antigenen zou inzicht kunnen geven in de antigenen die *in vivo* door het huidmicrobioom tot expressie worden gebracht en zal tevens openbaren hoe het immuunsysteem in patiënten met CE tegen

deze antigenen reageert. In **Hoofdstuk 5** werd de prevalentie van menselijke antilichaamreacties tegen *S. aureus* antigenen bij patiënten met CE geëvalueerd en vergeleken met gezonde controles. Hiervoor werd een systematisch literatuuronderzoek en meta-analyse uitgevoerd. Patiënten met CE vertoonden significant vaker een IgE-antilichaamrespons gericht tegen immuunmodulerende superantigenen van *S. aureus* (SEA, SEB en TSST-1) in vergelijking met gezonde controles. Gegevens van andere antistoffen evenals andere *S. aureus* antigenen waren beperkt. Dit suggereert dat *S. aureus* epitheliale schade stimuleert via directe T-lymfocyten stimulatie en daaropvolgende T-lymfocyten proliferatie en cytokine-afgifte.

De specifieke bijdrage van elk van de drie factoren aan het CE-fenotype is nog onbekend. Echter, er wordt gesuggereerd dat *S. aureus* een cruciale rol speelt in CE. Een behandeling gericht tegen *S. aureus* zou daarom interessant kunnen zijn om te bestuderen. **Hoofdstuk 6.1** beschrijft het protocol van een gerandomiseerde vehikel gecontroleerde studie, die het effect van een topicale behandeling middels een endolysine gericht tegen *S. aureus* in patiënten met CE onderzoekt. Vervolgens worden de resultaten van dit onderzoek gerapporteerd in **Hoofdstuk 6.2** van het proefschrift. In deze studie bleek een langdurige endolysine behandeling gericht tegen *S. aureus* goed te worden verdragen. Er was echter geen statistisch significant verschil in de waarschijnlijkheid van topicaal gebruik van corticosteroiden (TCS) per dag tussen de behandelgroepen, noch een verschil in vermindering van de klinische ernstscores, wat het gevolg zou kunnen zijn van een afname van de ernstscores van CE tijdens de inloop periode door het gebruik van triamcinoloncrème gecombineerd met een emollient. Bovendien was er geen verschil in de geobserveerde vermindering van de *S. aureus*-belasting tussen de met endolysine en met vehikel behandelde groepen, wat werd vastgesteld middels een semi-kwantitatieve kweek en qPCR, wat overeenkomst met de resultaten van de klinische uitkomsten. Daarom heeft de behandeling met endolysine zoals onderzocht in dit gerandomiseerde gecontroleerde onderzoek, in patiënten met realtief mild en niet-geïnfecteerd CE aan het begin van de behandeling, geen bijdrage gehad aan de ontrafeling van de rol van *S. aureus* binnen de pathogenese van CE. Desalniettemin werd de behandeling met endolysine goed verdragen en geeft deze studie voorspellingen van CE-symptomen, gebruik van TCS's en het percentage persisterende *S. aureus*-dragers, welke kunnen worden gebruikt voor toekomstige klinische studies.

Ten slotte wordt in **Hoofdstuk 7** een algemeen overzicht gegeven van de belangrijkste bevindingen en suggesties voor klinische implicaties en toekomstig onderzoek. Recente identificatie van nieuwe causatieve genen heeft het spectrum van klinische kenmerken die geassocieerd zijn met PID's verbreed. Patiënten met een PID en niet-infectieuze complicaties worden steeds vaker gediagnosticeerd met kenmerken van immuundysregulatie, waaronder auto-immuniteit, auto-inflammatie, lymfoproliferatie en maligniteiten. Daarom

stellen we voor om PID's te beschouwen als immuundysregulatie syndromen in plaats van immuundeficiëntie syndromen. Afhankelijk van de processen, genetische varianten en het aantal en functionaliteit van de betrokken immuuncellen, worden het klinisch beeld gedomineerd door een hogere gevoeligheid voor infecties of een verhoogd risico op auto-immuun of autoinflammatoire manifestaties. In dit proefschrift wordt daarom immuundysregulatie gevisualiseerd als een spectrum met infecties aan de ene kant en auto-immuniteit aan de andere kant van het spectrum. Patiënten met een PID vertonen doorgaans verschillende symptomen binnen dit spectrum, echter dominantie van specifieke manifestaties kan PID-specifiek zijn. Bovendien ondersteunen de resultaten van onze studies de hypothese dat immuundysregulatie ook een rol speelt in de multifactoriële pathogenese van huidaandoeningen en atopie. In zowel CE als andere atopische ziekten kunnen patiënten worden gekenmerkt door een overwegend infectieus fenotype of door een auto-immuun fenotype. Aangezien immuundysregulatie kan resulteren in een diversiteit aan klinische manifestaties binnen dezelfde patiënt, wordt in dit proefschrift een model voorgesteld dat veronderstelt dat er overlap is tussen immuundysregulatie aandoeningen (PID's, huidaandoeningen en atopie). Onze aanname benadrukt dat meer aandacht zou moeten worden besteed aan de gelijktijdige aanwezigheid van verschillende symptomen gerelateerd aan immuundysregulatie in de klinische zorg. Bijvoorbeeld om (specifieke) huidaandoeningen verder te overwegen als potentieel alarmsignaal voor een onderliggende PID. Daarnaast zouden gen-gerichte en/of proces-gerichte behandelstrategieën, die zijn geregistreerd voor specifieke immuundysregulatie aandoeningen, effectief kunnen zijn bij andere ziekten met klinisch overlappende symptomen waarbij immuundysregulatie een rol speelt.

Appendices

Abbreviations
Publications
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Dankwoord

ABBREVIATIONS

AD	atopic dermatitis
AD-HIES	autosomal dominant hyper IgE syndrome
AE	adverse event
APECED	autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
AR	allergic rhinitis
AR-HIES	autosomal recessive hyper IgE syndrome
AT	ataxia-telangiectasia
CDLQI	Children's Dermatology Life Quality Index
CGD	chronic granulomatous disease
CI	confidence interval
CID	combined immunodeficiency
ClfA	clumping factor A
CVID	common variable immunodeficiency disorder
DLQI	Dermatology Life Quality Index
EASI	Eczema Area and Severity Index
ELISA	enzyme-linked immunosorbent assay
ESID	European Society for Immunodeficiencies
ET	exfoliative toxin
FA	food allergy
FBP	fibronectin-binding protein
GLME	generalized linear mixed-effect
HIES	hyper IgE syndrome
HOME	Harmonising Outcome Measures for Eczema
HR-QoL	health-related quality of life
IDQOL	Infant's Dermatitis Quality of Life Index
Ig	immunoglobulin
IGA	Investigators Global Assessment
IHE	Institute of Health Economics
IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked
IQR	interquartile range
ISAAC	International Study of Asthma and Allergies in Childhood
IUIS	International Union of Immunological Societies
LAD	leukocyte adhesion defect
LME	linear mixed-effect
LTA	lipoteichoic acid
MOOSE	Meta-analysis Of Observational Studies in Epidemiology
MSCRAMM	Microbial Surface Components Recognizing Adhesive Matrix Molecule

n	number
NGS	next-generation sequencing
NOS	Newcastle Ottawa Scale
NRS	Numeric Rating Scale
OR	odds ratio
PAD	predominant antibody deficiency
PID	primary immunodeficiency disease
PLAID	PLCG2 associated antibody deficiency and immune dysregulation
PLCG2	Phospholipase C Gamma 2
POEM	Patient Oriented Eczema Measure
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
QoL	quality of life
qPCR	quantitative polymerase chain reaction
RIA	radioimmunoassay
S. aureus	*Staphylococcus aureus*
SCC	squamous cell carcinoma
SCID	severe combined immunodeficiency
SCORAD	SCORing Atopic Dermatitis
SD	standard deviation
SE	staphylococcal enterotoxin
SF36	Short Form 36
SIgAD	selective IgA deficiency
sIgE	specific IgE
SR-QoL	skin-related quality of life
Staphefekt	Staphefekt SA.100
TAPQOL	TNO-AZL Preschool Children's Quality of Life questionnaire
TCS	topical corticosteroid
TSST	toxic shock syndrome toxin
WAS	Wiskott-Aldrich syndrome

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L. Liu, R. van Wijck, Y. Li, S.M.A. Swagemakers, **J. de Wit**, H.R. Langeveld-Benders, P.C.J. de Laat, P.M. van Hagen, S.G.M.A. Pasmans, P.J. van der Spek
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Basiscursis Regelgeving en Organisatie voor Klinisch Onderzoek (BROK)	2016	1.0 ECTS
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Specific courses / workshops		
Systematisch Literatuuronderzoek in PubMed	2016	0.1 ECTS
Werken met Endnote	2016	0.1 ECTS
OpenClinica	2016	0.3 ECTS
Biostatistical Methods I: Basic Principles (NIHES)	2016	5.7 ECTS
Biomedical English Writing Course for MSc and PhD (MolMed)	2017	2.0 ECTS
Basic Course on "R" (MolMed)	2017	1.8 ECTS
Diagnostic Data for Dummies: The Untapped Potential of Data Re-use (Rotterdam Summer School)	2017	0.7 ECTS
Cursus Medische Immunologie	2018	1.4 ECTS
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Symposium Maintaining Oral Health, ACTA, Amsterdam, the Netherlands	2017	1.0 ECTS
5th PhD weekend Dermatology Erasmus MC, Den Bosch, the Netherlands	2017	1.0 ECTS
19th Annual Scientific Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands	2018	1.0 ECTS
6th PhD weekend Dermatology Erasmus MC, Den Bosch, the Netherlands	2018	1.0 ECTS
18th Biennial Meeting of European Society for Immunodeficiencies (ESID), Lisbon, Portugal	2018	1.0 ECTS
Scientific Meeting of the Nederlandse Vereniging voor Dermatologie en Venereologie (NVDV), Rotterdam, the Netherlands	2018	0.3 ECTS
20th Annual Scientific Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands	2019	1.0 ECTS
7th PhD weekend Dermatology Erasmus MC, Scheveningen, the Netherlands	2019	1.0 ECTS
28th Congress of the European Acady for Dermatology and Venereology (EADV), Madrid, Spain	2019	1.0 ECTS
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Skin disorders in primary immunodeficiency diseases; Werkgroep Genodermatosen, UMC Utrecht, Utrecht, the Netherlands	2019	1.0 ECTS
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The prevalence of antibody responses against Staphylococcus aureus antigens in patients with atopic dermatitis: a systematic review and meta-analysis; 19th Annual Scientific Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands	2018	1.0 ECTS
Skin disorders are prominent features in primary immunodeficiency diseases: a systematic overview of current data; 18th Biennial Meeting of European Society for Immunodeficiencies (ESID), Lisbon, Portugal	2018	1.0 ECTS
Skin disorders are prominent features in primary immunodeficiency diseases: a systematic overview of current data; 20th Annual Scientific Meeting of the Dutch Society for Experimental Dermatology (NVED), Lunteren, the Netherlands	2019	1.0 ECTS
Skin disorders are prominent features in primary immunodeficiency diseases: a questionnaire-based study in pediatric and adult patients; 28th Congress of the European Acady for Dermatology and Venereology (EADV), Madrid, Spain	2019	1.0 ECTS
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Research project Joyce van Velhuizen	2016-2017	1.0 ECTS
Research project Fleur van Osnabrugge	2017-2018	1.0 ECTS
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Research meeting Pediatric Dermatology Erasmus MC	2016-2019	2.5 ECTS
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CURRICULUM VITAE

Jill de Wit is geboren op 9 janurari 1991 te Nijmegen. Zij groeide op in Beuningen, een dorp in de buurt van Nijmegen. In 2009 behaalde zij haar Gymniasium diploma aan het Dominicus College te Nijmegen. In hetzelfde jaar werd zij via decentrale selectie aangenomen voor de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Tijdens wetenschapsstages als Bachelor en Master studente werd haar interesse in (klinisch) onderzoek gewekt. In het jaar voorafgaand aan haar afstuderen in 2016 nam zij deel aan diverse onderzoeksprojecten betreffende de rol van het microbioom binnen de pathognenese van constitutioneel eczeem. Deze projecten vormden de basis van haar huidige promotieonderzoek op de afdeling Dermatologie van het Erasmus MC Rotterdam, welke zij per mei 2016 startte onder begeleiding van promotor prof. dr. S.G.M.A. Pasmans en copromotoren dr. V.A.S.H. Dalm en dr. J.E.E. Totté. Sinds januari 2020 is zij in opleiding tot dermatoloog in het Erasmus MC Rotterdam.

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