Hidradenitis Suppurativa

Clinical and Translational Studies

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CONTENTS

Chapter 1

General introduction and aims of this thesis

Parts of the introduction are based on:

Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model
Frontiers in Immunology – accepted for publication

Hidradenitis suppurativa/acne inversa: a practical framework for treatment optimisation – systematic review and recommendations from the HS ALLIANCE working group
J Eur Acad Dermatol Venereol – in press

Surgical approaches to hidradenitis suppurativa management
Dermatologic Surgery, New York: McGraw Hill, 2018

BACKGROUND

Hidradenitis suppurativa (HS), also known as acne in versa, is a common chronic, recurrent, inflammatory follicular occlusive disease. Estimated prevalence of HS in Europe and North America range from <1% to 4%.[1,2] The disease usually presents after puberty with painful, deep-seated inflamed lesions, predominantly at inverse body sites carrying terminal hairs such as the axillae, inguinal and anogenital regions.[3] Atypical areas including the nape, retro-auricular areas and the back can also be affected.[4]

Key symptoms of HS include chronic pain, discomfort, and a purulent, malodorous discharge.[5] The major factors influencing the patients well-being are disease severity, the number of flares or affected skin areas, and the lesion location.[6] The physical and psychological consequences of HS can profoundly reduce several aspects of patient's quality of life. This is demonstrated by the affected scores of questionnaires for general health (EQ-5D and SF-36), dermatologic-specific quality of life (DLQI and Skindex), and sexual health.[6-8] The impact of HS on general health (EQ-5D) can be compared with cerebrovascular stroke, diabetes mellitus or severe chronic obstructive pulmonary disease.[6] In addition, rates of depression and anxiety among HS patients are significantly higher than in healthy controls.[9] Collectively, this might explain the significantly increased completed suicide risk is in patients with HS.[10]

The pathogenesis of HS is not fully understood. Several factors contribute to the onset and maintenance of the disease. Genetic predisposition is a well-known endogenous factor, as demonstrated by a positive family history being reported by 30-40% of the patients.[3] Exogenous risk factors include a positive smoking status and obesity.[11] In addition, HS is linked to a number of comorbid diseases. Data suggest that HS is most convincingly associated with the metabolic syndrome including the report of higher rates of diabetes mellitus, which may explain the significantly increased risk of adverse cardiovascular events.[12-14] More recently, several immune mediated inflammatory diseases have been linked to HS, notably inflammatory bowel disease and spondyloarthropathy.[12,15] HS can also occur in the context of auto-inflammatory syndromes such as pyoderma gangrenosum, acne and suppurative hidradenitis (PASH).[16]

PATHOPHYSIOLOGY

Current evidence highlights a complex multifactorial pathogenesis.[17] A key triggering factor is the occlusion of the hair follicle, caused by keratosis and hyperplasia of the follicular epithelium leading to cyst development.[18,19] Subsequently, the cyst will rupture, causing a fierce immune response and inflammation that, depending on the

severity, may progress to abscess and sinus tract development and scarring.[18,19] The name of the disease implies that sweating and bacterial infection are a fundamental part of the disease process. This is misleading and now considered a misnomer as no evidence has been found that HS is triggered by events in the apocrine or eccrine glands. Recent findings on the pathogenesis of HS and its syndromic forms are largely derived from four main lines of investigation: genetics, inflammatory markers, bacteriology including the microbiome, and physiological and environmental factors.

Genetics

Mutations in γ-secretase genes whose gene products act on many substrates including Notch,[20] suggest that Notch or other substrates of γ-secretase and/or phosphoinositide 3-kinase (PI3K) may play a role in the pathogenesis of HS.[21] However, the functional significance of γ-secretase remains elusive. Interestingly, γ-secretase knock-out mice are characterised by a phenotype of multiple cutaneous cysts, a key feature of HS.[22] However, these mice did not exhibit skin inflammation,[22] and nicastrin (*NCSTN)* mutations in HS did not enhance cytokine production in LPS-stimulated peripheral blood mononuclear cells.[23]

There is also evidence of mutations to the proline-serine-threonine phosphatase interacting protein 1 *(PSTPIP1)* gene in cases of PASH and pyogenic arthritis, pyoderma gangrenosum, acne and suppurative hidradenitis (PAPASH) syndromes.[24,25] A mutation in the *PSTPIP1* gene resulted in a case of pyoderma gangrenosum (PG), acne and ulcerative colitis (PAC), in which the associated elevated interleukin (IL)-1β levels were responsive to the IL-1 receptor antagonist anakinra.[26] PSTPIP1 is a cytoskeleton-associated adaptor protein, highly expressed in hemopoietic cells.[26] The PSTPIP1 protein manifests its immunomodulatory effects through downregulation of CD2 adhesion, regulation of c-Abl tyrosine kinase activity, and interaction with other immunity-related proteins including the Wiskott–Aldrich syndrome protein (WASp)[27], and pyrin and the familial Mediterranean fever (FMF) protein.[26] Furthermore, a genetic analysis of auto-inflammation in PG and the syndromic form PASH identified mutations in a range of auto-inflammatory genes (*MEFV*, *NLRP3*, *NLRP12*, *NOD2*, *LPIN2* and *PSTPIP1*), suggesting the involvement of inflammatory pathways such as NLRP inflammasomes, cystolic pattern recognition sensors, the innate immune system, and IL-1β signalling (*PSTPIP1*).[16]

The majority of HS cases appear to be non-familial, suggesting the existence of separate subsets and the need for stratification of patients diagnosed with HS.[28] In a study of 139 unrelated patients with non-familial HS, single nucleotide polymorphisms of the *IL-12Rb1* gene coding for the IL-12Rb1 receptor subunit did not genetically predispose to HS.[29] However, their carriage was directly associated with the phenotype of HS, indicating the importance of the IL-12/23 pathway for the pathogenesis of

HS. Findings from a case-control study of two independent and genetically diverse cohorts of patients with non-familial HS from Greece (n = 163) and Germany (n = 98) suggested that the copy number of the β-defensin gene cluster (DEFB) confers susceptibility for HS and modulates the disease phenotype.[30]

Inflammatory markers

Clear evidence suggests the involvement of pro-inflammatory cytokines in the immune dysregulation of HS, with elevated levels of tumour necrosis factor (TNF)-α, IL-1ß, IL-6, IL-17 and interferon (IFN)-γ observed in HS lesions.[17,31,32] The immune dysregulation is initiated by caspase-1 activity in the inflammasome which lead to the secretion of the pleiotropic cytokine IL-1β, thereby stimulating the infiltration of inflammatory cells and the induction of chemokines.[33] As a result, a number of inflammatory markers, most of them related to the IL-17 pathway, have been found elevated in HS skin and serum on the mRNA and/or protein level.

Alterations in the skin have recently been reported for IL-1β[16], CXCL-8/IL-8[16,34], IL-17/IL-17A[16], IL-23p40[35], IL-32[36], and IL-36/IL-36α/IL-36β/IL-36γ[34,37]. Data also indicate the involvement of T helper (Th) cells, which accumulate in HS lesions, in the pathogenesis of HS.[32,38] In addition, studies have shown that antimicrobial peptides (AMPs) are increased in HS lesions compared with the normal skin of HS patients.[39] Keratinocytes isolated from HS patients exhibited a pro-inflammatory profile and a dysregulated production of AMPs such as HBD-2, psoriasin (S100A7) and calgranulin B (S100A8), indicating that the skin immune system is already activated in the steady state.[40]

Alterations in the serum have recently been reported for IL-1β[41], IL-6[41], CXCL-8/IL-8[41], IL-10[41], IL-12p70[41] and IL-17/IL-17A[41,42]. In addition, TNF-α, S100A8, and S100A9 have been found to be upregulated in the circulation of HS patients.[43,44] Systemic inflammation is also demonstrated by elevated levels of c-reactive protein (CRP), erythrocyte sedimentation rate, neutrophils, and monocytes.[41,45] A significant association between CRP levels and neutrophil count with HS disease severity has been reported.[46] Lastly, the use of TNF-α inhibitors such as adalimumab and infliximab have been associated with improvements in immune dysregulation in HS, which supports the importance of (local) molecular drivers in the pathogenesis of HS.[3,47,48]

Bacteriology and the microbiome

A number of studies investigated bacterial cultures from HS lesions and generated evidence for the involvement of microbes in the disease pathogenesis. A histological study of 42 patients with chronic HS identified bacterial aggregates (biofilms) in 67% of chronic lesional samples and in 75% of perilesional samples.[49] The same author group conducted a case-control study of punch biopsy specimens and demonstrated

that the microbiome in patients with HS differs significantly from that in healthy controls in both lesional and non-lesional skin.[50] A microbial analysis of lesional versus unaffected skin from 65 patients with HS identified anaerobic microbes in 83% lesions versus 53% control samples, and the microbiome varied with disease severity.[51] These bacteria were associated with low pathogenicity. An extensive prospective microbiological study identified two opportunistic bacterial pathogens associated with HS lesions (*S.lugdunensis* and anaerobic actinomycetes).[52] These pathogens can cause abscesses and severe infections. A cross-sectional study of 50 patients reported that bacterial colonisation was correlated with severity and localisation of HS lesions.[53] Over two thirds (68.8%) of patients with both aerobic and anaerobic bacteria had the most severe grade of HS (Hurley stage III).

Physiological and environmental factors

Recent literature supports the involvement of previously suggested physiological and environmental risk factors, such as smoking and obesity, in HS.[46,54,55] A postal follow-up survey study (N = 212) found the chance of remission from HS may be improved in non-smokers versus smokers, and in non-obese (body mass index [BMI] <30) versus obese patients.[54] In contrast, a retrospective study of inflammatory serum markers in HS patients found no association between smoking status and HS severity, but smoking was associated with increased neutrophil counts.[46] This study did find an association between increased BMI and HS severity whereas there was no correlation between BMI and neutrophil counts.

Related to obesity, an analysis of 14 obese patients with HS described the role of mechanical stress (for example on the abdomen at the level of the waistband) in promoting a 'Koebner-like phenomenon' in HS.[55] The development of lesions at sites of traumatised but previously uninvolved skin highlights the importance of localised environmental factors in HS development. A hospital-based cross-sectional study conducted in the Netherlands reported a significantly higher average BMI in 106 patients with HS than in 212 general dermatological patients.[56] Among those patients identified as obese, bodyweight distribution was more peripheral in patients with HS than those without, consistent with enhanced friction due to overlapping skin folds.

The influence of hormones has been suspected in women with HS for more than 60 years yet has not been proven.[57] Kromann and colleagues reported no clear effect of pregnancy or menopause on HS symptoms.[54] However, a substantial subset of women did experience HS-related alterations, with deterioration of HS around menses and amelioration of symptoms during pregnancy reported in 43% (n = 80) and 30% (n = 29) of the respondents, respectively.[58] This study found a significant correlation between perimenstrual deterioration of HS symptoms and amelioration during pregnancy.

Integrated viewpoint on HS pathogenesis 'sequence of events'

On the basis of the latest evidence, we are able to propose a three-stage sequence of events that contribute to the pathogenesis of HS. This integrated viewpoint is illustrated schematically in Figure 1.

The first event is follicular occlusion with subsequent dilation. This may be driven by endogenous factors in individuals harbouring a genetic predisposition for an enhanced risk of infundibular keratinisation and cyst formation and/or follicular fragility. Exogenous factors such as smoking, mechanical friction and metabolic changes such as obesity – which is associated with pseudoacanthosis – also contribute to occlusion of the follicular isthmus. Furthermore, occlusion of the hair follicle may lead to a dysregulation of the homeostatic keratinocyte symbiosis and microbial dysbiosis, making the skin prone to a Th1/Th17-driven inflammatory disease.

The second event is rupture of the dilated follicle. The scattering of follicle content in the dermis including keratin fibres, commensal flora or pathogen- and damage-associated molecular patterns (PAMPs/DAMPs) triggers an acute and severe immune response. The anatomical location, i.e. the inverse body areas, and enhanced mechanical friction at these predilection sites facilitates the inward rupture and extension of inflammation. We argue that the release of the follicular debris into the dermis results in simultaneous activation of multiple inflammatory pathways, particularly Th17/IL-23, the NLRP inflammasomes and innate receptors (toll-like receptors, TLRs such as TLR2). This is accompanied by histological alterations with a diverse cell infiltrate characterised by the mixed participation of monocytes, neutrophils, eosinophils, multinucleated giant cells, B-cells, plasma cells, T-cells, and natural killer cells, leading to an erythematous nodule or fluctuating abscess.

The third event is chronic inflammation with sinus tract or tunnel formation. Following follicular rupture, sequestered proliferating Ki-67+ epithelial strands promote continuous activation of the immune system. The presence of epithelial strands in the dermis, in addition to an imbalance in matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), and increased activity of fibrotic factors such as transforming growth factor (TGF)-ß 1-2-3, may lead to scarring and the development of sinuses/tunnels or fistulae, a hallmark of chronic HS. These (partly) epithelialised intracutaneous cavities provide an excellent habitat for biofilm-producing bacteria, which are able to continuously trigger inflammation with associated purulent drainage. Furthermore, we hypothesise that circulating pro-inflammatory cytokines and chemokines from chronic lesions may activate the immune system of the hair follicle in distant predilection sites.

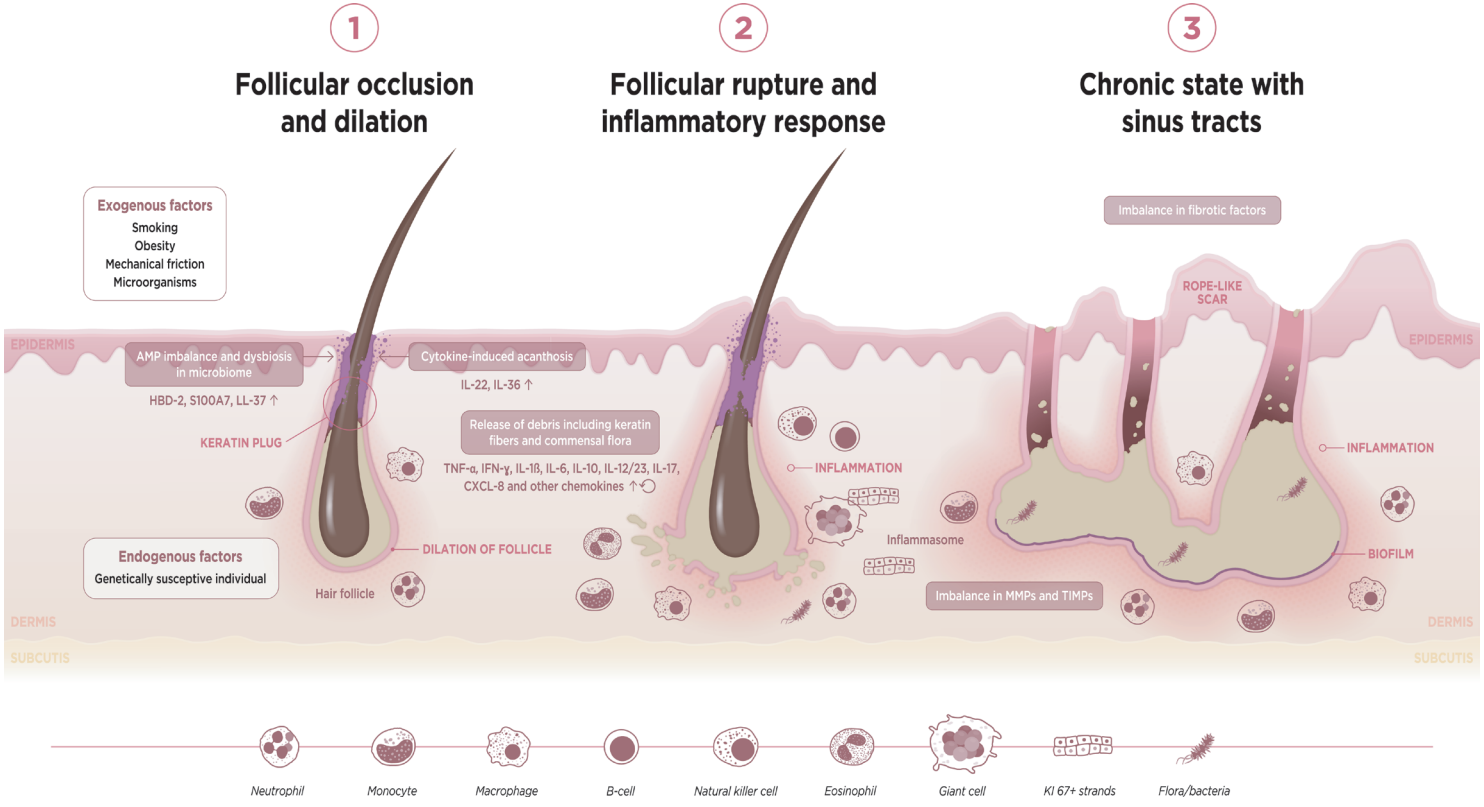

Figure 1. Schematic diagram to illustrate postulated sequence of events underlying HS pathophysiology. Adapted from Saunte and Jemec, 2017. AMP: antimicrobial peptide. HBD: human ß-defensin. IFN: interferon. IL, interleukin. MMP: matrix metalloproteinase. TIMP: tissue inhibitor of metalloproteinase. TNF: tumour necrosis factor.

TREATMENT

To date, there is no long-term cure for HS. In general, the main treatment goal is to improve patients' quality of life. This can be achieved by reducing the inflammation-related pain and purulent discharge, limiting the incidence and duration of flares, and removing chronic lesions using surgical techniques. As there are limited effective treatment options, there remains a large unmet medical need in this area. To date, only eight randomised controlled trials (RCTs) have investigated the efficacy of anti-inflammatory agents. The highest-level evidence available addresses the use of biologic therapies, especially adalimumab (anti-TNF-α), and topical and systemic antibiotics. The majority of the remaining evidence to guide management decisions is based on case reports, small cohort studies and expert opinion.

Anti-inflammatory antibiotics

The literature available on the use of antibiotics in HS is limited and largely restricted to retrospective studies. For Hurley stage I, topical clindamycin 1% is a possible therapy, especially in the absence of abscesses.[59,60] If there are several lesions and frequent exacerbations, the therapeutic group of systemic tetracyclines may be considered.[60] In Hurley stage II/III patients who have several active lesions, systemic clindamycin and rifampicin (dosage: 300 mg twice daily) should be administered.[61-64] A triple regimen of rifampicin (10 mg/kg once daily), moxifloxacin (400 mg once daily) and metronidazole (500 mg thrice daily) administered for up to 12 weeks, with metronidazole discontinuation after 6 weeks, may be an alternative option.[65] To limit resistance, only one antibiotic of the same class should be used for a maximum of 12 weeks. The S1 European and HS ALLIANCE guidelines recommend that antibiotics should be reintroduced in case of recurrence under the requirement that they were effective at the last time of use.[3] Of note, in HS, traditional microbiological sampling is not necessary as there is no evidence for infection as a causal factor. Moreover, antibiotics are prescribed in HS for their anti-inflammatory properties, rather than for their antibacterial action.

Biologics

Biologics targeting TNF-α have been most widely investigated in HS and adalimumab is the only registered drug for HS. Adalimumab should be considered as a first-choice biologic agent in moderate-to-severe HS after failure of conventional treatments.[66-68] Infliximab has also been shown to be effective and should be considered as a second-line biologic for moderate-to-severe HS.[69] Targeting IL-1 gave ambiguous clinical outcomes and is considered as third-line option. On the one hand anakinra proved to be efficacious in moderate-to-severe HS in a RCT, but on the other hand cases of

failure to anakinra therapy have been reported.[70-72] Ustekinumab (anti-IL-12/23p40) is potentially effective for the treatment of moderate-to-severe HS.[73] Secukinumab (anti-IL-17A) has demonstrated clinical improvement of HS in single cases,[74,75] and the results of two RCTs (ClinicalTrials.gov Identifier: NCT02421172, NCT03248531) investigating IL-17 antagonists are being awaited.

Surgical treatment

Surgery is indicated throughout all stages of the disease.[76] The presence of inflammation and suppuration determines the need for anti-inflammatory treatment before surgery, e.g. oral antibiotics or biologics. The required surgical intervention is chosen based on the nature of the symptoms, the type of lesions, the presence of sinus tracts, and the size of the area. The evidence for surgical therapies in HS is based on case series and cohort studies with differing methodologies and outcome definitions, impeding the mutual comparison of studies that investigate surgical techniques in HS.

To ensure the best patient outcomes, surgeons should select the appropriate surgical technique based upon operator experience and the individual needs of the patient. A small excision or deroofing can be used for recurrent nodules at fixed locations or sinus tracts in limited areas.[77,78] Wide excision of an entire affected area (body surface are >1%), with removal of (non-)inflamed sinuses, nodules and scar tissue, is indicated for patients suffering Hurley III stage disease.[79-81] Special attention should be paid to patients with perianal and/or perineal HS due to the possible existence of fistulas.[82,83] In addition to electrosurgical (or cold steel techniques), ablative CO_2 laser treatment is an effective alternative method.[84,85]

Secondary intention healing is the preferred management after excisions in HS. Theoretically, secondary intention healing may reduce the rate of recurrence by allowing the remaining aberrant keratinocytes or residual keratin fibres to escape from the wound. Trapping these remnant foci of diseased tissue by primary closure or reintroduction of hair follicles in a predilection site by flap reconstruction may induce recurrence in the operated area. However, to date there is no literature available to support this hypothesis.

Other therapies

Various other treatments have been investigated in HS, but their applicability to widespread practice and outcomes is currently unknown.[86] Further research is warranted for these therapies. Systemic acitretin may be considered as a third-line therapy for patients with mild-to-moderate HS.[87-90] The combination of oral zinc gluconate 30 mg thrice daily and topical triclosan 2% twice daily is a treatment option in Hurley I-II patients.[91] Systemic dapsone 50-200 mg daily induced improvement of HS in 38% (9/24) of treated patients.[92] Metformin at a maximum dose of 500 mg thrice

daily showed clinical amelioration of HS,[93] and could therefore be an adjuvant treatment in obese HS patients at risk for developing diabetes mellitus or the metabolic syndrome. Low-dose systemic corticosteroids (10 mg prednisolone equivalent per day) may be an effective adjunct in recalcitrant HS.[94] Lastly, laser- and light-based treatments have shown promising results for patients with HS in different disease stages.[86] These treatments include the use of intense pulsed light or Nd:YAG laser and external or intralesional photodynamic therapy.

Acute management of flares

Flares of disease, characterised by the acute onset of painful nodules or abscesses, are a hallmark of HS. Adequate management of flares is an essential part of the treatment strategy because acute lesions can be extremely painful and interfere heavily with daily life. Self-treatment of acute lesions can be performed by the application of resorcinol 15% cream thrice daily.[95] Both the keratolytic and mild antiseptic properties of this topical agent has the potential to reduce levels of pain and achieve early clinical resolution of a treated boil. In a clinical setting, inflammatory nodules often benefit from intralesional corticosteroids by inhibiting the synthesis of pro-inflammatory cytokines,[96] whereas abscesses require incision and drainage to rapidly relieve symptoms of pain and pressure.[97-99] Of note, incision and drainage should not be considered as a sole treatment because recurrence is almost inevitable.

AIMS OF THIS THESIS

In HS, rapidly evolving understanding of pathogenic mechanisms and clinical perspectives are needed to improve disease awareness, disease management, and ultimately improve patient outcomes. Because multiple facets of HS are not yet known, the outline of this thesis is not limited to only one aspect of the disease. Using a translational approach, we focused on clinical features and (immuno)pathogenic mechanisms as a rationale for the development of novel treatment strategies.

In our clinical experience a substantial proportion of patients reports itch, also known as pruritus. Therefore, the aim of **Chapter 2** was to investigate the significance of HS-related pruritus by determining the prevalence of pruritus, and exploring its impact on daily activities in a cohort of HS patients. In addition, a selection of serological and histological markers of pruritus were evaluated in a subpopulation.

Extensive inflammation is a clinical hallmark of HS and identification of important inflammatory markers in the pathogenesis of HS may help both therapeutic stratifica-

tion. Consequently, in **Chapter 3** we investigated the cytokine and chemokine profile in the plasma and lesional skin of HS patients.

Biologics targeting inflammatory mediators are now widely used for the treatment of HS in daily practice, but their clinical efficacy shows great inter-patient variability. For that reason, the aim of **Chapter 4** was to determine the anti-inflammatory potency of currently available biologics for the treatment of HS in an *ex vivo* skin model using lesional HS biopsies.

High-quality evidence on HS treatment is limited, highlighting a significant unmet need for novel effective anti-inflammatory therapies. The aim of **Chapter 5** was to investigate the efficacy of apremilast in patients with moderate HS using a randomised placebo-controlled trial design. In **Chapter 5.1** the clinical efficacy, and short-term safety and tolerability of apremilast were evaluated. In **Chapter 5.2,** in a mode of action study, we analysed the change in expression of inflammatory markers in lesional skin of patients receiving apremilast compared with placebo.

As follicular occlusion is the primary event in the HS pathophysiology, we hypothesised that reducing the number of hair follicles would ameliorate the disease course. The aim of **Chapter 6** was to evaluate two non-invasive techniques that (primarily) target the hair follicle in patients with mild HS. In **Chapter 6.1** we assessed the effect of hair removal using a long-pulsed 1064-nm neodymium-doped yttrium aluminium garnet (Nd:YAG) laser. Lastly, in **Chapter 6.2**, the efficacy and safety of microwave ablation for mild axillary HS was evaluated in a randomised intrapatient-controlled trial.

REFERENCES

1 Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. *JAMA* 2017; **318**: 2019-32.

2 Dany M, Elston D. Gene expression of sphingolipid metabolism pathways is altered in hidradenitis suppurativa. *Journal of the American Academy of Dermatology* 2017; **77**: 268-73.

3 Zouboulis CC, Desai N, Emtestam L *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619-44.

4 Canoui-Poitrine F, Revuz JE, Wolkenstein P *et al.* Clinical characteristics of a series of 302 French patients with hidradenitis suppurativa, with an analysis of factors associated with disease severity. *J Am Acad Dermatol* 2009; **61**: 51-7.

5 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

6 Matusiak L. Profound consequences of hidradenitis suppurativa: a review. *Br J Dermatol* 2018.

7 Jemec GB. Quality of life considerations and pain management in hidradenitis suppurativa. *Semin Cutan Med Surg* 2017; **36**: 75-8.

8 Janse IC, Deckers IE, van der Maten AD *et al.* Sexual health and quality of life are impaired in hidradenitis suppurativa: a multicentre cross-sectional study. *Br J Dermatol* 2017; **176**: 1042-7.

9 Shavit E, Dreiher J, Freud T *et al.* Psychiatric comorbidities in 3207 patients with hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2015; **29**: 371-6.

10 Thorlacius L, Cohen AD, Gislason GH *et al.* Increased Suicide Risk in Patients with Hidradenitis Suppurativa. *J Invest Dermatol* 2018; **138**: 52-7.

11 Sartorius K, Emtestam L, Jemec GB *et al.* Objective scoring of hidradenitis suppurativa reflecting the role of tobacco smoking and obesity. *Br J Dermatol* 2009; **161**: 831-9.

12 Kohorst JJ, Kimball AB, Davis MD. Systemic associations of hidradenitis suppurativa. *J Am Acad Dermatol* 2015; **73**: S27-35.

13 Bui TL, Silva-Hirschberg C, Torres J *et al.* Hidradenitis suppurativa and diabetes mellitus: A systematic review and meta-analysis. *J Am Acad Dermatol* 2018; **78**: 395-402.

14 Egeberg A, Gislason GH, Hansen PR. Risk of Major Adverse Cardiovascular Events and All-Cause Mortality in Patients With Hidradenitis Suppurativa. *JAMA Dermatol* 2016; 152: 429-34.

15 Deckers IE, Benhadou F, Koldijk MJ *et al.* Inflammatory bowel disease is associated with hidradenitis suppurativa: Results from a multicenter cross-sectional study. *J Am Acad Dermatol* 2017; **76**: 49-53.

16 Marzano AV, Damiani G, Ceccherini I *et al.* Autoinflammation in pyoderma gangrenosum and its syndromic form (pyoderma gangrenosum, acne and suppurative hidradenitis). *Br J Dermatol* 2017; **176**: 1588-98.

17 Banerjee A, McNish S, Shanmugam VK. Interferon-gamma (IFN-gamma) is Elevated in Wound Exudate from Hidradenitis Suppurativa. *Immunological investigations* 2017; **46**: 149-58.

18 von Laffert M, Helmbold P, Wohlrab J *et al.* Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. *Exp Dermatol* 2010; **19**: 533-7.

19 von Laffert M, Stadie V, Wohlrab J *et al.* Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. *Br J Dermatol* 2011; **164**: 367-71.

20 Wang B, Yang W, Wen W *et al.* Gamma-secretase gene mutations in familial acne inversa. *Science* 2010; **330**: 1065.

21 Xiao X, He Y, Li C *et al.* Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signalling pathways. *Br J Dermatol* 2016; **174**: 522-32.

22 Pan Y, Lin MH, Tian X *et al.* gamma-secretase functions through Notch signaling to maintain skin appendages but is not required for their patterning or initial morphogenesis. *Dev Cell* 2004; **7**: 731–43.

23 Xu H, He Y, Hui Y *et al.* NCSTN mutations in hidradenitis suppurativa/acne inversa do not influence cytokine production by peripheral blood mononuclear cells. *Br J Dermatol* 2017; **176**: 277–9.

24 Marzano AV, Trevisan V, Gattorno M *et al.* Pyogenic arthritis, pyoderma gangrenosum, acne, and hidradenitis suppurativa (PAPASH): a new autoinflammatory syndrome associated with a novel mutation of the PSTPIP1 gene. *JAMA Dermatol* 2013; **149**: 762-4.

25 Calderon-Castrat X, Bancalari-Diaz D, Roman-Curto C *et al.* PSTPIP1 gene mutation in a pyoderma gangrenosum, acne and suppurative hidradenitis (PASH) syndrome. *Br J Dermatol* 2016; **175**: 194–8.

26 Zeeli T, Padalon-Brauch G, Ellenbogen E *et al.* Pyoderma gangrenosum, acne and ulcerative colitis in a patient with a novel mutation in the PSTPIP1 gene. *Clinical and Experimental Dermatology* 2015; **40**: 367-72.

27 Starnes TW, Bennin DA, Bing X *et al.* The F-BAR protein PSTPIP1 controls extracellular matrix degradation and filopodia formation in macrophages. *Blood* 2014; **123**: 2703-14.

28 Nomura Y, Nomura T, Sakai K *et al.* A novel splice site mutation in NCSTN underlies a Japanese family with hidradenitis suppurativa. *Br J Dermatol* 2013; **168**: 206-9.

29 Giatrakos S, Huse K, Kanni T *et al.* Haplotypes of IL-12Rbeta1 impact on the clinical phenotype of hidradenitis suppurativa. *Cytokine* 2013; **62**: 297-301.

30 Giamarellos-Bourboulis EJ, Platzer M, Karagiannidis I *et al.* High Copy Numbers of beta-Defensin Cluster on 8p23.1, Confer Genetic Susceptibility, and Modulate the Physical Course of Hidradenitis Suppurativa/Acne Inversa. *J Invest Dermatol* 2016; **136**: 1592-8.

31 van der Zee HH, de Ruiter L, van den Broecke DG *et al.* Elevated levels of tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-α and IL-1β. *British Journal of Dermatology* 2011; **164**: 1292-8.

32 Kelly G, Hughes R, McGarry T *et al.* Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. *Br J Dermatol* 2015; **173**: 1431-9.

33 Feldmeyer L, Werner S, French LE *et al.* Interleukin-1, inflammasomes and the skin. *Eur J Cell Biol* 2010; **89**: 638-44.

34 Di Caprio R, Balato A, Caiazzo G *et al.* IL-36 cytokines are increased in acne and hidradenitis suppurativa. *Archives of Dermatological Research* 2017; **309**: 673-8.

35 Schlapbach C, Hanni T, Yawalkar N *et al.* Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**: 790-8.

36 Thomi R, Yerly D, Yawalkar N *et al.* Interleukin-32 is highly expressed in lesions of hidradenitis suppurativa. *Br J Dermatol* 2017; 177: 1358-1366.

37 Thomi R, Kakeda M, Yawalkar N *et al.* Increased expression of the interleukin-36 cytokines in lesions of hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2017; 12: 2091-2096.

38 Moran B, Sweeney CM, Hughes R *et al.* Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. *J Invest Dermatol* 2017; **137**: 2389-95.

39 Emelianov VU, Bechara FG, Glaser R *et al.* Immunohistological pointers to a possible role for excessive cathelicidin (LL-37) expression by apocrine sweat glands in the pathogenesis of hidradenitis suppurativa/acne inversa. *Br J Dermatol* 2012; **166**: 1023-34.

40 Hotz C, Boniotto M, Guguin A *et al.* Intrinsic Defect in Keratinocyte Function Leads to Inflammation in Hidradenitis Suppurativa. *J Invest Dermatol* 2016; **136**: 1768-80.

41 Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L *et al.* The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa. *Mediators Inflamm* 2017; **2017**: 2450401.

42 Matusiak L, Szczech J, Bieniek A *et al.* Increased interleukin (IL)-17 serum levels in patients with hidradenitis suppurativa: Implications for treatment with anti-IL-17 agents. *Journal of the American Academy of Dermatology* 2017; **76**: 670-5.

43 Matusiak L, Bieniek A, Szepietowski JC. Increased serum tumour necrosis factor-alpha in hidradenitis suppurativa patients: is there a basis for treatment with anti-tumour necrosis factor-alpha agents? *Acta Derm Venereol* 2009; **89**: 601-3.

44 Wieland CW, Vogl T, Ordelman A *et al.* Myeloid marker S100A8/A9 and lymphocyte marker, soluble interleukin 2 receptor: biomarkers of hidradenitis suppurativa disease activity? *Br J Dermatol* 2013; **168**: 1252-8.

45 Miller IM, Ring HC, Prens EP *et al.* Leukocyte Profile in Peripheral Blood and Neutrophil-Lymphocyte Ratio in Hidradenitis Suppurativa: A Comparative Cross-Sectional Study of 462 Cases. *Dermatology* 2016.

46 Hessam S, Sand M, Gambichler T *et al.* Correlation of inflammatory serum markers with disease severity in patients with hidradenitis suppurativa (HS). *Journal of the American Academy of Dermatology* 2015; **73**: 998-1005.

47 Grant A, Gonzalez T, Montgomery MO *et al.* Infliximab therapy for patients with moderate to severe hidradenitis suppurativa: A randomized, double-blind, placebo-controlled crossover trial. *Journal of the American Academy of Dermatology*; **62**: 205-17.

48 van der Zee HH, Laman JD, de Ruiter L *et al.* Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

49 Ring HC, Bay L, Nilsson M *et al.* Bacterial biofilm in chronic lesions of hidradenitis suppurativa. *Br J Dermatol* 2017; **176**: 993-1000.

50 Ring HC, Thorsen J, Saunte DM *et al.* The Follicular Skin Microbiome in Patients With Hidradenitis Suppurativa and Healthy Controls. *JAMA dermatology* 2017; **153**: 897-905.

51 Guet-Revillet H, Jais JP, Ungeheuer MN *et al*. The Microbiological Landscape of Anaerobic Infections in Hidradenitis Suppurativa: A Prospective Metagenomic Study. *Clinical Infectious Diseases* 2017; **65**: 282-91.

52 Guet-Revillet H, Coignard-Biehler H, Jais JP *et al*. Bacterial pathogens associated with hidradenitis suppurativa, France. *Emerg Infect Dis* 2014; **20**: 1990-8.

53 Nikolakis G, Liakou AI, Bonovas S *et al*. Bacterial Colonization in Hidradenitis Suppurativa/Acne Inversa: A Cross-sectional Study of 50 Patients and Review of the Literature. *Acta Derm Venereol* 2017; **97**: 493-8.

54 Kromann CB, Deckers IE, Esmann S *et al*. Risk factors, clinical course and long-term prognosis in hidradenitis suppurativa: a cross-sectional study. *Br J Dermatol* 2014; **171**: 819-24.

55 Boer J. Should Hidradenitis Suppurativa Be Included in Dermatoses Showing Koebnerization? Is It Friction or Fiction? *Dermatology (Basel, Switzerland)* 2017; **233**: 47-52.

56 Vossen A, van der Zee HH, Onderdijk AJ *et al*. Hidradenitis suppurativa is not associated with the metabolic syndrome based on body type: A cross-sectional study. *The Journal of Dermatology* 2017; **44**: 154–9.

57 Cornbleet T. Pregnancy and apocrine gland diseases: hidradenitis, Fox-Fordyce disease. *AMA Arch Derm Syphilol* 1952; **65**: 12-9.

58 Vossen AR, van Straalen KR, Prens EP *et al*. Menses and pregnancy affect symptoms in hidradenitis suppurativa: A cross-sectional study. *J Am Acad Dermatol* 2017; **76**: 155-6.

59 Clemmensen OJ. Topical treatment of hidradenitis suppurativa with clindamycin. *Int J Dermatol* 1983; **22**: 325-8.

60 Jemec GB, Wendelboe P. Topical clindamycin versus systemic tetracycline in the treatment of hidradenitis suppurativa. *J Am Acad Dermatol* 1998; **39**: 971-4.

61 Bettoli V, Zauli S, Borghi A *et al*. Oral clindamycin and rifampicin in the treatment of hidradenitis suppurativa-acne inversa: a prospective study on 23 patients. *J Eur Acad Dermatol Venereol* 2014; **28**: 125-6.

62 Gener G, Canoui-Poitrine F, Revuz JE *et al*. Combination therapy with clindamycin and rifampicin for hidradenitis suppurativa: a series of 116 consecutive patients. *Dermatology* 2009; **219**: 148-54.

63 Mendonca CO, Griffiths CE. Clindamycin and rifampicin combination therapy for hidradenitis suppurativa. *Br J Dermatol* 2006; **154**: 977-8.

64 van der Zee HH, Boer J, Prens EP *et al*. The effect of combined treatment with oral clindamycin and oral rifampicin in patients with hidradenitis suppurativa. *Dermatology* 2009; **219**: 143-7.

65 Join-Lambert O, Coignard H, Jais JP *et al*. Efficacy of rifampin-moxifloxacin-metronidazole combination therapy in hidradenitis suppurativa. *Dermatology* 2011; **222**: 49-58.

66 Kimball AB, Kerdel F, Adams D *et al*. Adalimumab for the treatment of moderate to severe Hidradenitis suppurativa: a parallel randomized trial. *Ann Intern Med* 2012; **157**: 846-55.

67 Kimball AB, Okun MM, Williams DA *et al*. Two Phase 3 Trials of Adalimumab for Hidradenitis Suppurativa. *N Engl J Med* 2016; **375**: 422-34.

68 Miller I, Lynggaard CD, Lophaven S *et al*. A double-blind placebo-controlled randomized trial of adalimumab in the treatment of hidradenitis suppurativa. *Br J Dermatol* 2011; **165**: 391-8.

69 Grant A, Gonzalez T, Montgomery MO *et al.* Infliximab therapy for patients with moderate to severe hidradenitis suppurativa: a randomized, double-blind, placebo-controlled crossover trial. *J Am Acad Dermatol* 2010; **62**: 205-17.

70 Menis D, Maronas-Jimenez L, Delgado-Marquez AM *et al.* Two cases of severe hidradenitis suppurativa with failure of anakinra therapy. *Br J Dermatol* 2015; **172**: 810-1.

71 Russo V, Alikhan A. Failure of Anakinra in a Case of Severe Hidradenitis Suppurativa. *J Drugs Dermatol* 2016; **15**: 772-4.

72 Tzanetakou V, Kanni T, Giatrakou S *et al.* Safety and Efficacy of Anakinra in Severe Hidradenitis Suppurativa: A Randomized Clinical Trial. *JAMA Dermatol* 2016; **152**: 52-9.

73 Blok JL, Li K, Brodmerkel C *et al.* Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; **174**: 839-46.

74 Schuch A, Fischer T, Boehner A *et al.* Successful Treatment of Severe Recalcitrant Hidradenitis Suppurativa with the Interleukin-17A Antibody Secukinumab. *Acta Derm Venereol* 2018; **98**: 151-2.

75 Thorlacius L, Theut Riis P, Jemec GBE. Severe hidradenitis suppurativa responding to treatment with secukinumab: a case report. *Br J Dermatol* 2017; 179: 182-185.

76 Horvath B, Janse IC, Blok JL *et al.* Hurley Staging Refined: A Proposal by the Dutch Hidradenitis Suppurativa Expert Group. *Acta Derm Venereol* 2016.

77 van der Zee HH, Prens EP, Boer J. Deroofing: a tissue-saving surgical technique for the treatment of mild to moderate hidradenitis suppurativa lesions. *J Am Acad Dermatol* 2010; **63**: 475-80.

78 van Rappard DC, Mooij JE, Mekkes JR. Mild to moderate hidradenitis suppurativa treated with local excision and primary closure. *J Eur Acad Dermatol Venereol* 2012; **26**: 898-902.

79 Alharbi Z, Kauczok J, Pallua N. A review of wide surgical excision of hidradenitis suppurativa. *BMC Dermatology* 2012; **12**:.

80 Mitchell KM, Beck DE. Hidradenitis suppurativa. *Surg Clin North Am* 2002; **82**: 1187-97.

81 Rompel R, Petres J. Long-term results of wide surgical excision in 106 patients with hidradenitis suppurativa. *Dermatol Surg* 2000; **26**: 638-43.

82 Balik E, Eren T, Bulut T *et al.* Surgical approach to extensive hidradenitis suppurativa in the perineal/perianal and gluteal regions. *World J Surg* 2009; **33**: 481-7.

83 Bocchini SF, Habr-Gama A, Kiss DR *et al.* Gluteal and perianal hidradenitis suppurativa: surgical treatment by wide excision. *Dis Colon Rectum* 2003; **46**: 944-9.

84 Lapins J, Sartorius K, Emtestam L. Scanner-assisted carbon dioxide laser surgery: a retrospective follow-up study of patients with hidradenitis suppurativa. *J Am Acad Dermatol* 2002; **47**: 280-5.

85 Mikkelsen PR, Dufour DN, Zarchi K *et al.* Recurrence rate and patient satisfaction of CO2 laser evaporation of lesions in patients with hidradenitis suppurativa: a retrospective study. *Dermatol Surg* 2015; **41**: 255-60.

86 van Straalen KR, Schneider-Burrus S, Prens EP. Current and future treatment of hidradenitis suppurativa. *Br J Dermatol* 2018.

87 Boer J, Nazary M. Long-term results of acitretin therapy for hidradenitis suppurativa. Is acne inversa also a misnomer? *Br J Dermatol* 2011; **164**: 170-5.

88 Matusiak L, Bieniek A, Szepietowski JC. Acitretin treatment for hidradenitis suppurativa: a prospective series of 17 patients. *Br J Dermatol* 2014; **171**: 170-4.

89 Puri N, Talwar A. A study on the management of hidradenitis suppurativa with retinoids and surgical excision. *Indian J Dermatol* 2011; **56**: 650-1.

90 Tan MG, Shear NH, Walsh S *et al.* Acitretin: Monotherapy or Combined Therapy for Hidradentis Suppurativa? *J Cutan Med Surg* 2017; **21**: 48-53.

91 Hessam S, Sand M, Meier NM *et al.* Combination of oral zinc gluconate and topical triclosan: An anti-inflammatory treatment modality for initial hidradenitis suppurativa. *J Dermatol Sci* 2016; **84**: 197-202.

92 Yazdanyar S, Boer J, Ingvarsson G *et al.* Dapsone therapy for hidradenitis suppurativa: a series of 24 patients. *Dermatology* 2011; **222**: 342-6.

93 Verdolini R, Clayton N, Smith A *et al.* Metformin for the treatment of hidradenitis suppurativa: a little help along the way. *J Eur Acad Dermatol Venereol* 2013; **27**: 1101-8.

94 Wong D, Walsh S, Alhusayen R. Low-dose systemic corticosteroid treatment for recalcitrant hidradenitis suppurativa. *J Am Acad Dermatol* 2016; **75**: 1059-62.

95 Boer J, Jemec GB. Resorcinol peels as a possible self-treatment of painful nodules in hidradenitis suppurativa. *Clin Exp Dermatol* 2010; **35**: 36-40.

96 Riis PT, Boer J, Prens EP *et al.* Intralesional triamcinolone for flares of hidradenitis suppurativa (HS): A case series. *J Am Acad Dermatol* 2016; **75**: 1151-5.

97 Ellis LZ. Hidradenitis suppurativa: surgical and other management techniques. *Dermatol Surg* 2012; **38**: 517-36.

98 Danby FW, Hazen PG, Boer J. New and traditional surgical approaches to hidradenitis suppurativa. *J Am Acad Dermatol* 2015; **73**: S62-5.

99 Revuz J. Hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2009; **23**: 985-98.

Chapter 2

Assessing pruritus in hidradenitis suppurativa: a cross-sectional study

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ABSTRACT

Background

Pruritus is still a forgotten aspect of hidradenitis suppurativa (HS) and, to date, has never been adequately studied.

Objective

The aim of this study was to determine the prevalence, and explore the characteristics, of pruritus in a well-defined cohort of HS patients.

Setting

An academic hospital-based cross-sectional study in The Netherlands.

Methods

A numeric rating scale (NRS, 0-10) was used to determine the prevalence of HS-related itch (NRS score ≥3). Candidate predictors for pruritus were subsequently determined using logistic regression models, and the impact of pruritus was assessed using a modified five-dimensional (5-D) itch scale. Associated serological and histological markers of pruritus were (semi-)quantitatively investigated in a subpopulation.

Results

The prevalence rate of pruritus in 211 HS patients was 57.3%, with a mean NRS score of 6.1 ± 2.0. Patients with a pruritus NRS score ≥3 had more HS-affected body sites than patients with a score <3 ($p < 0.001$). The occurrence of a pruritus NRS score ≥3 was associated with Hurley III disease (odds ratio [OR] 7.73; $p = 0.003$) and HS-related pain (OR 1.34; $p < 0.001$). Pruritus affected sleep and activities of daily living (ADL) in the majority of cases, with a mean (± SD) associated modified 5-D itch score of 13.7 ± 3.6 (on a scale from 5 to 25) in 52 HS patients. Histological examination showed that eosinophilic granulocytes were present in 25% (2/8) of the perilesional skin and 63% (10/16) of the lesional skin, while a perineural infiltrate was found in 25% (2/8) and 69% (11/16) of the perilesional and lesional skin, respectively.

Conclusion

Pruritus is a frequent but underreported symptom in patients with HS. Its moderate-to-severe intensity and significant impact on daily activities have great potential to impair patients' quality of life.

INTRODUCTION

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic, recurrent, inflammatory skin disease that mostly develops after puberty and predominantly occurs in women, with a female-to-male ratio of 3 : 1.[1,2] The estimated prevalence rate in Europe is approximately 1%.[1] Risk factors for developing HS are smoking and overweight or obesity.[3] The disease is characterised by deep-seated, inflamed nodules and abscesses, often followed by sinus tract formation, and is most commonly located in the flexural body sites carrying terminal hairs.[4]

Key symptoms of HS include acute and chronic pain, discomfort, and a purulent, foul-smelling discharge, which, overall, contribute to a poor quality of life.[5,6] Previous clinical studies have mainly focused on these well-known symptoms,[1,4,5] while less is known about the itch sensation, also known as pruritus. In 2011, a small qualitative study in which 12 HS patients were interviewed for the first time revealed that not only drainage, pain, and scarring but also itching have a significant psychosocial impact in HS patients.[7]

To date, one cross-sectional study demonstrated a positive association between disutility and itch in a cohort of 294 HS patients, using the EuroQoL-5D (EQ-5D) and a visual analogue scale (VAS) for itch, respectively.[8] However, the prevalence and accompanying (psychosocial) factors of pruritus in HS have not been adequately investigated. The aim of this study was to determine the prevalence, and explore the characteristics, of pruritus in a cohort of HS patients. In addition, serological and histological markers of pruritus were evaluated in a subpopulation.

MATERIALS AND METHODS

Study design and population

This cross-sectional study consisted of consecutive male and female patients with a physician-verified diagnosis of HS who visited the department of Dermatology of the Erasmus University Medical Center in Rotterdam, The Netherlands. Each patient filled out a questionnaire relating to the intensity of pruritus and the pain caused by their HS. Patients with a limited understanding of the Dutch language, as well as patients with a concomitant skin disease that might cause pruritus (e.g. psoriasis, atopic dermatitis, chronic urticaria) were excluded.

Epidemiological and clinical parameters

Patients separately assessed the highest intensity of their HS-related pruritus and pain over the past 7 days on an 11-point numeric rating scale (NRS) ranging from 0 (no

itch/pain) to 10 (unbearable/extreme itch/pain).[9] All patients with an NRS score ≥3 were included in the prevalence analysis. It has previously been demonstrated that the minimal clinically important difference (MCID) for clinical improvement in itch ranks as a decrease of 2.7 points rated on the NRS in the last 3 days.[10]

Clinical parameters were collected during routine care and were derived from medical charts. HS severity was assessed using the Hurley classification of the worst affected body area,[11] and the extent of disease activity was evaluated by the number of anatomical skin regions with inflammation. The presence of three or more papules/pustules, one or more inflammatory nodules, one or more draining sinuses, or one or more abscesses per region (left and right separate) was considered as inflammatory active HS disease. Additionally, the NRS relating to pain was used as the patient-reported outcome measure (PROM) of disease activity.

The impact of pruritus

Randomly selected patients with a pruritus NRS score ≥3 were asked to fill in a five-dimensional (5-D) itch scale, which is used to evaluate the impact of pruritus on daily activities and includes five domains – duration, degree, direction, disability, and distribution.[12] The questionnaire was adapted through (1) translation into Dutch, and (2) explicitly mentioning the axillary and genital regions, with removal (i.e. points of contact with clothing) and merging (i.e. tops of feet and soles; palms and tops of hands; thighs and lower legs; forearms and upper arms) of non-relevant sections in the distribution domain. The latter was carried out to obtain better insight into the HS predilection areas, and resulted in 12, instead of 16, answer options (Supplementary Material). The outcomes of domains 1 to 4 are measured on a 5-point Likert scale.[12] The scoring system for the distribution domain was not adapted as it was not expected that HS patients without concomitant skin disease that might cause pruritus would have more than 12 skin regions with symptoms of itch. The overall 5-D score was calculated by adding up the individual scores of the five domains, resulting in scores ranging from 5 (no impact) to 25 (most severe impact on daily life).[12]

Serological analysis

A random subset of HS patients aged ≥18 years with a pruritus NRS score ≥3 was asked for a one-time collection of a blood sample in order to screen for other possible causes of pruritus.[13,14] Tryptase, haemoglobin, bilirubin, creatinine, urea, thyroid-stimulating hormone (TSH), and glycated haemoglobin (HbA1c) were assessed in serum.

Histological analysis

Histopathological analysis was performed on 24 random HS skin samples (including eight perilesional skin samples, and six early and ten chronic lesions) in order to evaluate potential skin-related mediators of pruritus. Large specimens of chronic inflamed skin were obtained from the excised skin of patients who had radical excision of their HS under general anaesthesia as this was considered waste material. In our clinic, HS lesions are excised with a healthy-appearing skin margin of 2 cm; this normal-appearing skin is denoted as perilesional skin. All early (i.e. newly emerging) HS lesions, as judged by both the HS patient and the dermatologist, were biopsied within 4 days after onset.

Three independent observers (ARJVV, KRvS, and EPP), blinded to the disease stage, assessed all skin samples, stained by haematoxylin and eosin (H&E), on the density of the infiltrate, followed by evaluation of eosinophilic granulocytes and perineural involvement within the infiltrate. The outcomes were scored in a semiquantitative manner using a global assessment on an ordinal scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe).[15] Disagreements between the three observers were resolved through discussion until consensus was reached. The average overall score per category was calculated from the consensus score of the observers.

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 21.0 (IBM Corporation, Armonk, NY). Patient characteristics were analysed using descriptive statistics, with continuous data presented as the mean ± standard deviation (SD) or median and interquartile range (IQR), and categorical data presented as number (%). A Shapiro–Wilk test was performed to test whether continuous data were normally distributed. For the primary objective, i.e. the evaluation of patient characteristics between patients with and without pruritus, the parametric *t* test for normally distributed independent samples (two-sided), the non-parametric Mann–Whitney U test for non-normally distributed samples, and the Chi-square test or Fisher's exact test for categorical data were applied. Candidate predictors for pruritus, based on both clinical experience and the literature, were subsequently determined using both univariable and multivariable logistic regression models, with pruritus (NRS score ≥3) as the dependent variable. In order to prevent overfitting of the regression analysis, we were restricted to using 9 degrees of freedom in the multivariate model, as the limiting sample size of patients without pruritus was 90 (Table 1).[15] Data were presented as odds ratio (OR) with 95% confidence intervals (CIs). In all comparisons, a two-sided *p*-value of 0.05 was considered significant.

Ethical Statement

The Medical Ethical Committee of the Erasmus University Medical Center in Rotterdam, The Netherlands, reviewed and approved the study protocol (reference MEC-2016-092). Written informed consent for the serological and histological analysis was obtained from all subjects in accordance with the Declaration of Helsinki principles.

Table 1. Patient characteristics.

Characteristic	Total (N = 211)	Pruritus NRS ≥3 (n = 121)	Pruritus NRS <3 (n = 90)	*p*-value
Age, years	38.0 [29-49]	38.0 [29-48]	39.0 [29-52]	0.39
Female sex	135 (64%)	78 (65%)	57 (63%)	0.87
BMI, kg/m^2	28.5 ± 5.9	28.7 ± 5.8[a]	28.3 ± 6.0[b]	0.67
Diabetes mellitus	18 (9%)	10 (8%)	8 (9%)	0.87
Smoking status[c]				0.05
Never smoked	37 (20%)	16 (13%)	21 (23%)	
Present smoker	42 (62%)	83 (70%)	48 (54%)	
Past smoker	131 (18%)	21 18%)	21 (23%)	
Pack-years[d]	12.0 [7-23]	12.9 [6-22]	12.0 [7-26]	0.65
Positive family history[e]	80 (38%)	49 (41%)	31 (34%)	0.37
Use of systemic medication	64 (32%)	36 (30%)	28 (31%)	0.92
Skin type (Fitzpatrick)[f]				0.49
I	18 (9%)	14 (12%)	4 (5%)	
II	126 (60%)	69 (57%)	57 (63%)	
III	17 (8%)	11 (9%)	6 (7%)	
IV	30 (14%)	17 (14%)	13 (14%)	
V	11 (5%)	5 (4%)	6 (7%)	
VI	9 (4%)	5 (4%)	4 (4%)	
Duration of HS, years	14.0 [7-25]	15.0 [8-27]	13.0 [5-23]	0.22
HS disease severity[g]				<0.001*
Hurley I	32 (15%)	13 (11%)	19 (21%)	
Hurley II	140 (66%)	75 (62%)	65 (72%)	
Hurley III	39 (19%)	33 (27%)	6 (7%)	
Currently inflamed areas[h]	1.5 ± 1.3	1.7 ± 1.4	0.9 ± 1.1	<0.001**
HS-related pain on NRS	6.3 ± 3.0	6.9 ± 2.5	5.0 ± 3.5	<0.001**

Data are expressed as mean ± SD, median [IQR], or n (%). * indicates significant at $p = 0.05$. ** indicates significant at $p = 0.001$. [a] Missing n = 6. [b] Missing n = 9. [c] Missing n = 1. [d] Pack-year = (number of cigarettes smoked per day × number of years smoked) / 20. [e] A positive family history for HS-symptoms in first and second degree relatives. [f] Fisher's exact test. [g] χ^2-test. [h] Inflamed area = ≥3 papules/pustules or ≥1 inflammatory nodule or ≥1 draining sinus or ≥1 abscess. BMI: body mass index. HS: hidradenitis suppurativa. NRS: numeric rating scale.

RESULTS

Epidemiology of pruritus

A total of 231 HS patients were screened, of whom 20 were excluded (13 had a concomitant dermatological comorbidity causing itch, 5 had a limited understanding of the Dutch language, and 2 patients declined to take part in the study). The 211 patients included in the study (64% female) had a median age of 38 years (range 15-71) (Table 1). The prevalence rate (NRS score ≥3) of pruritus was 57.3% (121/211), with a mean (± SD) NRS score of 6.1 ± 2.0. For comparison, applying a cut-off value of a NRS score ≥1 resulted in a rate of 67.3% (mean NRS score of 5.4 ± 2.5). The mean intensity of itch in all 211 patients was rated at an NRS score of 3.7 ± 3.3. The occurrence of pain (NRS score ≥3) was more frequently reported, resulting in a prevalence rate of 83.4% and associated mean NRS score of 7.4 ± 1.8.

Patient characteristics

Comparison of the patient characteristics between patients with pruritus (NRS score ≥3) and patients reporting no or negligible pruritus (NRS score <3) revealed no significant differences, with the exception of HS disease activity/severity (Table 1). Patients with a pruritus NRS score ≥3 reported a higher level of HS-related pain ($p < 0.001$), had more affected body areas ($p < 0.001$), and had more severe disease according to the Hurley classification ($p < 0.001$) compared with patients reporting a pruritus NRS

Table 2. Associations between patient characteristics and the occurrence of itch in 211 HS patients.

		Univariable[a]		Multivariable[a]	
Characteristic	**Coding**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Age	Continuous	0.99 (0.97-1.01)	0.33	0.97 (0.94-1.01)	0.10
Gender	Male	1.05 (0.60-1.86)	0.87	0.75 (0.37-1.53)	0.75
Smoking status	Never smoked	Reference variable		Reference variable	
	Present smoker	2.27 (1.08-2.76)	0.03*	2.02 (0.82-4.95)	0.13
	Past smoker	1.31 (0.54-3.19)	0.55	1.26 (0.43-3.68)	0.67
Duration of HS (years)	Continuous	1.02 (0.99-1.04)	0.20	1.04 (1.00-1.07)	0.38
HS severity	Hurley I	Reference variable		Reference variable	
	Hurley II	1.69 (0.77-3.68)	0.19	1.78 (0.72-4.45)	0.22
	Hurley III	8.04 (2.62-25)	<0.001**	7.73 (2.01-27)	0.003*
Currently inflamed areas	Continuous	1.70 (1.32-2.19)	<0.001**	1.21 (0.92-1.58)	0.18
HS-related pain on NRS	Continuous	1.35 (1.12-1.50)	<0.001**	1.34 (1.18-1.52)	<0.001**

* indicates significant at $p = 0.05$. ** indicates significant at $p = 0.001$. [a] Logistic regression analysis with pruritus NRS ≥3 as dependent variable: univariable model, unadjusted; and multivariable model, adjusted for factors and covariates in the model (OR > 1 is a predictor for the occurrence of itch). CI: confidence interval. HS: hidradenitis suppurativa. NRS: numeric rating scale. OR: odds ratio.

score <3 (Table 1). Candidate predictors for patients reporting a pruritus NRS score ≥3 were Hurley stage III (OR 7.73; $p = 0.003$) and HS-related pain, with an OR of 1.34 for each additional point on the NRS ($p < 0.001$) (Table 2). A higher number of currently inflamed areas was only associated with pruritus in the univariable analysis (OR 1.70; $p < 0.001$). In addition, patients who smoked tended to complain more about itch than patients who have never smoked (univariable analysis; OR 2.27; $p = 0.03$).

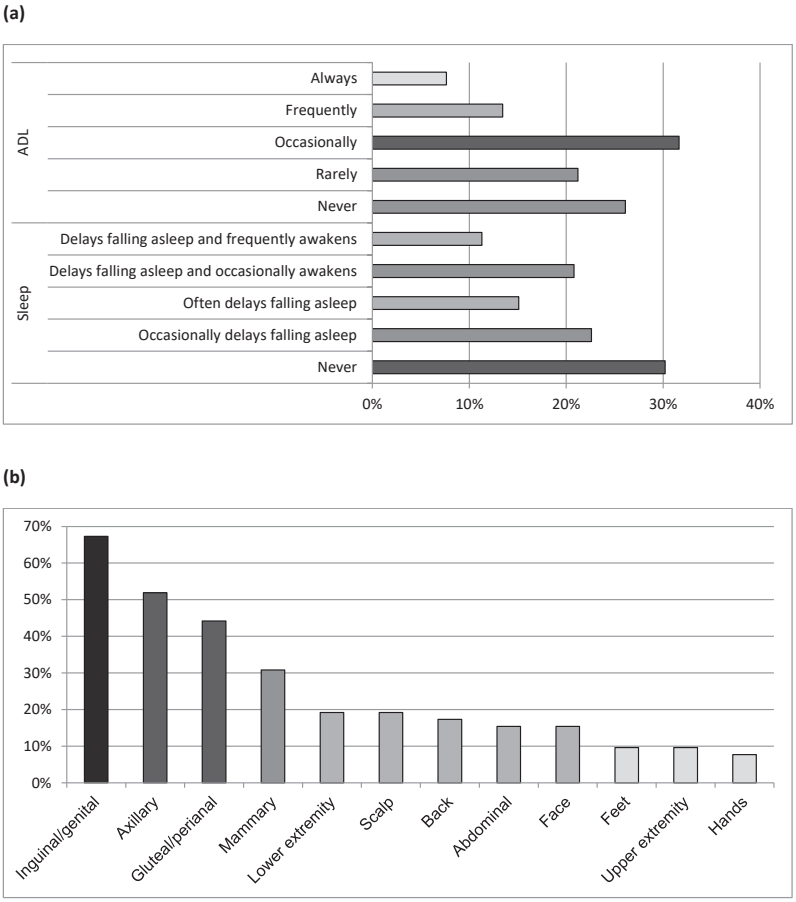

Figure 1. The Modified 5-Dimensional Itch Scale of 51 hidradenitis suppurativa patients with a pruritus numeric rating scale score of ≥3. **(a)** Disability: the impact of itch on ADL and sleep in the past 2 weeks. **(b)** Distribution of itch per body area in the past 2 weeks. ADL: activities of daily living, based on the highest outcome of the three subcategories in the questionnaire.

The impact of pruritus

Fifty-two patients in the pruritus NRS score ≥3 group (n = 121) completed the 5-D itch scale; however, one incorrectly filled out questionnaire was excluded from the analysis. First, the most commonly reported pruritus characterisation was moderate (54%) to severe (27%) itching sensations for less than 6 hours per day (56%), which had not changed in the previous 2 weeks (48%). Second, pruritus, at least occasionally, affected sleep and activities of daily living (ADL), i.e. leisure, social contact, housework, errands, and work/school, in 70% and 53% of patients, respectively (Figure 1a). The mean number of body areas affected by pruritus was 3.1 ± 2.5, with the inguinal/genital (67%) and axillary (52%) regions most frequently involved (Figure 1b). The overall modified 5-D itch score was 13.7 ± 3.6, i.e. HS-related pruritus had a moderate impact on daily activities.

Serological analysis

Serum pruritus markers were evaluated in 24 patients in the pruritus NRS score ≥3 group (n = 121). The mean serum values were within the normal range, with the exception of a lower haemoglobin level found in male patients (n = 5) (Table 3). Three outliers were detected: tryptase (19.3 µg/L) in one patient with a pruritus NRS score of 7, and HbA1c (91.0 and 57.0 mmol/mol) in two patients with pruritus NRS scores of 3 and 5, respectively.

Table 3. General serum pruritus markers of 24 HS patients with itch.

Serum pruritus marker		Unit	Mean ± SD	Reference
Bilirubin		µmol/L	6.0 ± 2.6	<17
Creatinine		µmol/L	69.4 ± 16.8	55 - 115[a]
HbA1c[b]		mmol/mol	38.9 ± 12.7	26 - 42
Haemoglobin	men (n = 5)	mmol/L	8.4 ± 1.2	8.6 - 10.5
	women (n = 19)	mmol/L	8.4 ± 0.6	7.5 - 9.5
Tryptase		µg/L	5.4 ± 3.5	<11.4
TSH		mU/L	1.6 ± 1.0	0.4 - 4.3
Urea[b]		mmol/L	4.0 ± 1.5	2.5 - 7.5

[a] Upper limit dependent on ethnic origin, gender and age. [b] n = 1 missing. HbA1c: glycated haemoglobin. SD: standard deviation. TSH: thyroid-stimulating hormone.

Histological analysis

The average semiquantitative scores for the three types of HS skin specimens are presented in Figure 2. Histological evaluation showed that inflammatory cell infiltration was present (i.e. score of 1-3) in 75% (6/8) of the perilesional samples, and 100% (16/16) of both early and chronic lesional samples. Eosinophilic granulocytes

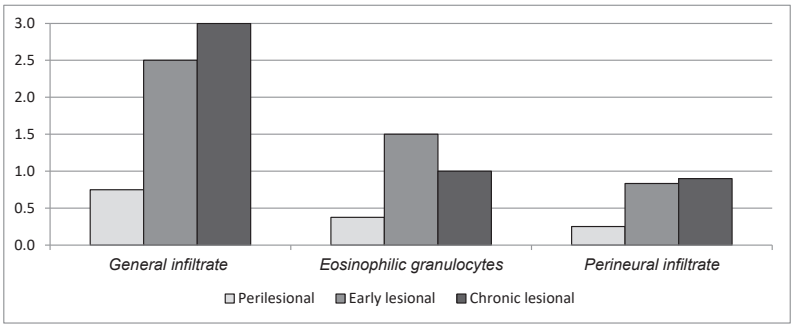

Figure 2. Semiquantitative scores for histological markers of pruritus in three types of hidradenitis suppurativa lesions. A total of 24 samples were assessed: perilesional, n = 8; early lesional, n = 6; chronic lesional, n = 10. The y-axis indicates the average semiquantitative scores on an ordinal scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe).

were present (i.e. score of 1–3) in 25% (2/8) of perilesional skin and 63% (10/16) of lesional skin (Figure 3a/b). A perineural infiltrate, mainly consisting of lymphocytes and a few neutrophils, was found in 25% (2/8) and 69% (11/16) of perilesional and lesional skin, respectively (Figure 3c/d).

DISCUSSION

For the first time, this explorative study evaluated the prevalence and clinical characteristics of pruritus in a well-defined cohort of HS patients. We demonstrated a high prevalence rate of 57% (121/211 with an NRS score ≥3), with a mean NRS score of 6.1 ± 2.0. This prevalence rate is substantially higher than the prevalence of itch in a healthy French population (29%) and a Norwegian national sample with several ethnicities (7%).[16,17] A recently conducted study involving a general dermatological population of a German clinical practice reported a 36% prevalence rate of itch.[18] Compared with other inflammatory skin conditions, the 57% prevalence rate of pruritus in HS is similar to the rate in patients with psoriasis (49-90%),[18,19] and lower than patients with burn injuries (67-93%)[20,21] or chronic idiopathic urticaria (79%).[22]

The overall mean intensity of itch in this study (NRS score 3.7 ± 3.3) was similar to the pruritus VAS score of 3.7 ± 3.2 in a cohort of 294 HS patients.[8] In addition, the mean modified 5-D itch score of 13.7 ± 3.6 in 51 HS patients is comparable with the 5-D score in 51 patients with an inflammatory skin condition such as burn wounds (13.5 ± 3.2).[12] Systemic diseases such as HIV/AIDS (n = 28; 16.8 ± 5.3), chronic liver disease (n = 63; 16.9 ± 4.7), and chronic kidney disease (n = 36; 18.2 ± 4.1) have shown higher 5-D itch scores.[12] However, the majority (81%) of HS patients ranked

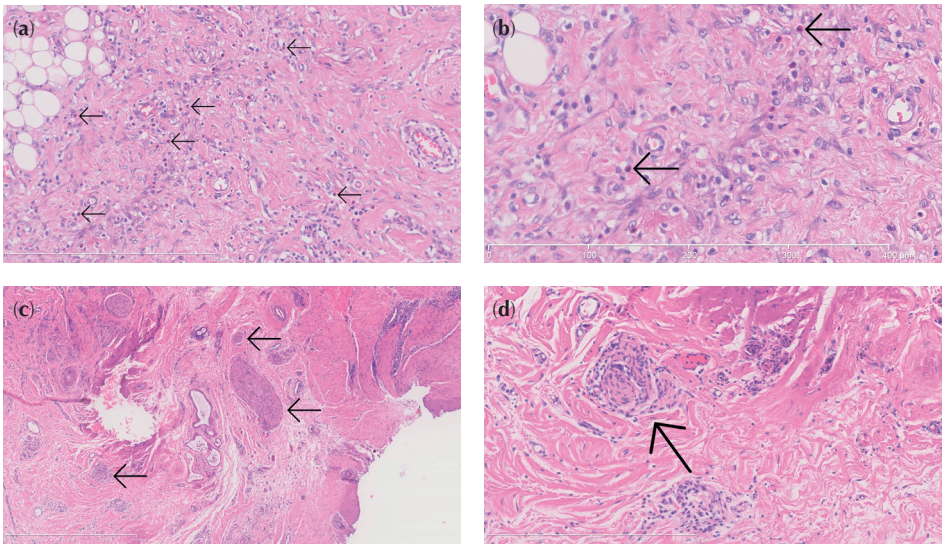

Figure 3. Two histological pruritus markers in hidradenitis suppurativa lesional skin. (**a**) Inflammatory infiltrate with influx of eosinophilic granulocytes (*arrows*); H&E, magnification ×20. (**b**) Closer view of eosinophilic granulocytes (*arrows*); H&E. (**c**) Perineural infiltration of the peripheral nerves (*arrows*) in overview; H&E, magnification ×5. (**d**) Infiltration of a peripheral nerve (*arrow*) by lymphocytes and a few neutrophils in detail; H&E, magnification ×20. H&E: hematoxylin and eosin.

the severity of pruritus as moderate-to-severe on a 5-point Likert scale. Moreover, sleep and ADL were negatively impacted by pruritus in more than half of the patients.

Pruritus is a multidimensional phenomenon and is thought to signal danger from various environmental factors or physiological abnormalities. Therefore, it frequently accompanies various inflammatory skin conditions, including atopic dermatitis, psoriasis, chronic urticaria, and burn wound healing.[23] To date, the aetiology of pruritus in skin diseases is only partially understood. A possible explanation for itching in HS patients is the presence of tryptase-positive mast cells, which were found to be increased in all stages of the disease, including perilesional skin.[24] Increased serum levels of immunoglobulin (Ig)E have recently been reported in patients with HS.[25] The latter, in combination with a dense infiltration of mast cells in HS, could trigger degranulation of these cells, releasing histamine and other mediators, such as proteases, causing pruritus. In addition, we found an influx of eosinophilic granulocytes and the presence of a perineural infiltrate (when nerve fibres were present) in the majority of prototypic HS lesions. An important phenomenon supporting the hypothesis of neurogenic inflammation is the finding of an abnormal innervation of the skin in psoriasis.[26] As demonstrated in cutaneous T-cell lymphoma, interleukin (IL)-31 may play a role by exerting indirect effects on sensory nerves through keratinocytes to transmit itch signals.[27] However, itch in HS patients could also result from a small

fibre neuropathy due to scar formation in the course of HS. Recurrent and chronic inflammation will destroy dermal nerves and subsequently enhance nerve regeneration and neovascularisation when inflammation has subsided. This type of neuropathic itch coincides with pain and may be caused by a disproportionate number of regenerating, unmyelinated C nerve fibres within HS lesions.[28]

The major strength of this explorative cross-sectional study is the recruitment of a relatively large number of physician-verified HS patients. As the occurrence of itch at a specific point in time is susceptible to confounders such as mental distress, use of systemic medication, and comorbidities,[29] a comparator group and quality-of-life measurements, e.g. Dermatology Quality of Life Index (DLQI) or SKINDEX-29, are indicated for future research. Another strength is screening for common systemic causes of chronic recurrent itch that could interfere with the occurrence of HS-related itch. In addition, histopathological evaluation of two important skin-related mediators of pruritus have been performed, although the evaluation lacks information relating to the associated presence of pruritus. For future analysis, it would be interesting to evaluate specific itch-related biomarkers in the serum or skin lesions, such as eosinophilic granulocytes, thymus and activation-regulated chemokine (TARC), IL-2, IL-31, and substance P.

A limitation of this study could be the use of a non-validated, HS-modified 5-D itch scale. From a clinical point of view, the alteration of the distribution section was not expected to influence scoring as HS patients rarely have notable itch in distal, unaffected body sites such as the hands and feet. Moreover, the body areas mentioned in the distribution domain of the adapted scale are more specific to HS-related itch (Supplementary Material). The modified 5-D itch scale, which could be validated for this application in future research, could therefore be a specialised and very useful tool for analysing pruritus in HS patients.

CONCLUSION

Pruritus is a frequent but underreported aspect of HS. Its moderate-to-severe intensity and significant impact on sleep and ADL have great potential to impair patients' quality of life. Therefore, assessment of pruritus in both daily practice and clinical research settings, e.g. by using an NRS or VAS, together with the DLQI and EQ-5D, may form a helpful additional PROM to evaluate disease severity/activity and treatment outcome.

REFERENCES

1 Zouboulis CC, Desai N, Emtestam L *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619-44.

2 Miller IM, McAndrew RJ, Hamzavi I. Prevalence, Risk Factors, and Comorbidities of Hidradenitis Suppurativa. *Dermatol Clin* 2016; **34**: 7-16.

3 Sartorius K, Emtestam L, Jemec GB *et al.* Objective scoring of hidradenitis suppurativa reflecting the role of tobacco smoking and obesity. *Br J Dermatol* 2009; **161**: 831-9.

4 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

5 Dufour DN, Emtestam L, Jemec GB. Hidradenitis suppurativa: a common and burdensome, yet under-recognised, inflammatory skin disease. *Postgrad Med J* 2014; **90**: 216-21.

6 Matusiak L, Bieniek A, Szepietowski JC. Hidradenitis suppurativa markedly decreases quality of life and professional activity. *J Am Acad Dermatol* 2010; **62**: 706-8.

7 Esmann S, Jemec GB. Psychosocial impact of hidradenitis suppurativa: a qualitative study. *Acta Derm Venereol* 2011; **91**: 328-32.

8 Riis PT, Vinding GR, Ring HC *et al.* Disutility in Patients with Hidradenitis Suppurativa: A Cross-sectional Study Using EuroQoL-5D. *Acta Derm Venereol* 2016; **96**: 222-6.

9 Phan NQ, Blome C, Fritz F *et al.* Assessment of pruritus intensity: prospective study on validity and reliability of the visual analogue scale, numerical rating scale and verbal rating scale in 471 patients with chronic pruritus. *Acta Derm Venereol* 2012; **92**: 502-7.

10 Reich A, Riepe C, Anastasiadou Z *et al.* Itch Assessment with Visual Analogue Scale and Numerical Rating Scale: Determination of Minimal Clinically Important Difference in Chronic Itch. *Acta Derm Venereol* 2016; **96**: 978-80.

11 Revuz J. Hidradenitis suppurativa. *J Eur Acad Dermatol Venereol* 2009; **23**: 985-98.

12 Elman S, Hynan LS, Gabriel V *et al.* The 5-D itch scale: a new measure of pruritus. *Br J Dermatol* 2010; **162**: 587-93.

13 Pereira MP, Kremer AE, Mettang T *et al.* Chronic Pruritus in the Absence of Skin Disease: Pathophysiology, Diagnosis and Treatment. *Am J Clin Dermatol* 2016; **17**: 337-48.

14 Yosipovitch G, Greaves MW, Schmelz M. Itch. *Lancet* 2003; **361**: 690-4.

15 Whitehead J. Sample size calculations for ordered categorical data. *Stat Med* 1993; **12**: 2257-71.

16 Dalgard F, Holm JO, Svensson A *et al.* Self reported skin morbidity and ethnicity: a population-based study in a Western community. *BMC Dermatol* 2007; **7**: 4.

17 Misery L, Rahhali N, Duhamel A *et al.* Epidemiology of pruritus in France. *Acta Derm Venereol* 2012; **92**: 541-2.

18 Kopyciok ME, Stander HF, Osada N *et al.* Prevalence and Characteristics of Pruritus: A One-Week Cross-sectional Study in a German Dermatology Practice. *Acta Derm Venereol* 2016; **96**: 50-5.

19 Remrod C, Sjostrom K, Svensson A. Pruritus in psoriasis: a study of personality traits, depression and anxiety. *Acta Derm Venereol* 2015; **95**: 439-43.

20 Van Loey NE, Bremer M, Faber AW *et al.* Itching following burns: epidemiology and predictors. *Br J Dermatol* 2008; **158**: 95-100.

21 Carrougher GJ, Martinez EM, McMullen KS *et al.* Pruritus in adult burn survivors: postburn prevalence and risk factors associated with increased intensity. *J Burn Care Res* 2013; **34**: 94-101.

22 Yosipovitch G, Ansari N, Goon A *et al.* Clinical characteristics of pruritus in chronic idiopathic urticaria. *Br J Dermatol* 2002; **147**: 32-6.

23 Weisshaar E, Szepietowski JC, Darsow U *et al.* European guideline on chronic pruritus. *Acta Derm Venereol* 2012; **92**: 563-81.

24 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

25 Pascual JC, Garcia-Martinez FJ, Martorell A *et al.* Increased total serum IgE levels in moderate-to-severe hidradenitis suppurativa. *Br J Dermatol* 2016; **175**: 1101-2.

26 Szepietowski JC, Reich A. Pruritus in psoriasis: An update. *Eur J Pain* 2016; **20**: 41-6.

27 Nattkemper LA, Martinez-Escala ME, Gelman AB *et al.* Cutaneous T-Cell Lymphoma and Pruritus: the Expression of IL-31 and its Receptors in the Skin. *Acta Derm Venereol* 2016; 96 894-898..

28 Chuquilin M, Alghalith Y, Fernandez KH. Neurocutaneous disease: Cutaneous neuroanatomy and mechanisms of itch and pain. *J Am Acad Dermatol* 2016; **74**: 197-212.

29 Halvorsen JA, Dalgard F, Thoresen M *et al.* Itch and mental distress: a cross-sectional study among late adolescents. *Acta Derm Venereol* 2009; **89**: 39-44.

SUPPLEMENTARY MATERIAL

The modified 5D itch scale.

1.	**DURATION** *During the last two weeks, how many hours have you been itching?*	< 6 hours per day	□ **1**		
		6-12 hours per day	□ **2**		
		12-18 hours per day	□ **3**		
		18-23 hours per day	□ **4**		
		Whole day	□ **5**		
2.	**DEGREE** *Please rate the intensity of your itching in the past 2 weeks?*	Not present	□ **1**		
		Mild	□ **2**		
		Moderate	□ **3**		
		Severe	□ **4**		
		Unbearable	□ **5**		
3.	**DIRECTION** *Over the past 2 weeks, has your itching gotten better or worse compared to the previous month?*	Completely resolved	□ **1**		
		Much better, but still present	□ **2**		
		Somewhat better, but still present	□ **3**		
		Unchanged	□ **4**		
		Worsened	□ **5**		
4.	**DISABILITY** *A. What was the impact of itching on your sleep over the last two weeks?*	Never affects sleep	□ **1**		
		Occasionally delays falling asleep	□ **2**		
		Often delays falling asleep	□ **3**		
		Delays falling asleep and occasionally wakes me up at night	□ **4**		
		Delays falling asleep and frequently wakes me up at night	□ **5**		
	B. Did the itching influence your leisure or social activities over the last two weeks?	Not applicable	□		
		Never	□ **1**		
		Rarely	□ **2**		
		Occasionally	□ **3**		
		Frequently	□ **4**		
		Always	□ **5**		
	C. Did the itching influence your homework or errands over the last two weeks?	Not applicable	□		
		Never	□ **1**		
		Rarely	□ **2**		
		Occasionally	□ **3**		
		Frequently	□ **4**		
		Always	□ **5**		
	D. Did the itching influence your work or school over the last two weeks?	Not applicable	□		
		Never	□ **1**		
		Rarely	□ **2**		
		Occasionally	□ **3**		
		Frequently	□ **4**		
		Always	□ **5**		
5.	**DISTRIBUTION** *Mark whether itching has been present in the following bodyparts over the past 2 weeks.* *If a body part is not listed, choose the one that is closest anatomically.*	Head or scalp	□	Abdomen	□
		Face	□	Back	□
		Chest	□	Groins and genitals	□
		Armpits	□	Buttocks	□
		Arms	□	Legs	□
		Hands	□	Feet	□

Chapter 3

Novel cytokine and chemokine markers of hidradenitis suppurativa reflect chronic inflammation and itch

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Allergy – accepted for publication in modified form

ABSTRACT

Background

A key element of hidradenitis suppurativa (HS) is an aberrant immune response. Identification of inflammatory markers is important for the clinical stratification of HS and may help refining treatment choices.

Objective

To simultaneously detect important cytokines and chemokines in respectively the plasma and lesional skin of patients with HS.

Methods

A multiplex electrochemiluminescent immunoassay platform (Meso Scale Discovery) was used to quantify the *in vivo* protein levels of 30 cytokines and chemokines in twenty HS patients and ten healthy controls. Immunohistochemistry was performed for newly identified markers. Additionally, a correlation between individual plasma and lesional skin protein levels was calculated within the HS patients.

Results

In the circulation of HS patients, CCL-26 (eotaxin-3) was significantly elevated and CXCL-10 significantly lower compared with healthy controls. In the skin, protein levels of IL-16, IL-17A, CXCL-8, IL-12/23p40, CCL-4, and CXCL-10 were significantly higher in HS patients than in controls. Immunohistochemistry demonstrated overexpression of CCL-4, CXCL-10, and CCL-26 in the HS infiltrate. There was no significant correlation between protein levels in patient plasma and lesional skin with correlation coefficients varying between –0.53 and +0.42.

Conclusion

The cytokine and chemokine profile of HS patients, including newly identified IL-16, CCL-4, CXCL-10 and CCL-26, reflects the ongoing skin inflammation in HS. The local and systemic upregulation of CCL-26 in HS patients can be linked to the high pruritus score in HS. Furthermore, our results demonstrate that plasma gives a limited reflection of the activated local cutaneous inflammatory milieu.

INTRODUCTION

Hidradenitis suppurativa (HS) is an auto-inflammatory skin disease characterised by recurrent or chronic painful and pruritic inflammatory nodules, abscesses and sinus tracts in predominantly the axillary, inguinal and gluteal areas.[1] Diagnosis at present is based largely on the clinical appearance and location of lesions, and their chronicity. Well-known symptoms are acute and chronic pain, discomfort, and a purulent, foul-smelling discharge, which, overall, contribute to a decreased quality of life.[2,3] More recently, itch, also known as pruritus, was found to be a frequent and bothersome symptom in HS patients.[4,5]

The pathogenesis of HS is complex with multiple factors contributing to the onset and progression of the disease including genetics, smoking, obesity, and mechanical friction.[6] A key element of the HS pathophysiology is occlusion of the follicular infundibulum and subsequent cyst formation, followed by rupture of the cyst inducing an acute inflammatory response. Hereby a broad range of immune cells such as T cells, natural killer (NK) cells, neutrophils, eosinophils, monocytes, and dendritic cells are recruited.[4,7] Accordingly, elevated levels of multiple pro-inflammatory cytokines such as TNF-α, IL-1ß, IL-6, IL-8 (CXCL-8), and IL-17 have been detected in the skin and/or serum of HS patients.[8-11] Unravelling the role of cytokines and chemokines in disease initiation and progression is important for the clinical and therapeutic stratification of HS. Identification of biomarkers in HS could help in personalising treatments, and may aid in monitoring the efficacy of treatments.

To date, no studies have investigated the inflammatory protein levels in the serum/plasma and skin *in parallel* in a cohort of HS patients at a single time point. Therefore, the primary aim of this study was to simultaneously detect important cytokines and chemokines in respectively the plasma and lesional skin of patients with HS.

MATERIALS AND METHODS

Ethical statement

The research protocol was approved by the local Institutional Review Board (reference MEC-2013-337/NL45264.078.13). The collection of plasma and skin was conducted in the Department of Dermatology of the Erasmus University Medical Center and Sint Franciscus Hospital in Rotterdam, The Netherlands. All participants provided written informed consent in accordance with the Declaration of Helsinki principles for using their plasma and skin tissue in this study.

Hidradenitis suppurativa patient and healthy control samples

Blood and skin samples were prospectively collected from 20 patients with a dermatologist-verified diagnosis of HS and 10 healthy controls. HS patients, of which 10 were female, had a mean (± SD) age of 41.5 ± 10.6 years, a mean body mass index of 32.1 ± 7.6kg/m^2, and a mean HS duration of 16.4 ± 10.9 years. Seventeen patients were current or past smokers. Disease severity was assessed according to Hurley staging: two patients suffered Hurley I, 14 patients suffered Hurley II, and four patients suffered Hurley III. The healthy control subjects consisted of otherwise healthy patients undergoing dermatologic and plastic surgery who had no family history of HS. Participants receiving any systemic immunomodulators or immunosuppressants, and non-HS patients with any significant inflammatory disease were excluded. Punch biopsies of 4 mm in diameter were obtained and immediately snap-frozen in liquid nitrogen. Skin samples of HS patients were taken from actively inflamed, non-fluctuating, indurated, erythemous lesions or plaques recurring on fixed locations. Venous blood was collected in vacuum EDTA tubes under sterile conditions, and after separation of the plasma samples were aliquoted and stored at −80°C until analysis.

In vivo protein quantification in skin homogenates

Plasma and skin samples were analysed using the Meso Scale Discovery (MSD) V-PLEX™ Human Cytokine 30-plex kit (K15054D; Meso Scale Discovery, Gaithersburg, MD) according to the instructions of the manufacturer. MSD assays use electrochemiluminescent labels that allows for analysis of multiple analytes in a variety of sample types.[12]

The plasma and skin samples were thawed at room temperature. Skin biopsies were weighed, thereafter homogenised in 250 µL phosphate-buffered saline (PBS) and grinded using micro pestles. The homogenates were subsequently transferred to 1.5 mL Eppendorf tubes and centrifuged at 13,000 g for 5 minutes at room temperature. Just before analysis, the plasma and supernatant samples were diluted two times for inflammatory cytokines and four times for chemokine analysis in sample diluents. The diluted samples were incubated on the MSD plates for two hours at room temperature with gentle shaking. The plates were rinsed and incubated for an additional two hours with detection antibodies. For CXCL/IL-8 two antibodies were used, both recognizing the same domain of its target. The difference between these antibodies relies on the dynamic range being measured. In addition to the regular antibody, the IL-8 HA (human antibody) has been validated for the MSD V-PLEX™ kit, and is recommended when high CXCL/IL-8 levels are anticipated.

After rinsing twice, read buffer T was added to each well and the plate was analysed using the Sector Imager 6000. Calibrator concentrations and skin and plasma samples were analysed in duplicates. The calibrator concentrations were plotted as log signal

unit on the vertical (Y) axis versus log concentration on the horizontal (X) axis using the MSD Workbench software. A weighted four parameter logistic fit (4PL) equation was used for curve fitting and back calculation of sample concentrations.

Immunohistochemistry

Three chemokines, which have not previously been reported to be overexpressed in HS patients, were additionally analysed by immunohistochemistry (IHC). First, paraffin embedded tissue sections were heated at 60°C for 30 minutes, de-paraffinised, and rehydrated. Slides were subsequently placed in pH6 antigen retrieval buffer and heated at 95°C for 20 minutes in a hot water bath. After cooling, slides were treated with 3% H_2O_2 (5 minutes) and blocked using 10% goat serum (30 minutes). Overnight incubation (4°C) was then performed using the primary antibody at a concentration of 10µg/mL. Lastly, slides were washed, treated with secondary antibody, peroxidase (30 minutes) and diaminobenzidine substrate. The following antibodies were used: anti-CXCL10 (Thermofisher Scientific, cat#701225), anti-CCL4 (ThermoFisher Scientific, cat#PA5-23681), anti-CCL26 (Thermofisher Scientific, cat# PA5-75690), and Rabbit IgG control (Lifespan Biosciences, cat#LS-C149375).

Statisical analyses

Plasma protein concentrations were expressed as picogram (pg) per mL. Protein levels of the skin samples were normalised for mg tissue dry weight (pg/mg). In case a protein level was below the detection limit, the lowest limit of quantification (LLOQ) was used for further calculations. If more than 50% of the samples per analysed protein in either the HS or healthy control group had values below the LLOQ, values were substituted by two categories: detectable versus non-detectable, i.e. above versus below the LLOQ, respectively.

For the primary objective either the Mann Whitney U test or Fisher's exact test was used to assess the null-hypothesis that there was no difference in the levels of individual markers between healthy control and HS samples. For the secondary objective, to assess the correlation between individual plasma and skin protein levels within the HS patients, we used the Spearman's rho for the non-parametrically continuous variables and categorical variables (detectable versus non-detectable).[13] Alternatively, in case of a mismatch in the type of variable (continuous, categorical) between protein levels in skin and plasma, logistic regression models were used to estimate a Nagelkerke's R-squared.

Statistical analyses were conducted using SPSS Statistics 24.0 (IBM Corporation, Armonk, NY). A two-sided *p*-value below 0.05 was considered significant. The level of significance was corrected by a false discovery rate using the Benjamini Hochberg test for multiple comparisons in the plasma and skin samples separately.

RESULTS

Detection of cytokines and chemokines

In total, 30 plasma samples and 30 skin samples from 20 HS patients and 10 healthy controls were analysed by MSD multiplex assay. The protein levels for 30 inflammatory markers in plasma and skin are summarised in Table 1 and Table 2, respectively.

Table 1. Inflammatory protein expression in the plasma of healthy control subjects and HS patients.

	Protein pg/mL	NN (n = 10) median (IQR) or x/total	HS (n = 20) median (IQR) or x/total	LLOQ pg/mL	Unadjusted *p*-value
1	**CXCL-10 (IP-10)**	402.7 (328.7-550.5)	277.4 (236.0-328.8)	2.40	0.003*
2	**CCL-26 (Eotaxin-3)**	2/10	16/20	18	0.0041*
3	**IL-12/23p40**	132.7 (97.7-182.5)	104.0 (74.6-127.2)	1.30	0.055
4	**IL-1α**	1.8 (1.7-3.6)	4.2 (2.4-10.2)	0.62	0.055
5	**CCL-4 (MIP-1β)**	119.4 (66.6-176.0)	78.5 (59.9-102.5)	2.10	0.091
6	**TNF-β**	2/10	0/20	0.28	0.103
7	**IL-1β**	3/10	13/20	0.24	0.122
8	**CCL-22 (MDC)**	926.2 (716.5-1212.4)	1312.7 (1000.8-1538.6)	38	0.155
9	**INF-γ**	8.8 (5.3-14.0)	6.9 (4.8-8.9)	2.20	0.155
10	**IL-15**	2.0 (1.7-2.3)	1.7 (1.5-2.1)	0.32	0.198
11	**IL-7**	18.6 (14.8-24.2)	22.3 (17.0-30.3)	0.32	0.214
12	**IL-10**	0.3 (0.2-0.4)	0.2 (0.2-0.3)	0.16	0.231
13	**CCL-3 (MIP-1α)**	2/10	1/20	15.60	0.251
14	**CXCL-8 (IL-8)**	8.7 (7.2-9.7)	7.1 (6.0-9.1)	3.80	0.286
15	**IL-16**	208.0 (191.9-287.3)	257.9 (186.1-317.9)	4.20	0.475
16	**CCL-11 (Eotaxin-1)**	135.9 (95.6-181.4)	151.6 (118.5-210.1)	5.60	0.502
17	**IL-6**	1.3 (0.9-2.6)	1.1 (0.7-2.6)	0.36	0.530
18	**IL-13**	1/10	1/20	0.98	0.532
19	**IL-17A**	4/10	6/20	2.10	0.690
20	**CCL-17 (TARC)**	385.8 (222.6-511.6)	325.9 (259.9-653.7)	2.80	0.713
21	**TNF-α**	2.6 (2.3-3.2)	2.5 (2.2-3.0)	0.64	0.779
22	**CCL-13 (MCP-4)**	188.0 (160.3-234.8)	210.6 (120.9-238.3)	4.80	0.880
23	**CCL-2 (MCP-1)**	85.0 (75.3-99.1)	83.0 (62.9-114.9)	0.22	0.983
24	**VEGF**	140.1 (116.0-200.0)	155.0 (103.6-250.7)	7	0.983
25	**IL-2**	ND	ND	0.68	-
26	**IL-4**	ND	ND	0.38	-
27	**IL-5**	ND	ND	0.40	-
28	**IL-12p70**	ND	ND	0.74	-
29	**GM-CSF**	ND	ND	1.80	-
30	**IL-8 HA**	ND	ND	344	-

* Significant after correction with the Benjamini Hochberg test ($p < 0.0042$). HS: hidradenitis suppurativa patients. IQR: interquartile range. LLOQ: lowest level of quantification. ND: not detected. NN: healthy controls. x: number of samples with a detectable value.

In plasma, 20 of 30 (66.7%) analytes were detected. In the skin 25 of 26 (96.2%) proteins were detected, while four proteins (IL-4, IL-7, VEGF, GM-CSF) were not analysed because they have not been validated for skin-derived samples.

Table 2. Inflammatory protein expression in the skin of healthy control subjects and HS patients.

	Protein pg/mg skin tissue	NN (n = 10) median (IQR) or x/total	HS (n = 20) median (IQR) or x/total	Unadjusted *p*-value
1	**IL-16**	10.90 (7.67-13.09)	57.54 (38.50-120.81)	<0.001*
2	**IL-17A**	0/10	15/20	<0.001*
3	**CXCL-8 (IL-8)**	0.30 (0.21-1.30)	5.90 (1.25-19.48)	0.001*
4	**IL-12/23p40**	0.10 (0.08-0.17)	0.25 (0.14-0.47)	0.007*
5	**CCL-4 (MIP-1β)**	0.13 (0.08-0.15)	0.62 (0.19-1.83)	0.011*
6	**CXCL-10 (IP-10)**	0.66 (0.18-1.10)	1.80 (1.07-3.32)	0.011*
7	**IL-8 HA**	0/10	10/20	0.011*
8	**TNF-β**	1/10	9/20	0.101
9	**CCL-3 (MIP-1α)**	2/10	11/20	0.119
10	**INF-γ**	3/10	13/20	0.122
11	**TNF-α**	0/10	5/20	0.140
12	**IL-1β**	0.13 (0.07-0.18)	0.21 (0.08-0.73)	0.155
13	**CCL-13 (MCP-4)**	0.66 (0.53-0.72)	0.36 (0.25-0.66)	0.172
14	**IL-10**	0.009 (0.005-0.011)	0.006 (0.004-0.008)	0.183
15	**CCL-17 (TARC)**	2/10	9/20	0.246
16	**IL-5**	0.024 (0.019-0.039)	0.017 (0.013-0.029)	0.322
17	**IL-1α**	1.28 (0.92-2.10)	1.54 (0.86-4.40)	0.350
18	**IL-2**	0.035 (0.016-0.081)	0.031-0.023-0.039)	0.530
19	**IL-6**	0.26 (0.02-0.41)	0.08 (0.03-0.54)	0.530
20	**CCL-2 (MCP-1)**	3.13 (0.30-4.82)	1.43 (0.42-3.35)	0.588
21	**IL-15**	0.029 (0.026-0.039)	0.035 (0.026-0.045)	0.588
22	**CCL-11 (Eotaxin-1)**	4/10	11/20	0.700
23	**CCL-22 (MDC)**	1.80 (1.44-3.44)	1.82 (1.23-3.25)	0.983
24	**IL-13**	0/10	1/20	1.000
25	**IL-12p70**	3/10	6/20	1.000
26	**CCL-26 (Eotaxin-3)**	ND	ND	-
27	**IL-7**	NA	NA	-
28	**VEGF**	NA	NA	-
29	**IL-4**	NA	NA	-
30	**GM-CSF**	NA	NA	-

* Significant after correction with the Benjamini Hochberg test ($p < 0.014$). IL-8 HA (human antibody) has been validated for the MSD V-PLEX™ kit, and is recommended when high CXCL/IL-8 levels are anticipated. HS: hidradenitis suppurativa patients. IQR: interquartile range. LLOQ: lowest level of quantification. NA: not analysed, not validated for skin samples. ND: not detected. NN: healthy controls. x: number of samples with a detectable value.

Seven inflammatory markers were significantly elevated in HS patients

In plasma, CCL-26 was detected significantly more often in HS patients (16 of 20) compared with healthy controls (2 of 10), $p = 0.004$ (Table 1). Accordingly, the median CCL-26 level in HS patients was 24.9 pg/mL, interquartile range (IQR) 19.1-37.0 (Figure 1). In contrast, CXCL-10 levels were significantly lower in HS patients, $p = 0.003$. In addition, there was a trend for higher protein levels of IL-1α and lowered levels of IL-12/23p40 in HS patients. In lesional skin, IL-16 ($p < 0.001$), IL-17A ($p < 0.001$), CXCL-8 ($p = 0.001$), plus IL-8 HA ($p = 0.011$), representing very high CXCL-8 concentrations, IL-12/23p40 ($p = 0.007$), CCL-4 ($p = 0.011$), CXCL-10 ($p = 0.011$) showed higher levels in HS patients compared with healthy controls (Table 2, Figure 2). The median IL-17A protein concentration in lesional HS skin was 0.18 pg/mg tissue (IQR 0.10-0.39). Besides CCL-4, CCL-3 (MIP-1α) was also detected in the majority (11/20) of HS lesional skin samples versus two of ten in the healthy control samples ($p = 0.119$). The elevated CCL-4 and CXCL-10 protein levels in HS lesions were confirmed by IHC (Figure 3). A strong staining of CCL-26 was observed in lesional skin, despite the fact that CCL-26 protein was not detected in lesional HS skin by the MSD multiplex assay (Table 2, Figure 3).

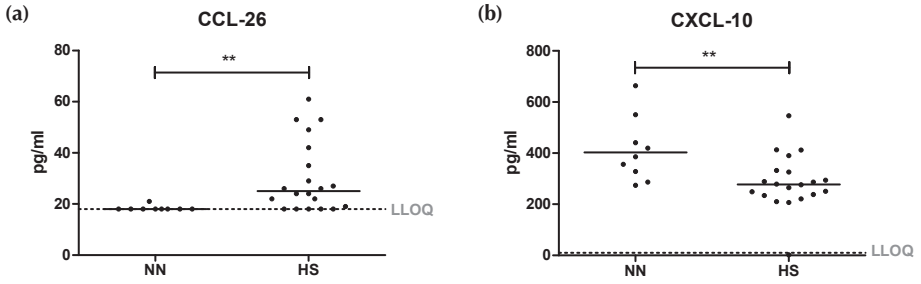

Figure 1. Levels of six elevated inflammatory proteins in lesional skin of HS patients in comparison with healthy control patients. **(a)** CCL-26 ($p = 0.004$). **(b)** CXCL-10 ($p = 0.003$), for NN one data point is out of the y-axis range. Horizontal bars display the median. ** $p < 0.01$. HS: hidradenitis suppurativa patients. LLOQ: lowest limit of quantification. NN: healthy controls.

Weak correlations between protein levels in the plasma and lesional skin of HS patients

Of the 30 proteins, nine were not detected/analysed in either plasma or skin. In 16 analytes, a correlation coefficient (*r*) for protein concentrations in HS plasma and skin samples was calculated. In general, weak correlations between the protein levels in plasma and skin samples were observed: 13 analytes displayed a negative *r* (range –0.053 to –0.532), and three analytes had a positive *r* (range 0.198 to 0.423) (Table 3). The top-3 upregulated

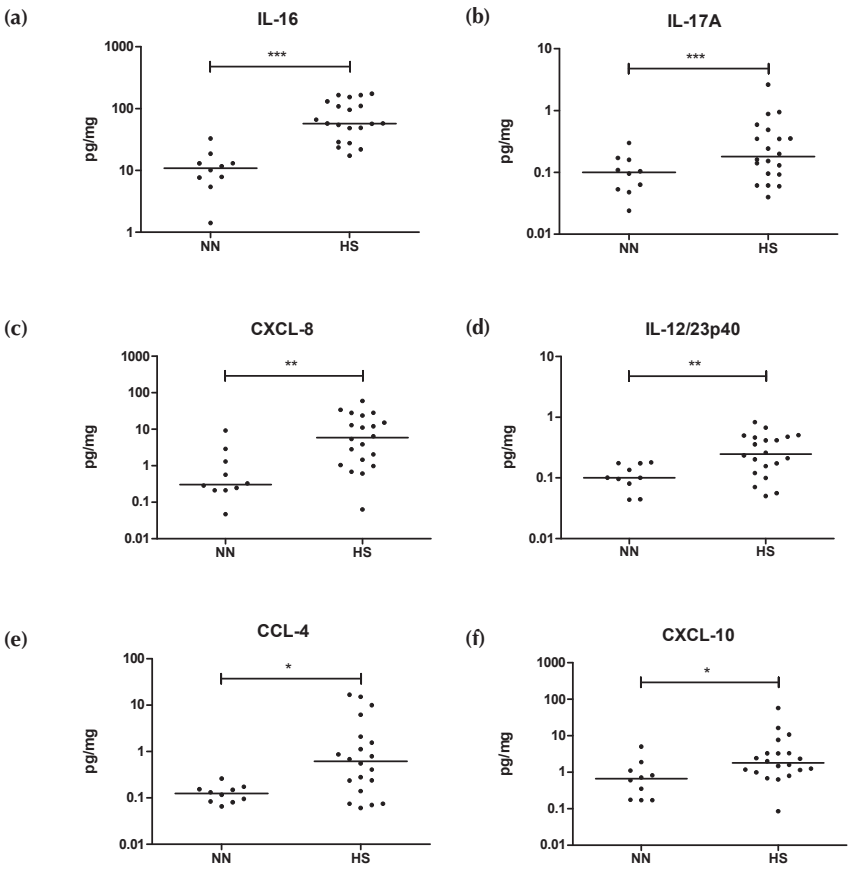

Figure 2. Levels of six elevated inflammatory proteins in lesional skin of HS patients in comparison with healthy control patients. **(a)** IL-16 ($p < 0.001$). **(b)** IL-17A ($p < 0.001$). The LLOQ was used to calculate the concentration of IL-17A in the healthy controls (0 of 10 detected). **(c)** CXCL-8 ($p = 0.001$). **(d)** IL-12/23p40 ($p = 0.007$). **(e)** CCL-4 ($p = 0.011$). **(f)** CXCL-10 ($p = 0.011$). The Y-axis displays a logarithmic scale with the concentrations expressed as pg per mg skin tissue. Horizontal bars display the median. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. HS: hidradenitis suppurativa patients. NN: healthy controls.

cytokines/chemokines in the lesional HS skin in which a correlation was calculated, showed a plasma-skin correlation coefficient of respectively $r = 0.409$ (CCL-4), $r = -0.358$ (CXCL-8), $r = -0.340$ (CXCL-10). None of the correlations was statistically significant ($p > 0.05$). Of note, CCL-2 ($p = 0.016$, unadjusted) and CCL-13 ($p = 0.028$, unadjusted) had no statistically significant plasma-skin correlations after correction for multiple testing. For IL-1ß, TNF-α, IFN-γ, CCL-11, CCL-17 there was a mismatch in the type of variable (continuous, categorical) in plasma and skin. The R-squared values for these five inflammatory markers ranged from 0.004 to 0.096, all statistically nonsignificant.

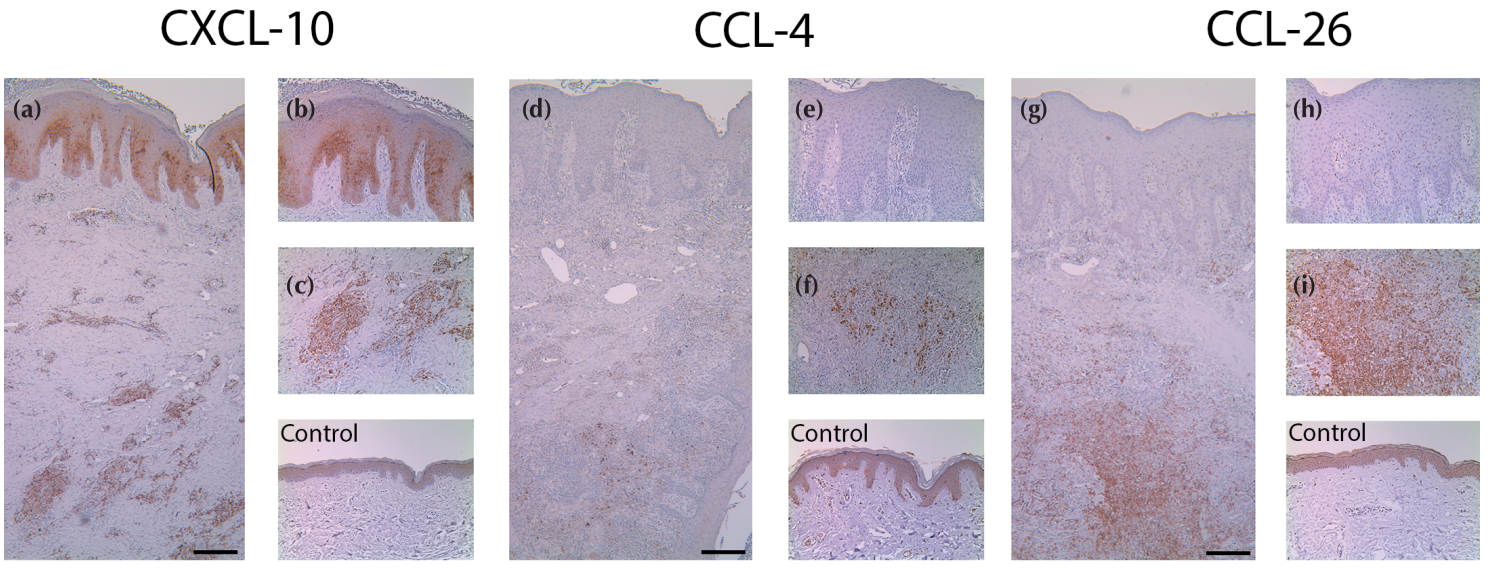

Figure 3. Immunohistochemical detection of CCL-4, CXCL-10 and CCL-26 in HS lesional skin and healthy control skin. CCL-4, CXCL-10 and CCL-26 were not expressed in healthy skin (control panels). CCL-4 **(d, e ,f)** was localised to the inflammatory HS infiltrate, whereas CXCL-10 **(a, b, c)** and CCL-26 **(g, h ,i)** were expressed at a higher intensity. DAB with haematoxylin counterstain. Bar inserted corresponds with 100μM.

Table 3. Correlation between protein expression in plasma and skin of HS patients. None of the inflammatory markers were statistically significant after Benjamini Hochberg correction.

	Protein	Coefficient (r) plasma vs skin	Unadjusted *p*-value
1	**CCL-2 (MCP-1)**	–0.532	0.016
2	**CCL-13 (MCP-4)**	–0.490	0.028
3	**CCL-22 (MDC)**	0.423	0.063
4	**CCL-4 (MIP-1β)**	0.409	0.073
5	**CXCL-8 (IL-8)**	–0.358	0.121
6	**CXCL-10 (IP-10)**	–0.340	0.143
7	**IL-1α**	–0.275	0.240
8	**CCL-3 (MIP-1α)**	–0.254	0.281
9	**IL-12/23p40**	0.198	0.402
10	**TNF-ß**	–0.189	0.317
11	**IL-17A**	–0.126	0.597
12	**IL-6**	–0.101	0.672
13	**IL-16**	–0.098	0.682
14	**IL-10**	–0.090	0.705
15	**IL-15**	–0.078	0.743
16	**IL-13**	–0.053	0.826

r: correlation coefficient (range -1, +1) calculated using the Spearman rho test. Nine markers were not tested for a correlation as these markers were not detected in either skin or plasma: IL-2, IL-4, IL-5, IL-7, IL-12p70, IL-8-HA, CCL-26, VEGF, GM-CSF.

DISCUSSION

In this study we simultaneously measured the protein levels of 30 important inflammatory markers, including Th1 and Th17 cytokines and chemokines, in the plasma and lesional skin of a well-defined cohort of HS patients. In the circulation, CCL-26 was detected significantly more often in HS patients. In lesional skin, the levels of IL-16, IL-17A, IL-23p40, CCL-4, CXCL-8, and CXCL-10 were significantly elevated compared with healthy controls. Remarkably, only weak correlations were found between the protein levels in the plasma and skin of HS patients.

In plasma, the chemokine CCL-26 (also known as eotaxin-3 or MIP-4α) was a newly identified inflammatory marker in HS patients. Significant elevation of this chemokine in the serum has previously been reported in atopic dermatitis, cutaneous T-cell lymphoma and HIV-associated eosinophilic folliculitis, which are characterised by the infiltration of eosinophils, basophils, and specific subpopulations of T cells,[14-16] and all, like HS, diseases characterised by high pruritus scores.[4]

No cytokines were found upregulated in the circulation despite the inclusion of severe cases of HS with 18 of 20 patients with Hurley stage II and III disease severity. Previously, increased serum levels of general inflammatory markers including (high sensitive) CRP, erythrocyte sedimentation rate, neutrophils, monocytes, and IgE have been demonstrated in HS patients.[8,10,17,18] In addition, six studies have reported on specific inflammatory serum markers in HS, of which IL-6, IL-17A, TNF-α, TNF-α receptor I, soluble IL-2 receptor, S100A8, S100A9 and MMP-8 have been found to be upregulated compared with healthy controls.[8,10,19-22]

Our results obtained in the skin confirm previous findings demonstrating overexpression of IL-17 pathway-associated cytokines and chemokines such as IL-17A, IL-23p40 and CXCL-8 in HS.[11,23,24] However, some previously published results, that showed significant upregulation of TNF-α, IL-1ß and IL-10 in (peri)lesional HS skin, could not be confirmed statistically.[24,25] This can be explained by the different approaches as in the current study biopsies were homogenised for in situ assessment, while van der Zee *et al.* and Kelly *et al.* cultured the skin biopsies for 24 and three hours respectively. This step of *ex vivo* culturing of skin samples allows for a prolonged production of cytokines that may lead to higher cytokine levels in the culture media. Interestingly, CCL-26 was found in abundance in the HS infiltrate by IHC, but was not detected in skin homogenates, possibly because CCL-26 is too strongly bound to its receptor on the many eosinophils present in the HS infiltrate.[4]

Upregulation of CXCL-10 in the skin is a remarkable finding, because only limited levels of IFN-γ, despite the significant T cell infiltration, have been detected in lesional HS skin. The interleukin IL-16 and chemokines CCL-4 and CXCL-10 are produced by many cell types including macrophages, dendritic cells, B cells, mast cells, eosinophils and T lymphocytes, and play a crucial role in the induction and modulation of immune responses during infection and inflammation.[26,27] In the context of a strong upregulation of CXCL-8, IL-17A and IL-23, we hypothesise that IL-16, CCL-4 and CXCL-10 may participate in the recruitment of leucocyte subsets, especially neutrophils, eosinophils, monocytes and dendritic cells, into the inflamed lesional HS skin.[28,29] The importance of neutrophils in the pathophysiology of HS is underlined by the increased levels of CXCL-8 that can be cleaved by neutrophil elastase to activate Th17 cells to produce bioactive IL-17.[30] Furthermore, it has been demonstrated that activated neutrophils induce chemotaxis of Th17 cells by a reciprocal cross-talk.[31]

The poor correlation between cutaneous cytokine and chemokine protein levels was reflected by all coefficients except for CCL-2 being below (+/–)0.50, i.e. a low to negligible correlation.[13] Moreover, most of the analytes demonstrated a negative (inverse) correlation between expression in plasma and skin. The overexpression of CXCL-10 in HS lesions might explain the lower plasma levels as CXCL-10 could have been attracted from the circulation into lesional skin and bound by target cells to fuel

local inflammation. A similar observation was reported in a recent study, in which plasma concentrations of complement factor C5a and C5b9 were higher among patients with mild disease than those with moderate or severe HS.[32] Collectively, our findings indicate that enhanced levels of cytokines and chemokines in lesions of HS patients is generally not reflected in the plasma or serum.

Our study has several strengths including the *in parallel* assessment of inflammatory markers in skin and plasma using a sensitive and accurate detection technique. Limitations of this study are the limited sample size, which did not allow for a subgroup analysis by Hurley disease severity, and the use of a predefined panel of 30 cytokines and chemokines, which did not measure all previously reported HS-biomarkers including antimicrobial peptides. Lastly, the cross-sectional study design did not allow for analysis of cytokine- and chemokine modulation by anti-inflammatory therapies.

In conclusion, CCL-26 is a newly identified inflammatory marker that is upregulated in the circulation of HS patients. Besides previously demonstrated overexpression of IL-17A, IL-23p40, CXCL-8 in HS lesions, this study found IL-16, CCL-4, CXCL-10 and CCL-26 as novel and potentially important players in the pathogenesis of HS. The local and systemic upregulation of CCL-26 in HS patients can be linked to the high pruritus score in HS. Furthermore, our results demonstrate that plasma gives a limited reflection of the activated local cutaneous inflammatory milieu.

REFERENCES

1 Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. *JAMA* 2017; **318**: 2019-32.

2 Dufour DN, Emtestam L, Jemec GB. Hidradenitis suppurativa: a common and burdensome, yet under-recognised, inflammatory skin disease. *Postgrad Med J* 2014; **90**: 216-21.

3 Matusiak L, Bieniek A, Szepietowski JC. Hidradenitis suppurativa markedly decreases quality of life and professional activity. *J Am Acad Dermatol* 2010; **62**: 706-8, 8 e1.

4 Vossen A, Schoenmakers A, van Straalen KR *et al.* Assessing Pruritus in Hidradenitis Suppurativa: A Cross-Sectional Study. *Am J Clin Dermatol* 2017; **18**: 687-95.

5 Matusiak L, Szczech J, Kaaz K *et al.* Clinical Characteristics of Pruritus and Pain in Patients with Hidradenitis Suppurativa. *Acta Derm Venereol* 2018; **98**: 191-4.

6 Prens E, Deckers I. Pathophysiology of hidradenitis suppurativa: An update. *J Am Acad Dermatol* 2015; **73**: S8-S11.

7 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

8 Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L *et al.* The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa. *Mediators Inflamm* 2017; **2017**: 2450401.

9 Hessam S, Sand M, Gambichler T *et al.* Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop. *Br J Dermatol* 2018; **178**: 761-7.

10 Matusiak L, Szczech J, Bieniek A *et al.* Increased interleukin (IL)-17 serum levels in patients with hidradenitis suppurativa: Implications for treatment with anti-IL-17 agents. *J Am Acad Dermatol* 2017; **76**: 670-5.

11 van der Zee HH, Laman JD, de Ruiter L *et al.* Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

12 Chowdhury F, Williams A, Johnson P. Validation and comparison of two multiplex technologies, Luminex and Mesoscale Discovery, for human cytokine profiling. *J Immunol Methods* 2009; **340**: 55-64.

13 Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J* 2012; **24**: 69-71.

14 Kagami S, Kakinuma T, Saeki H *et al.* Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis. *Clin Exp Immunol* 2003; **134**: 309-13.

15 Yokobayashi H, Sugaya M, Miyagaki T *et al.* Analysis of serum chemokine levels in patients with HIV-associated eosinophilic folliculitis. *J Eur Acad Dermatol Venereol* 2013; **27**: 212-6.

16 Suga H, Sugaya M, Miyagaki T *et al.* Association of nerve growth factor, chemokine (C-C motif) ligands and immunoglobulin E with pruritus in cutaneous T-cell lymphoma. *Acta Derm Venereol* 2013; **93**: 144-9.

17 Pascual JC, Garcia-Martinez FJ, Martorell A *et al.* Increased total serum IgE levels in moderate-to-severe hidradenitis suppurativa. *Br J Dermatol* 2016; **175**: 1101-2.

18 Miller IM, Ring HC, Prens EP *et al.* Leukocyte Profile in Peripheral Blood and Neutrophil-Lymphocyte Ratio in Hidradenitis Suppurativa: A Comparative Cross-Sectional Study of 462 Cases. *Dermatology* 2016; **232**: 511-9.

19 Matusiak L, Bieniek A, Szepietowski JC. Soluble interleukin-2 receptor serum level is a useful marker of hidradenitis suppurativa clinical staging. *Biomarkers* 2009; **14**: 432-7.

20 Matusiak L, Bieniek A, Szepietowski JC. Increased serum tumour necrosis factor-alpha in hidradenitis suppurativa patients: is there a basis for treatment with anti-tumour necrosis factor-alpha agents? *Acta Derm Venereol* 2009; **89**: 601-3.

21 Wieland CW, Vogl T, Ordelman A *et al.* Myeloid marker S100A8/A9 and lymphocyte marker, soluble interleukin 2 receptor: biomarkers of hidradenitis suppurativa disease activity? *Br J Dermatol* 2013; **168**: 1252-8.

22 Tsaousi A, Witte E, Witte K *et al.* MMP8 Is Increased in Lesions and Blood of Acne Inversa Patients: A Potential Link to Skin Destruction and Metabolic Alterations. *Mediators Inflamm* 2016; **2016**: 4097574.

23 Schlapbach C, Hanni T, Yawalkar N *et al.* Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**: 790-8.

24 Kelly G, Hughes R, McGarry T *et al.* Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. *Br J Dermatol* 2015; **173**: 1431-9.

25 van der Zee HH, de Ruiter L, van den Broecke DG *et al.* Elevated levels of tumour necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-alpha and IL-1beta. *Br J Dermatol* 2011; **164**: 1292-8.

26 Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine Growth Factor Rev* 2002; **13**: 455-81.

27 Lee EY, Lee ZH, Song YW. CXCL10 and autoimmune diseases. *Autoimmun Rev* 2009; **8**: 379-83.

28 Wolpe SD, Davatelis G, Sherry B *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J Exp Med* 1988; **167**: 570-81.

29 Tecchio C, Cassatella MA. Neutrophil-derived chemokines on the road to immunity. *Semin Immunol* 2016; **28**: 119-28.

30 Souwer Y, Groot Kormelink T, Taanman-Kueter EW *et al.* Human TH17 cell development requires processing of dendritic cell-derived CXCL8 by neutrophil elastase. *J Allergy Clin Immunol* 2018; **141**: 2286-9 e5.

31 Pelletier M, Maggi L, Micheletti A *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010; **115**: 335-43.

32 Kanni T, Zenker O, Habel M *et al.* Complement activation in hidradenitis suppurativa: a new pathway of pathogenesis? *Br J Dermatol* 2018; **179**: 413-9.

Chapter 4

The anti-inflammatory potency of biologics targeting TNF-α, IL-17A, IL-12/23 and CD20 in hidradenitis suppurativa: an ex vivo study

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Submitted

ABSTRACT

Background

Biologics targeting inflammatory mediators are able to clinically improve hidradenitis suppurativa (HS). However, their clinical efficacy shows great inter-patient variability in daily practice.

Objective

To investigate the anti-inflammatory potency of currently available biologics for the treatment of HS in an *ex vivo* skin culture system using lesional HS biopsies.

Methods

Lesional skin samples of ten HS patients and normal skin samples of five healthy controls were cultured *ex vivo* and exposed to prednisolone or biologics targeting TNF-α, IL-17A, IL-12/23p40, or CD20, respectively adalimumab, infliximab, secukinumab, ustekinumab, and rituximab. Real-time quantitative PCR and cytokine bead arrays were used to measure the inhibitory effect of the biologics on cytokines and antimicrobial peptides (AMPs).

Results

The relative mRNA expression of all tested cytokines and AMPs was significantly downregulated by all anti-inflammatory agents ($p < 0.0001$). The release of the pro-inflammatory cytokines TNF-α, IFN-γ, IL-1ß, IL-6, and IL-17A was significantly inhibited by adalimumab, infliximab, ustekinumab, prednisolone (all $p < 0.0001$) and rituximab ($p = 0.0071$) but not by secukinumab ($p = 0.0663$). In addition, adalimumab, infliximab, and prednisolone reduced the levels of a broader mix of individual cytokines than secukinumab, ustekinumab, and rituximab.

Conclusion

This *ex vivo* study suggests that TNF-α inhibitors and prednisolone are the most powerful inhibitors of pro-inflammatory cytokines and AMPs in HS lesional skin, which is in accordance with our clinical experience in patients with HS.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic skin disease characterised by painful, deep-seated, inflamed nodules, abscesses, and in later stages sinus tracts.[1] Occlusion of the hair follicle with subsequent rupture, followed by a fierce local inflammatory response, are considered primary pathogenic events. The aberrant innate immune response is characterised by the upregulation of various pro-inflammatory cytokines and antimicrobial peptides (AMPs).[2]

Several studies have found elevated mRNA and protein levels of tumour necrosis factor (TNF)-α in lesional and perilesional HS skin.[3,4] Targeting TNF-α with adalimumab and infliximab has been shown to be clinically efficacious in HS in randomised placebo-controlled trials.[5-7] In addition, upregulation of interleukin (IL)-1ß, IL-17A, and IL-23 in HS lesions point to the importance of the IL-17 pathway in the pathophysiology of HS.[8-10] However, targeting IL-1 in HS gave ambiguous clinical outcomes. In a randomised controlled trial anakinra proved to be efficacious in moderate-to-severe HS, yet cases of failure on anakinra therapy have been reported.[11-13]

Most recently IL-17 has been targeted in two clinical trials in HS (NCT02421172, NCT03248531), but the results have not yet been released. Treatment with secukinumab or ustekinumab induced amelioration of HS in a few individual cases and case series.[14-16] In addition, enhanced levels of AMPs in lesional HS skin have been described for S100A7 (psoriasin), S100A8, S100A9, human ß-defensin (HBD)-2, HBD-3 and LL-37.[17-19] Upregulation of these AMPs in HS lesional skin is mainly driven by IL-17.[18]

Chronic HS lesions are characterised by a marked increase in the number of CD20+, CD79A+ B cells, and CD138+ plasma cells.[20] One case report described a clear improvement in HS after treatment with rituximab for concomitant idiopathic carpotarsal osteolysis syndrome.[21] This highlight the contribution of these B and plasma cells to the inflammatory process in HS.

Although biologics targeting inflammatory mediators are now widely used for the treatment of HS, their clinical efficacy shows great inter-patient variability. Our *ex vivo* skin culture system is a fast and simple method to simultaneously investigate and compare the effect of biologics in fresh human lesional skin samples.[22] It approaches the *in vivo* situation by maintaining the patients' skin architecture and allows close monitoring of the events following response to immunostimulators or suppressors in the same experiment.[23] Therefore, this study sought to investigate the anti-inflammatory potency of currently available biologics used for the treatment of HS in an *ex vivo* skin culture system using lesional HS biopsies.

MATERIALS AND METHODS

Ethics and informed consent

HS lesional skin was collected from excised skin after HS surgery conducted in the department of Dermatology of the Erasmus University Medical Center in Rotterdam, The Netherlands from October 2017 through February 2018. According to the opt-out principle used in the Erasmus University Medical Center no informed consent is required for the use of excised tissue for research purposes as this is considered waste material. Control skin samples were obtained from healthy individuals in the Sint Franciscus Hospital in Rotterdam, The Netherlands. All healthy volunteers provided written informed consent for the use of their excised skin in this study.

Hidradenitis suppurativa patients

Ten HS patients, five males and five females, with chronic, active disease (seven Hurley stage II, three Hurley stage III) requiring surgical excision under general or sedative anaesthesia were included. HS lesional skin samples were obtained from the inguinogenital area in 5 of these 10 patients, axillae in 4, and from the gluteal area in 1 patient. The mean (± SD) age was 43.7 ± 7.4 years, the mean body mass index was 30.3 ± 6.5 kg/m^2, and 7 of 10 patients were current smokers. Eight patients received a stable (≥ 28 days) dose of systemic antibiotics for their HS at the time of surgery. None of the patients had been using immunosuppressive or immunomodulatory therapies (e.g. biologics) for at least two months prior to surgery.

Healthy control skin

Healthy skin samples were obtained from the submammary and abdominal waste material of five women who underwent breast or abdominal reduction surgery. We argue that skin from these regions is suitable as a control samples because the inframammary and abdominal folds are predilection sites for HS. The control subjects were otherwise healthy and had no family history of HS, and were not using any immunosuppressive or immunomodulatory treatment at the time of surgery.

Biopsy procedure

A total of seven fresh 4-mm punch biopsies per subject were taken. HS lesional skin biopsies were obtained from the same palpable infiltrate, and at least 1 cm away from the excision border. Abscesses were not biopsied to avoid sampling only the roof of the abscess. Each biopsy was weighed prior to culture as described in an earlier publication.[4] HS biopsies had a mean weight of 32.4 ± 6.6 mg, which was significantly heavier than biopsies from healthy skin with a weight of mean 16.1 ± 3.0 mg ($p < 0.001$). This potential confounder was addressed by normalising all protein levels for the mg tissue.

Ex vivo skin culture

The 4-mm biopsies were immediately cultured in a transwell system (Netwell; Costar, Cambridge, MA) as described previously.[22,23] In brief, samples were placed in punched-out holes in the transwell membrane of a 12-well plate with the epidermis exposed to the air and the dermis immersed in 1 mL Iscove's modified Dulbecco's medium (Gibco, Paisley, U.K.) containing 0.5% human AB serum, penicillin (100 U/mL) and streptomycin (100 U/mL) with or without an anti-inflammatory agent. Biologics and prednisolone were separately added to the culture media resulting in the following seven conditions: (i) culture media as negative control; (ii) prednisolone 100 µg/ml as positive control; (iii) adalimumab 30 µg/ml; (iv) infliximab 20 µg/ml; (v) secukinumab 30 µg/ml; (vi) ustekinumab 10 µg/ml; (vii) rituximab 200 µg/ml. The concentrations of the monoclonal antibodies were a multiple of the reported trough levels in patients with plaque psoriasis (adalimumab[24,25], infliximab[25], ustekinumab[26], secukinumab[27]), and CD20+ B cell malignancies (rituximab[28]). Skin biopsies were incubated for 24h at 37°C in an atmosphere of 5% CO_2 and 98% humidity. Subsequently, the biopsies were placed in 250 µL lysis buffer containing 1% ß-mercaptoethanol. Both the supernatants and the biopsies were transferred to a polypropylene tube and stored at –20°C until further analysis.

Gene expression analysis

Total mRNA was extracted using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MA). RNA was treated with 0.1 U/µL DNAse (Invitrogen) and cDNA was subsequently synthesised using 1 µg total RNA template, with SuperScript II reverse transcriptase, random hexamer primers (Invitrogen) and oligo(dT)15 (Promega). Primers and probes were designed and chosen using ProbeFinder Sofware and the Universal Probe library (Roche Applied Science, Indianapolis, IN). *ABL1* was chosen as a reference housekeeping gene.[29] Real-time quantitative PCR (qPCR) was performed for 12 genes using the ViiA7 sequence-detection system (Applied Biosystems): *TNF-α*, *IL-1ß*, *IL-6*, *CXCL-8/IL-8*, *IL-12p19*, *IL-17A*, *IFN-α/MxA*, *S100A7*, *S100A8*, *S100A9*, *LL37*, and *HBD-2*. Gene expression was analysed with QuantStudio Real-Time quantitative PCR Software version 1.3 (Applied Biosystems, Waltham, MA).

Protein quantification

Eighteen inflammation-related cytokines were simultaneously measured in the supernatant using a customised bead-based Multi-Analyte Profiling assay (Luminex, R&D systems, Minneapolis, MN): CCL-20, TNF-α, IFN-γ, IL-1ß, IL-1R1, IL-5, IL-6, IL-10, IL-12/23p40, IL-17A, IL-17E, IL-18, IL-19, IL-22, IL-27, IL-31, IL-33, and IL-36ß. Assays were used according to the manufacturers' protocol. A dilution factor of 2 was used for all supernatants.

Statistical analyses

The relative mRNA expression and protein production per sample compared with culture media (negative control) was calculated for every condition. Protein levels were normalised according to input weight and expressed in picogram (pg)/mL/mg tissue weight. The lower limit of quantification was imputed when protein concentrations in the supernatant (pg/mL) were below limit of detection. The Kruskal-Wallis test, i.e. a one-way ANOVA on ranks, was used to compare the variance in mRNA and protein levels per inflammatory marker. If another condition stochastically dominated one other condition, the Dunnett's post-test was subsequently used to separately test conditions versus culture media (pairwise comparisons). We chose this approach in order to increase power. A two-sided *p*-value lower than 0.05 was considered significant. The level of significance for the relative mRNA expression and protein production was separately adjusted by the Benjamini Hochberg procedure for multiple comparisons. A correlation between relative mRNA and protein levels per cytokine was calculated using the Spearman rho test for non-normally distributed continuous variables. GraphPad Prism version 6 (GraphPad Software, La Jolla, CA) was used for all statistical analyses.

RESULTS

Sample flow for analysis

In total, samples of ten HS patients and five healthy controls were analysed by real-time qPCR. Supernatants of one HS patient were excluded from protein analysis because of erroneous sample processing, resulting in samples of nine HS patients with protein data. For control skin, supernatants of three healthy volunteers were analysed by Luminex assay. However, protein levels were below level of detection or in the very low range of the calibration line in these control samples (data not shown). Therefore, samples of the other two patients were not analysed.

Significant downregulation of gene expression of cytokines and AMPs by prednisolone and different biologics

The relative changes in mRNA expression of the 12 genes in HS samples including significance levels are shown in Table 1. The overall median inhibitory effect on the mix of cytokines and AMPs per condition was: prednisolone 0.57 (interquartile range [IQR] 0.34-1.03); adalimumab 0.51 (0.33-0.92); infliximab 0.53 (0.32-0.78); secukinumab 0.56 (0.29-0.99); ustekinumab 0.64 (0.44-1.00), rituximab 0.73 (0.50-1.19). Remarkably, the inhibitory impact on AMPs was stronger for the biologics than for prednisolone (Table 1, Figure 1). Prednisolone, adalimumab and infliximab

Table 1. Relative mRNA expression and their modulation by biologics in HS lesional skin.

	HS lesional skin (n = 10)											
	Prednisolone		Adalimumab		Infliximab		Secukinumab[1]		Ustekinumab		Rituximab	
	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value
IL-1β	0.27 (0.15-0.48)	**<0.0001***	0.53 (0.23-0.74)	**0.0073***	0.60 (0.50-0.73)	**0.0306**	0.67 (0.53-0.87)	0.2377	0.76 (0.39-1.06)	0.6194	0.49 (0.35-0.72)	0.0597
IL-6	0.42 (0.36-0.51)	**<0.0001***	0.62 (0.52-0.89)	0.0956	0.65 (0.41-0.71)	**0.0047***	0.70 (0.44-0.90)	0.1063	0.55 (0.37-0.90)	**0.0122***	0.75 (0.55-0.97)	0.4396
CXCL-8 (IL-8)	0.44 (0.34-0.60)	0.1675	0.30 (0.22-0.49)	**0.0042***	0.29 (0.26-0.38)	**0.0002***	0.28 (0.16-0.47)	**0.0015***	0.39 (0.32-0.48)	**0.0296**	0.69 (0.41-0.86)	0.9779
IL-17A	0.13 (0.07-0.37)	**0.0049***	0.93 (0.59-1.37)	1.0000	0.55 (0.43-0.84)	1.0000	0.95 (0.62-1.42)	1.0000	0.96 (0.56-1.29)	1.0000	0.99 (0.43-1.61)	1.0000
IL-23p19	0.92 (0.33-1.20)	1.0000	0.48 (0.34-0.66)	0.3934	0.49 (0.31-0.88)	0.4901	0.48 (0.14-1.01)	0.3836	0.72 (0.46-1.79)	1.0000	0.97 (0.51-1.99)	1.0000
TNF-α	1.09 (0.64-1.58)	1.0000	0.92 (0.52-1.02)	1.0000	0.74 (0.37-1.03)	0.7250	0.53 (0.28-1.15)	0.5127	0.83 (0.53-2.56)	1.0000	0.71 (0.60-1.11)	1.0000
IFN-MXA	0.75 (0.60-1.23)	1.0000	0.56 (0.35-1.08)	0.7914	0.84 (0.59-1.19)	1.0000	0.67 (0.43-0.81)	0.3345	0.83 (0.55-1.17)	1.0000	0.91 (0.57-1.17)	1.0000
S100A7	0.80 (0.51-1.02)	0.3934	0.56 (0.50-0.70)	**0.0266**	0.56 (0.45-0.72)	**0.0116***	0.50 (0.34-0.88)	**0.0105***	0.64 (0.54-0.68)	**0.0223**	0.56 (0.46-0.74)	**0.0180**
S100A8	0.74 (0.55-1.11)	1.0000	0.50 (0.39-0.54)	**0.0060***	0.52 (0.44-0.60)	**0.0248**	0.67 (0.52-0.73)	0.2826	0.79 (0.59-1.12)	1.0000	0.69 (0.56-0.93)	0.7091
S100A9	0.80 (0.51-1.07)	0.8442	0.35 (0.30-0.53)	**0.0005***	0.29 (0.27-0.42)	**<0.0001***	0.36 (0.25-0.68)	**0.0010***	0.52 (0.42-0.78)	**0.0296**	0.66 (0.52-0.93)	0.9779
LL-37	0.67 (0.14-1.28)	0.8997	0.40 (0.37-0.83)	0.5915	0.64 (0.38-1.10)	1.0000	0.51 (0.32-1.84)	1.0000	0.77 (0.48-1.37)	1.0000	0.89 (0.67-1.22)	1.0000
HBD-2	0.48 (0.19-0.87)	**0.0416**	0.43 (0.29-0.65)	**0.0306**	0.32 (0.20-0.91)	**0.0445**	0.69 (0.13-1.07)	0.1763	0.49 (0.43-0.76	0.1538	0.76 (0.68-1.13)	1.0000
All (12)	0.57 (0.34-1.03)	**<0.0001**	0.51 (0.33-0.92)	**<0.0001**	0.53 (0.32-0.78)	**<0.0001**	0.56 (0.29-0.99)	**<0.0001**	0.64 (0.44-1.00)	**<0.0001**	0.73 (0.50-1.19)	**<0.0001**
Cytokines[2] (7)	0.41 (0.20-0.77)	**<0.0001**	0.52 (0.32-0.95)	**<0.0001**	0.54 (0.34-0.75)	**<0.0001**	0.60 (0.37-0.98)	**<0.0001**	0.61 (0.39-1.08)	**0.0002**	0.76 (0.44-1.21)	0.4780
AMPs[3] (5)	0.75 (0.44-1.11)	**<0.0001**	0.48 (0.33-0.68)	**<0.0001**	0.47 (0.29-0.73)	**<0.0001**	0.52 (0.30-0.96)	**<0.0001**	0.64 (0.45-0.89)	**<0.0001**	0.73 (0.53-0.98)	**0.0001**

p-values marked in bold indicate analytes that are significant at the 0.05 level. * Significant after correction with the Benjamini Hochberg test ($p < 0.0156$). [1] One HS sample for secukinumab was excluded for Real-Time qPCR analysis as a result of a human error during the process of cDNA synthesis (n = 9). [2] Cytokines: pooled effect of IL-1ß, IL-6, IL-8, IL-17A, IL-23p19, TNF-α, IFN-MXA. [3] AMPs: pooled effect of S100A7, S100A8, S100A9, LL-37, HBD-2. AMPs: antimicrobial peptides. HS: hidradenitis suppurativa. IQR: interquartile range. NT: not tested. Unadj: unadjusted.

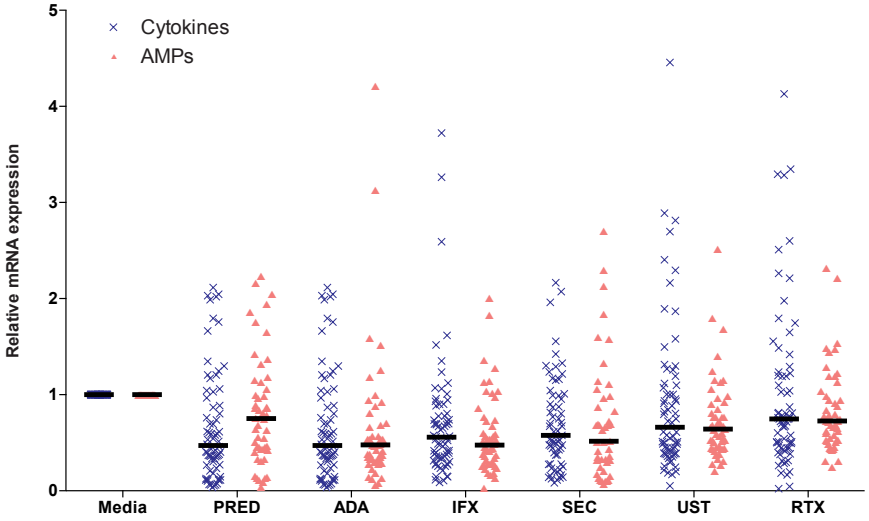


Figure 1. Anti-inflammatory impact on the relative mRNA expression of the mix of cytokines and AMPs in HS lesional skin, n = 10. All agents displayed significant inhibition of both cytokine and AMP mRNA expression except for the effect of RTX on cytokine mRNA expression. One data point is out of the y-axis range. Horizontal bars display the median. Media, culture media; PRED, prednisolone 100 µg/mL; ADA, adalimumab 30 µg/mL; IFX, infliximab 20 µg/mL; SEC, secukinumab 30 µg/mL; UST, ustekinumab 10 µg/mL; RTX, rituximab 200 µg/mL.

significantly inhibited *IL-1*β. Expression of *CXCL-8/IL-8* was significantly downregulated by adalimumab, infliximab, secukinumab, and ustekinumab. Messenger RNA levels of two other important pro-inflammatory cytokines, *TNF*-α and *IL-23p19*, were not significantly downregulated by any of the biologics or prednisolone. Gene expression of members of the *S100* family and *HBD-2* was significantly reduced by adalimumab, infliximab, secukinumab and ustekinumab (Table 1). In healthy control skin, only prednisolone, adalimumab and infliximab significantly downregulated mRNA expression of the mix of tested cytokines and AMPs (Supplementary Table 1, Supplementary Figure 1).

Significant *ex vivo* reduction in TNF-α, IFN-γ, IL-1ß, IL-6, and IL-17A protein levels of in HS lesional skin

In total, 18 cytokines were measured. Six cytokines were not detected in the majority of HS samples throughout all conditions: IL-1RI, IL-5, IL-12/23p40, IL-22, IL-31, and IL-33. Relative changes in protein production including significance levels for the other 12 cytokines are shown in Table 2. The overall median (IQR) effect on cytokines per condition was: prednisolone 0.51 (0.28-0.64); adalimumab 0.78 (0.54-0.91);

Table 2. Relative protein production and their modulation by biologics in HS lesional skin.

	HS lesional skin (n = 9)											
	Prednisolone		Adalimumab		Infliximab		Secukinumab		Ustekinumab		Rituximab	
	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value
TNF-α	0.20 (0.15-0.29)	**0.0022***	0.09 (0.07-0.20)	**<0.0001***	0.03 (0.02-0.05)	**<0.0001***	0.89 (0.53-0.93)	1.0000	0.54 (0.23-0.76)	0.3305	0.70 (0.65-1.10)	1.0000
IFN-γ	0.37 (0.11-0.53)	**0.0068***	0.43 (0.30-1.17)	0.4169	0.46 (0.13-0.91)	0.0673	0.56 (0.51-0.66)	0.6285	0.39 (0.33-0.61)	0.0673	0.49 (0.41-0.60)	0.2596
CCL-20	0.50 (0.20-0.81)	**0.2845**	0.58 (0.28-1.80)	1.0000	0.71 (0.53-1.13)	1.0000	0.94 (0.53-1.19)	1.0000	0.73 (0.62-1.28)	1.0000	0.95 (0.70-1.38)	1.0000
IL-1ß	0.23 (0.17-0.45)	**0.0049***	0.41 (0.29-0.73)	0.3114	0.44 (0.31-0.75)	0.3305	1.08 (0.46-1.43)	1.0000	0.59 (0.45-2.06)	1.0000	0.70 (0.52-1.03)	1.0000
IL-6	0.59 (0.46-0.73)	**0.0006***	0.81 (0.68-0.85)	0.0724	0.76 (0.64-0.91)	0.1035	0.99 (0.76-1.08)	1.0000	0.76 (0.69-0.99)	0.1556	0.87 (0.71-0.92)	0.1035
IL-17A	0.09 (0.07-0.19)	**<0.0001***	0.83 (0.68-1.38)	1.0000	0.50 (0.32-0.52)	0.2085	0.70 (0.60-1.60)	1.0000	0.54 (0.25-0.81)	0.3022	0.96 (0.34-1.24)	1.0000
IL-18	0.86 (0.59-1.03)	1.0000	0.75 (0.66-3.48)	1.0000	1.05 (0.74-1.11)	1.0000	0.90 (0.71-1.04)	1.0000	0.82 (0.78-0.90)	0.7341	0.93 (0.78-2.75)	1.0000
IL-36ß	0.94 (0.48-1.45	1.0000	0.98 (0.81-2.04)	1.0000	1.04 (0.82-1.47)	1.0000	1.88 (0.94-2.34)	1.0000	1.05 (0.78-1.37)	1.0000	1.21 (0.85-1.65)	1.0000
IL-10[1]	0.29 (0.20-0.41)	**0.0062***	1.12 (0.22-1.94)	1.0000	0.93 (0.37-1.05)	1.0000	0.81 (0.62-2.02)	1.0000	0.91 (0.55-1.42)	1.0000	0.62 (0.43-0.91)	0.6975
IL-25/17E[1]	0.52 (0.33-0.64)	**0.0043***	0.59 (0.48-0.95)	0.2517	0.50 (0.44-1.01)	0.0931	0.68 (0.55-0.88)	0.3208	0.67 (0.55-0.90)	0.2932	0.74 (0.52-0.90)	0.2440
IL-19[1]	0.68 (0.64-0.99)	0.9862	0.91 (0.51-1.67)	1.0000	0.69 (0.56-1.31)	1.0000	0.79 (0.48-0.92)	1.0000	0.77 (0.65-1.12)	1.0000	0.92 (0.51-1.52)	1.0000
IL-27[1]	0.63 (0.26-0.85)	0.0751	0.92 (0.72-1.01)	1.0000	0.95 (0.61-1.12)	1.0000	0.66 (0.64-1.02)	0.963	0.69 (0.62-0.83)	0.3609	0.72 (0.63-0.79)	0.2365
All[2] **(12)**	0.51 (0.28-0.64)	**<0.0001**	0.78 (0.54-0.91)	**0.0006**	0.70 (0.49-0.94)	**<0.0001**	0.85 (0.69-0.95)	0.0684	0.71 (0.57-0.79)	**0.0010**	0.81 (0.70-0.94)	**0.0121**
Pro-inflammatory (8)	0.45 (0.19-0.82)	NT	0.69 (0.29-1.18)	NT	0.64 (0.30-1.04)	NT	0.91 (0.53-1.43)	NT	0.71 (0.42-0.99)	NT	0.80 (0.58-1.22)	NT
Anti-inflammatory (4)	0.54 (0.29-0.70)	NT	0.87 (0.51-1.42)	NT	0.70 (0.46-1.11)	NT	0.69 (0.54-1.13)	NT	0.75 (0.61-1.05)	NT	0.74 (0.50-0.98)	NT
Important[3] **(5)**	0.23 (0.20-0.37)	**<0.0001**	0.43 (0.41-0.81)	**<0.0001**	0.46 (0.44-0.50)	**<0.0001**	0.89 (0.70-0.99)	0.0663	0.54 (0.54-0.59)	**<0.0001**	0.70 (0.70-0.87)	**0.0071**

p-values marked in bold indicate analytes that are significant at the 0.05 level. * Significant after correction with the Benjamini Hochberg test ($p < 0.0156$). [1] Cytokines with an anti-inflammatory function, note that IL-17E and IL-27 also have an immunoregulatory/pro-inflammatory function. [2] All: pooled effect of 12 cytokines. [3] Important: 5 key pro-inflammatory cytokines in the pathophysiology of HS; TNF-α ,IFN-γ, IL-1ß, IL-6, IL-17A. AMPs: antimicrobial peptides. HS: hidradenitis suppurativa. IQR: interquartile range. NT: not tested.Unadj: unadjusted.

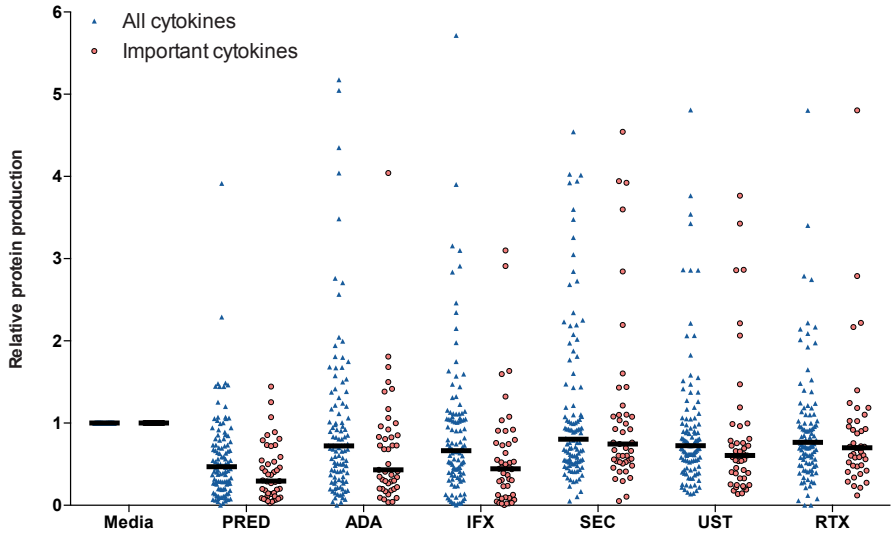


Figure 2. Anti-inflammatory impact on protein production of all cytokines and five important cytokines as measured by Luminex, n = 9. Important inflammatory cytokines: TNF-α, IFN-γ, IL-1ß, IL-6, IL-17A. Twelve data points are out of the y-axis range. Horizontal bars display the median. Media, culture media; PRED, prednisolone 100 µg/mL; ADA, adalimumab 30 µg/mL; IFX, infliximab 20 µg/mL; SEC, secukinumab 30 µg/mL; UST, ustekinumab 10 µg/mL; RTX, rituximab 200 µg/mL.

infliximab 0.70 (0.49-0.94); secukinumab 0.85 (0.69-0.95); ustekinumab 0.71 (0.57-0.79); rituximab 0.81 (0.70-0.94). Specifically the release of TNF-α, IFN-γ, IL-1ß, IL-6, and IL-17A was significantly inhibited by all tested drugs with the exception of secukinumab (Figure 2). In addition, prednisolone significantly inhibited the release of TNF-α, IFN-γ, IL-1ß, IL-6, IL-17A, IL-10, and IL-25/17E. As expected adalimumab and infliximab almost completely neutralised TNF-α levels in the supernatants (Figure 3). Of note, for the two important cytokines TNF-α and IL-17A we observed both a strong intra- and inter-patient variability among all biologic conditions (Figure 3).

Great variation in correlation between mRNA expression and protein production levels in HS samples

The cytokines IL-1ß, IL-6, IL-17A, and TNF-α, were measured by both real-time qPCR and Luminex. There was great variation in the correlation between mRNA and protein levels of these individual cytokines (Figure 4). A high correlation between mRNA and protein levels was found for IL-17A ($r = 0.86$; $p < 0.0001$), while the correlation for TNF-α was almost zero ($r = 0.04$; $p = 0.7480$).

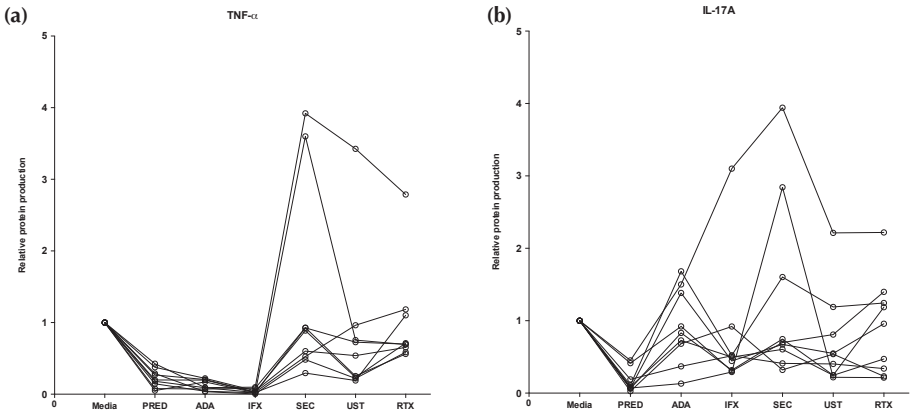


Figure 3. Inter- and intra-patient variability demonstrated by fluctuating protein levels of TNF-α and IL-17A, n = 9. **(a)** TNF-α. Connected dots represent one patient. High intra-patient variability as demonstrated by the fluctuating per patient protein levels between conditions. Low inter-patient variability for conditions PRED, ADA, IFX. **(b)** IL-17A. Connected dots represent one patient. Very high (more than TNF-α) intra-patient variability. Low inter-patient variability for conditions PRED. Media, culture media; PRED, prednisolone 100 µg/mL; ADA, adalimumab 30 µg/mL; IFX, infliximab 20 µg/mL; SEC, secukinumab 30 µg/mL; UST, ustekinumab 10 µg/mL; RTX, rituximab 200 µg/mL.

DISCUSSION

In this study we show that the commercially available biologics used in daily practice for the treatment of HS (adalimumab, infliximab, ustekinumab and rituximab), significantly inhibited mRNA and protein expression of various cytokines and AMPs in lesional HS skin, cultured *ex vivo* for 24 hours. Moreover, the anti-inflammatory effect of prednisolone and all biologics, except secukinumab, is the strongest on TNF-α, IFN-γ, IL-1ß, IL-6, and IL-17A, which are important cytokines in the pathogenesis of HS. In addition, prednisolone and TNF-α inhibitors seem the most effective in reducing the release of a broader range of pro-inflammatory cytokines and AMPs in lesional HS skin.

The strongest evidence in patients with moderate-to-severe HS has been documented for anti-TNF agents adalimumab and infliximab.[5,30-32] We confirmed the previously reported decrease of *ex vivo* IL-1ß protein levels after adalimumab treatment on the mRNA level.[7] As TNF-α is a multifunctional cytokine with numerous actions, a simultaneous downregulation of other cytokines, such as IL-6 and cytokines of the IL-1 family, could also explain the results of the anti-TNF agents in our study.[9,33] Our translational findings correspond with the observed efficacy of TNF-α inhibitors in HS daily practice. Inter-patient variability in the response to biologics could explain why some HS patients are clinically good responders while others show a lesser response.

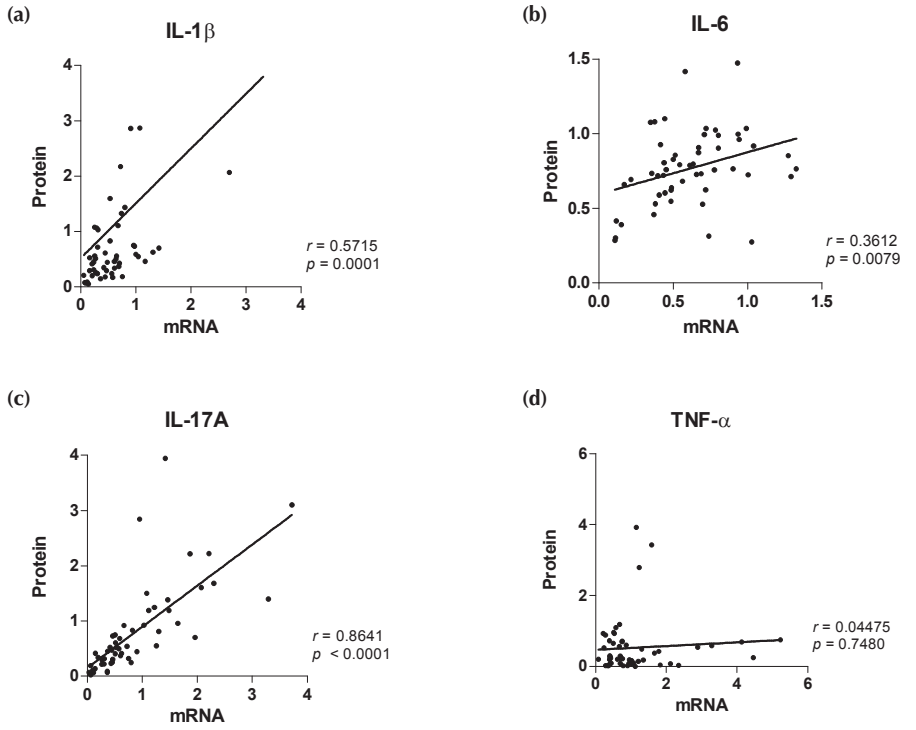


Figure 4. Correlation between the relative protein production (y-axis) and mRNA (x-axis) expression of HS lesional skin, n = 9. **(a)** IL-1ß. **(b)** IL-6. **(c)** IL-17A. **(d)** TNF-α. Values on x-axis and y-axis display the relative production and expression values of a cytokine. Abbreviations: r, correlation coefficient; *p*, *p*-value according to Spearman rho test.

Remarkably, secukinumab did not reduce the IL-17 protein levels in the same way as adalimumab and infliximab reduced that of TNF-α. Unfortunately, IL-12p40 protein, an important indicator of the IL-17 pathway, fell below the level of detection in the Luminex assay. It could be that other cytokines such as TNF-α and IL-1ß, not being blocked by secukimumab in this culture system were still able to induce production of IL-17. Moreover, levels of IL-17A were in the lower range of detection in most patients. Another explanation may be that the anti-IL-17A antibodies used in the Luminex assay detect a different epitope of IL-17A than recognised by secukinumab. The lower mRNA expression of AMPs, *IL-6* and *CXCL-8* may be considered as the result of blocking of IL-17A bioactivity by secukinumab.

The pan-cytokine inhibitory characteristics of prednisolone were demonstrated by inhibition of multiple cytokines on both the mRNA and protein level, which supports the efficacy of systemic and intralesional corticosteroids for acute HS flares in clinical practice.[34] However, prolonged high-dose systemic corticosteroids are not

recommended as HS rapidly flares after tapering, especially after a long course.[35] Nonetheless, low-dose systemic prednisolone could be a valuable adjunct therapy for recalcitrant HS.[36]

Rituximab was the only biologic without a significant inhibitory effect on individual inflammatory mRNA and protein levels. This is not surprising as B cell blockade in inflammatory diseases acts via inhibition of antibody production, antigen presentation and indirectly via cytokine reduction.[37] Therefore, the presence of the complete immune system is required for B cell blockade to be effective. Moreover, HS is considered primarily a disease of a deficient innate immunity. On the other hand, chronic HS lesions are full of B cells and plasma cells, indicating that adaptive humoral immunity is also activated in longstanding HS.[20,38]

The impact of biologics on AMP expression has never been investigated in lesional HS skin. Our findings indicate a potential supporting role for AMPs in HS pathophysiology as it is known that AMPs are capable of activating keratinocytes and attracting innate immune cells to amplify the local immune response.[39] Cytokines produced by innate and adaptive immune cells, such as TNF-α, IL-17, and IL-12, drive AMP production in the keratinocytes.[40-42] Moreover, AMPs can be activated by damage- and pathogen-associated molecular patterns (DAMPs/PAMPs) after follicle rupture with the release of keratin fibres and skin commensals in the dermis.

Although it is assumed that levels of mRNA and protein have a one-to-one correlation, the absence of correlation between TNF-α mRNA and its protein levels has been previously reported.[43-45] In addition, other factors such as the half-life of proteins and the degradation and stability of mRNA may vary widely.[43,46,47]

Major strengths of our study are the use of a standardised *ex vivo* transwell culture system and weighing each biopsy in order to normalise all protein levels for mg tissue weight. Cytokines were evaluated on both mRNA and protein level, including four cytokines that were assessed for validation purposes. Furthermore, the use of healthy control skin from regions that are suitable to function as control samples further increases the validity of our study. Possible limitations include the relatively small sample size, lack of dose-response relationships, and AMPs which have only been evaluated on the mRNA level.

In conclusion, prednisolone, adalimumab, infliximab, ustekinumab and rituximab significantly inhibited the expression levels of a selected panel of inflammatory cytokines and AMPs in *ex vivo* lesional skin of HS patients. The *ex vivo* model will enable studies with combinations of biologics, or targeting of novel important cytokines, alone or in combination with low-dose prednisolone.

REFERENCES

1 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

2 Kelly G, Sweeney CM, Tobin AM *et al.* Hidradenitis suppurativa: the role of immune dysregulation. *Int J Dermatol* 2014; **53**: 1186-96.

3 Mozeika E, Pilmane M, Nurnberg BM *et al.* Tumour necrosis factor-alpha and matrix metalloproteinase-2 are expressed strongly in hidradenitis suppurativa. *Acta Derm Venereol* 2013; **93**: 301-4.

4 van der Zee HH, de Ruiter L, van den Broecke DG *et al.* Elevated levels of tumour necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-alpha and IL-1beta. *Br J Dermatol* 2011; **164**: 1292-8.

5 Kimball AB, Okun MM, Williams DA *et al.* Two Phase 3 Trials of Adalimumab for Hidradenitis Suppurativa. *N Engl J Med* 2016; **375**: 422-34.

6 Grant A, Gonzalez T, Montgomery MO *et al.* Infliximab therapy for patients with moderate to severe hidradenitis suppurativa: a randomized, double-blind, placebo-controlled crossover trial. *J Am Acad Dermatol* 2010; **62**: 205-17.

7 van der Zee HH, Laman JD, de Ruiter L *et al.* Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

8 von Laffert M, Helmbold P, Wohlrab J *et al.* Hidradenitis suppurativa (acne inversa): early inflammatory events at terminal follicles and at interfollicular epidermis. *Exp Dermatol* 2010; **19**: 533-7.

9 Moran B, Sweeney CM, Hughes R *et al.* Hidradenitis Suppurativa Is Characterized by Dysregulation of the Th17:Treg Cell Axis, Which Is Corrected by Anti-TNF Therapy. *J Invest Dermatol* 2017; **137**: 2389-95.

10 Schlapbach C, Hanni T, Yawalkar N *et al.* Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**: 790-8.

11 Menis D, Maronas-Jimenez L, Delgado-Marquez AM *et al.* Two cases of severe hidradenitis suppurativa with failure of anakinra therapy. *Br J Dermatol* 2015; **172**: 810-1.

12 Russo V, Alikhan A. Failure of Anakinra in a Case of Severe Hidradenitis Suppurativa. *J Drugs Dermatol* 2016; **15**: 772-4.

13 Tzanetakou V, Kanni T, Giatrakou S *et al.* Safety and Efficacy of Anakinra in Severe Hidradenitis Suppurativa: A Randomized Clinical Trial. *JAMA Dermatol* 2016; **152**: 52-9.

14 Thorlacius L, Theut Riis P, Jemec GBE. Severe hidradenitis suppurativa responding to treatment with secukinumab: a case report. *Br J Dermatol* 2017; 179: 182-185.

15 Schuch A, Fischer T, Boehner A *et al.* Successful Treatment of Severe Recalcitrant Hidradenitis Suppurativa with the Interleukin-17A Antibody Secukinumab. *Acta Derm Venereol* 2018; **98**: 151-152.

16 Blok JL, Li K, Brodmerkel C *et al.* Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; **174**: 839-46.

17 Bechara FG, Sand M, Skrygan M *et al.* Acne inversa: evaluating antimicrobial peptides and proteins. *Ann Dermatol* 2012; **24**: 393-7.

18 Wolk K, Warszawska K, Hoeflich C *et al.* Deficiency of IL-22 contributes to a chronic inflammatory disease: pathogenetic mechanisms in acne inversa. *J Immunol* 2011; **186**: 1228-39.

19 Hofmann SC, Saborowski V, Lange S *et al.* Expression of innate defense antimicrobial peptides in hidradenitis suppurativa. *J Am Acad Dermatol* 2012; **66**: 966-74.

20 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

21 Takahashi K, Yanagi T, Kitamura S *et al.* Successful treatment of hidradenitis suppurativa with rituximab for a patient with idiopathic carpotarsal osteolysis and chronic active antibody-mediated rejection. *J Dermatol* 2018; **45**: e116-117.

22 Companjen AR, van der Wel LI, Wei L *et al.* A modified ex vivo skin organ culture system for functional studies. *Arch Dermatol Res* 2001; **293**: 184-90.

23 Wei L, Debets R, Hegmans JJ *et al.* IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J Pathol* 1999; **187**: 358-64.

24 Menting SP, Coussens E, Pouw MF *et al.* Developing a Therapeutic Range of Adalimumab Serum Concentrations in Management of Psoriasis: A Step Toward Personalized Treatment. *JAMA Dermatol* 2015; **151**: 616-22.

25 Takahashi H, Tsuji H, Ishida-Yamamoto A *et al.* Plasma trough levels of adalimumab and infliximab in terms of clinical efficacy during the treatment of psoriasis. *J Dermatol* 2013; **40**: 39-42.

26 Menting SP, van den Reek JM, Baerveldt EM *et al.* The correlation of clinical efficacy, serum trough levels and antidrug antibodies in ustekinumab-treated patients with psoriasis in a clinical-practice setting. *Br J Dermatol* 2015; **173**: 855-7.

27 Cosentyx. Summary of product characteristics. 2015.

28 Tran L, Baars JW, Aarden L *et al.* Pharmacokinetics of rituximab in patients with CD20 positive B-cell malignancies. *Hum Antibodies* 2010; **19**: 7-13.

29 Gabert J, Beillard E, van der Velden VH *et al.* Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. *Leukemia* 2003; **17**: 2318-57.

30 Delage M, Samimi M, Atlan M *et al.* Efficacy of infliximab for hidradenitis suppurativa: assessment of clinical and biological inflammatory markers. *Acta Derm Venereol* 2011; **91**: 169-71.

31 Lesage C, Adnot-Desanlis L, Perceau G *et al.* Efficacy and tolerance of prolonged infliximab treatment of moderate-to-severe forms of hidradenitis suppurativa. *Eur J Dermatol* 2012; **22**: 640-4.

32 Miller I, Lynggaard CD, Lophaven S *et al.* A double-blind placebo-controlled randomized trial of adalimumab in the treatment of hidradenitis suppurativa. *Br J Dermatol* 2011; **165**: 391-8.

33 Delves PJ. Tumor Necrosis Factor α. *Encyclopedia of Immunology* 1998: 2435-40.

34 Riis PT, Boer J, Prens EP *et al.* Intralesional triamcinolone for flares of hidradenitis suppurativa (HS): A case series. *J Am Acad Dermatol* 2016; **75**: 1151-5.

35 Alhusayen R, Shear NH. Scientific evidence for the use of current traditional systemic therapies in patients with hidradenitis suppurativa. *J Am Acad Dermatol* 2015; **73**: S42-6.

36 Wong D, Walsh S, Alhusayen R. Low-dose systemic corticosteroid treatment for recalcitrant hidradenitis suppurativa. *J Am Acad Dermatol* 2016; **75**: 1059-62.

37 Dorner T, Kinnman N, Tak PP. Targeting B cells in immune-mediated inflammatory disease: a comprehensive review of mechanisms of action and identification of biomarkers. *Pharmacol Ther* 2010; **125**: 464-75.

38 Giamarellos-Bourboulis EJ, Antonopoulou A, Petropoulou C *et al.* Altered innate and adaptive immune responses in patients with hidradenitis suppurativa. *Br J Dermatol* 2007; **156**: 51-6.

39 van der Zee HH, Laman JD, Boer J *et al.* Hidradenitis suppurativa: viewpoint on clinical phenotyping, pathogenesis and novel treatments. *Exp Dermatol* 2012; **21**: 735-9.

40 Kolls JK, McCray PB, Jr., Chan YR. Cytokine-mediated regulation of antimicrobial proteins. *Nat Rev Immunol* 2008; **8**: 829-35.

41 Liang SC, Tan XY, Luxenberg DP *et al.* Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006; **203**: 2271-9.

42 Hollox EJ, Huffmeier U, Zeeuwen PL *et al.* Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* 2008; **40**: 23-5.

43 Maier T, Guell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett* 2009; **583**: 3966-73.

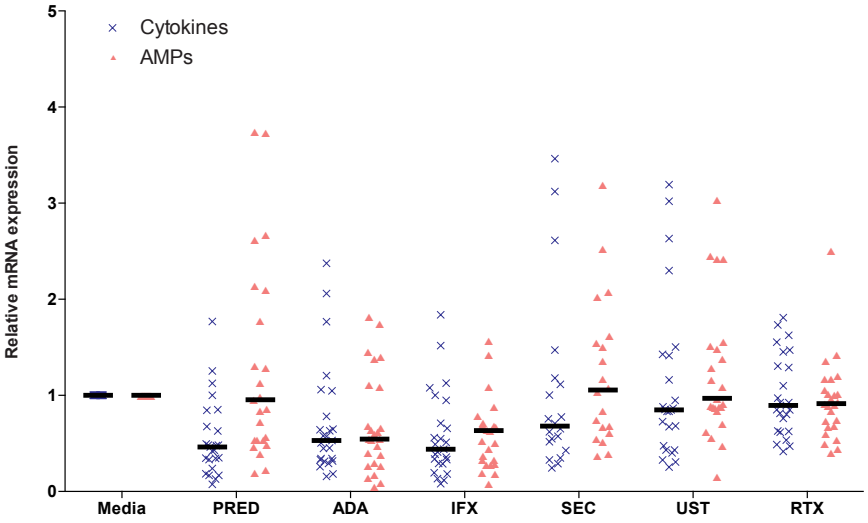
44 Schulz S, Schagdarsurengin U, Suss T *et al.* Relation between the tumor necrosis factor-alpha (TNF-alpha) gene and protein expression, and clinical, biochemical, and genetic markers: age, body mass index and uric acid are independent predictors for an elevated TNF-alpha plasma level in a complex risk model. *Eur Cytokine Netw* 2004; **15**: 105-11.

45 Rioja I, Bush KA, Buckton JB *et al.* Joint cytokine quantification in two rodent arthritis models: kinetics of expression, correlation of mRNA and protein levels and response to prednisolone treatment. *Clin Exp Immunol* 2004; **137**: 65-73.

46 Greenbaum D, Colangelo C, Williams K *et al.* Comparing protein abundance and mRNA expression levels on a genomic scale. *Genome Biol* 2003; **4**: 117.

47 Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* 2012; **13**: 227-32.

SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Relative mRNA expression of all cytokines (5) and all AMPs (5) in healthy control skin, n = 5. Two data points are out of the y-axis range. Horizontal bars display the median. Media, culture media; PRED, prednisolone 100 µg/mL; ADA, adalimumab 30 µg/mL; IFX, infliximab 20 µg/mL; SEC, secukinumab 30 µg/mL; UST, ustekinumab 10 µg/mL; RTX, rituximab 200 µg/mL.

Supplementary Table 1. Relative expression of mRNA and their modulation by biologics in healthy control skin.

	Healthy control skin (n = 5)											
	Prednisolone		Adalimumab		Infliximab		Secukinumab[1]		Ustekinumab		Rituximab	
	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value	Median (IQR)	Unadj. *p*-value
IL-1β	0.48 (0.43-0.63)	0.7616	0.50 (0.33-0.64)	0.9706	0.44 (0.34-0.66)	0.8102	0.90 (0.32-1.97)	1.0000	0.95 (0.43-2.63)	1.0000	0.62 (0.49-1.45)	1.0000
IL-6	0.18 (0.13-0.35)	**0.0168**	0.34 (0.31-1.05)	0.9146	0.36 (0.29-0.51)	0.4195	0.45 (0.32-0.61)	0.5504	0.43 (0.40-0.47)	1.0000	0.85 (0.63-1.10)	1.0000
CXCL-8 (IL-8)	0.35 (0.35-0.84)	0.2921	0.53 (0.45-0.57)	0.1324	0.39 (0.13-0.49)	**0.0187**	0.67 (0.54-0.85)	1.0000	0.85 (0.84-0.88)	1.0000	1.30 (0.97-1.55)	1.0000
IL-17A	NC		NC		NC		NC		NC		NC	
IL-23p19	NC		NC		NC		NC		NC		NC	
TNF-α	0.48 (0.33-0.48)	0.1324	0.65 (0.32-1.06)	1.0000	0.41 (0.33-0.71)	0.3639	0.86 (0.69-1.41)	1.0000	0.83 (0.73-1.43)	1.0000	0.93 (0.80-1.29)	1.0000
IFN-MXA	0.68 (0.49-1.13)	1.0000	0.63 (0.45-0.78)	0.4498	0.95 (0.56-1.00)	1.0000	0.92 (0.62-1.67)	1.0000	0.87 (0.85-1.50)	1.0000	0.85 (0.81-0.90)	1.0000
S100A7	1.28 (0.84-1.31)	1.0000	0.66 (0.63-1.11)	1.0000	0.79 (0.51-1.09)	1.0000	1.15 (0.73-1.79)	1.0000	1.09 (0.84-1.49)	1.0000	0.91 (0.74-1.01)	1.0000
S100A8	0.86 (0.72-0.99)	1.0000	0.55 (0.47-0.55)	0.3146	0.68 (0.29-0.88)	0.5516	1.15 (0.65-2.01)	1.0000	0.92 (0.88-1.39)	1.0000	1.20 (0.89-1.36)	1.0000
S100A9	0.95 (0.49-2.10)	1.0000	0.54 (0.30-0.60)	0.5695	0.81 (0.51-1.15)	0.5695	0.97 (0.89-1.52)	1.0000	1.02 (0.94-1.17)	1.0000	0.95 (0.49-2.10)	1.0000
LL-37	2.14 (0.40-3.73)	1.0000	0.64 (0.38-0.69)	0.5894	0.66 (0.65-0.68)	0.3146	1.11 (0.99-1.38)	1.0000	1.16 (0.88-1.28)	1.0000	0.74 (0.68-0.90)	1.0000
HBD-2	0.57 (0.54-1.13)	1.0000	0.40 (0.1-1.38)	0.7616	0.45 (0.38-0.53)	0.1989	1.06 (0.56-1.65)	1.0000	0.87 (0.47-2.46)	1.0000	0.69 (0.49-0.96)	1.0000
All[2] (10)	0.56 (0.40-1.13)	**0.0015**	0.55 (0.32-0.98)	**<0.0001**	0.51 (0.30-0.70)	**<0.0001**	0.77 (0.55-1.48)	0.7302	0.88 (0.69-1.47)	1.0000	0.91 (0.68-1.17)	0.9767
Cytokines (5)	0.46 (0.33-0.68)	NT	0.53 (0.33-0.78)	NT	0.44 (0.29-0.71)	NT	0.68 (0.49-1.13)	NT	0.85 (0.47-1.41)	NT	0.90 (0.63-1.30)	NT
AMPs[3] (5)	0.95 (0.54-2.10)	NT	0.55 (0.30-1.09)	NT	0.63 (0.33-0.68)	NT	1.06 (0.66-1.57)	NT	0.97 (0.87-1.52)	NT	0.91 (0.69-1.05)	NT

p-values marked in bold indicate analytes that are significant at the 0.05 level. None of the multiple tests performed with Benjamini Hochberg test yielded significant results. [1] One HS sample for secukinumab was excluded for Real-Time qPCR analysis as a result of a human error during the process of cDNA synthesis (n = 4). [2] Cytokines: pooled effect of IL-1ß, IL-6, IL-8, IL-17A, IL-23p19, TNF-α, IFN-MXA. [3] AMPs: pooled effect of S100A7, S100A8, S100A9, LL-37, HBD-2. IQR: interquartile range. NC: not calculated, non-reliable results due to low expression of these genes in healthy conditions. NT: not tested. Unadj: unadjusted.

Chapter 5.1

Apremilast for moderate hidradenitis suppurativa: results of a randomised controlled trial

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ABSTRACT

Background

Effective anti-inflammatory treatments for hidradenitis suppurativa (HS) are limited.

Objective

To evaluate the efficacy and short-term safety of apremilast in patients with moderate HS.

Methods

A total of 20 patients with moderate HS were randomised in a 3 : 1 ratio to receive blinded treatment with apremilast, 30 mg twice daily, or placebo for 16 weeks. The primary outcome was the Hidradenitis Suppurativa Clinical Response at week 16. Linear mixed effects modelling (analysis of covariance) was used to assess secondary clinical outcomes between treatment groups.

Results

The Hidradenitis Suppurativa Clinical Response was met in 8 of 15 patients in the apremilast group (53.3%) and none of 5 patients in the placebo group (0%) (p = 0.055) at week 16. Moreover, the apremilast-treated patients showed a significantly lower abscess and nodule count (mean difference, −2.6; 95% confidence interval, −6.0 to −0.9; p = 0.011), NRS for pain (mean difference, −2.7; 95% confidence interval, −4.5 to −0.9; p = 0.009), and itch (mean difference, −2.8; 95% confidence interval, −5.0 to −0.6; p = 0.015) over 16 weeks compared with the placebo-treated patients. There was no significant difference in the Dermatology Life Quality Index over time between the 2 treatment groups (mean difference, −3.4; 95% confidence interval, −9.0 to 2.3; p = 0.230). The most frequently reported adverse events in the apremilast-treated patients were mild-to-moderate headache and gastrointestinal symptoms, which did not result in dropouts.

Limitations

Small number of patients, relatively short study duration.

Conclusion

Apremilast, at a dose of 30 mg twice daily, demonstrated clinically meaningful efficacy and was generally well tolerated in patients with moderate HS.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, recurrent, autoinflammatory skin disorder with an estimated prevalence of approximately 1% of the general population.[1] The disease occurs more frequently in females and usually presents during adolescence with painful, itchy, deep-seated, inflamed nodules, abscesses, and sinus tracts predominantly in the axillae and inguinal and anogenital regions.[1,2] Disease severity can be categorised into 6 stages, ranging from clear (stage 0) to very severe (stage 6) according to the Hidradenitis Suppurativa Physician's Global Assessment (HS-PGA).[3] HS may progress over time, supporting the need for early intervention.

Currently, first-line therapies for moderate HS include anti-inflammatory oral antibiotics such as the tetracyclines and the combination of clindamycin and rifampicin.[4] Second-line treatment includes adalimumab, which is the only registered treatment for moderate-to-severe HS that provides clinically meaningful improvements.[5] Other biologic therapies have not been extensively studied in patients who have HS or are currently in the early stages of its clinical development.[6] Taken together, the high-quality evidence on HS treatment is limited, highlighting a significant unmet need for novel effective anti-inflammatory therapies.

Recently, in a small case series, 5 of 6 patients with moderate-to-severe HS treated with apremilast, 30 mg twice daily for 3 months, showed a promising response.[7] In addition, the clinical efficacy of apremilast for patients with moderate-to-severe psoriasis and psoriatic arthritis has previously been proved in multiple clinical trials.[8-10] Apremilast is an orally administered small molecule drug that specifically inhibits phosphodiesterase 4 and modulates the expression of a variety of proinflammatory and anti-inflammatory mediators.[11-13] As apremilast targets phosphodiesterase 4 in several inflammatory cell types involved in the pathogenesis of HS, such as T cells, natural killer cells, neutrophils, monocytes, and dendritic cells, we hypothesised that it might have a beneficial effect in patients with HS.

The objective of this double-blind randomised placebo-controlled trial was to investigate the clinical efficacy and short-term safety and tolerability of apremilast in patients with moderate HS. We specifically chose a study population with moderate HS because the clinical efficacy of apremilast in psoriasis (as measured by reaching a 90% improvement in baseline Psoriasis Area Severity Index score) is lower than that of most anti-tumour necrosis factor-α agents and certainly lower than that of the recently introduced anti-interleukin 17 agents and anti-interleukin 23p19 biologics.[14]

MATERIALS AND METHODS

Patients

Patients who were at least 18 years of age with moderate HS, defined as a HS-PGA score of 3, were enrolled. Additionally, patients were required to have at least 4 inflammatory lesions (i.e. abscesses, draining sinuses/tunnels, or inflammatory nodules in at least 2 anatomic locations). Patients receiving systemic antibiotics or immunosuppressive and/or immunomodulating therapy were required to stop treatment at least 28 days before baseline and until the end of study. Patients were excluded if they had minimal, mild, severe, or very severe disease severity according to the HS-PGA scale; any clinically significant active systemic disease or infection; a diagnosis of or suspected Crohn's disease; a history of major psychiatric illness requiring hospitalisation within the past 3 years; a malignancy or history of malignancy (except for treated basal or squamous cell skin carcinoma or early forms of cervical carcinoma with no recurrence in 5 years); open wounds remaining after surgical treatment; prior treatment with apremilast; or a contraindication for apremilast.

Screening

Once a patient was considered eligible for the study, the following screening procedures were performed: (1) medical history and physical examination; (2) chest radiograph; (3) serologic testing for HIV, hepatitis B and C virus, tuberculosis (QuantiFERON) and (in women) a serum pregnancy test; and (4) clinical laboratory analysis to determine haemoglobin level, white blood cell count, absolute neutrophil count, platelet count, serum creatinine level, alanine aminotransferase level, and alkaline phosphatase level.

Study design

This study was an investigator-initiated clinical trial (EudraCT 2016-000859-27; NCT03238469) that was conducted in the department of Dermatology of the Erasmus University Medical Center Rotterdam, The Netherlands, approved by the local medical ethics committee (NL.57003.078.16), and implemented according to the Declaration of Helsinki principles. All patients provided written informed consent before enrolment. Eligible patients were randomly assigned to treatment with apremilast at a dose of 30 mg twice daily (n = 15) or placebo tablets with an appearance, packaging, and labelling identical to that of apremilast (n = 5). An independent statistician generated the randomisation scheme on the basis of the anticipated group size with a ratio of 3 to 1 by using R Statistical Software (R Foundation for Statistical Computing, Vienna, Austria). Treatment was allocated by using the sequential randomisation code provided and guarded by the trial pharmacist. Patients, care providers, and

those assessing the outcomes were blinded to treatment allocation. Apremilast was dose-escalated over the first week of treatment (10 mg on day 1, with increases of 10 mg/d) until the target dose of 30 mg twice daily had been reached. The duration of the treatment period was 16 weeks. Study visits were scheduled for screening and at week 0 (baseline), week 2, week 4 (early response), week 8, week 12, and week 16 (end of study).

Outcome measures

The primary outcome was the percentage of patients reaching the Hidradenitis Suppurativa Clinical Response (HiSCR) at week 16, which was defined as at least a 50% reduction in total abscess and inflammatory nodule (AN) count, with no increase in abscess count and no increase in draining sinus count relative to baseline.[15,16] At every visit, a physical examination (including a lesion count) was performed. Secondary clinical outcomes included the HiSCR at week 4, total lesion count, AN count, and Dermatology Life Quality Index (DLQI) score[17]; the patient's assessment of pain, itch, and disease burden on a numeric rating scale (NRS) ranging from 0 (no itch, pain, or burden) to 10 (unbearable/extreme itch, pain, and/or burden); and clinical photographs. Both physician-reported outcomes and patient-reported outcomes were collected at all visits. Safety assessments were conducted from screening until the end of the study. Adverse events (AEs) and vital signs were evaluated at each visit, and clinical laboratory measurements were evaluated at weeks 2, 8, and 16.

Statistical analyses

No statistical sample size was calculated.[18] Sample size estimations were based on previous exploratory clinical trials in HS.[19,20] Clinical efficacy and safety outcomes were analysed in the intention-to-treat population according to the randomised group assignments. The proportion of patients achieving HiSCR was analysed by Fisher's exact test at week 4 and week 16 (primary outcome). Missing outcomes in terms of the HiSCR of 2 patients at week 16 were imputed by last observation carried forward. To assess response over 16 weeks of treatment, the total lesion count, AN count, and DLQI score, as well as the patient's global assessment on pain, itch, and disease burden, were analysed by linear mixed effects modelling using analysis of covariance with the fixed factor treatment*time, the baseline value as covariate, and first-order autoregressive as best fitting covariance structure. Exactly the same model was subsequently used to separately evaluate the treatment arms relative to baseline for an impression of the short-term (week 4) and final response to treatment (end of the study [week 16]). Secondary outcomes were not statistically compared at time points to increase the power. Missing data for the analysis of covariance modelling were not imputed, nor were they carried forward. Post hoc analysis did not show

significant differences between the approach of no data imputation and the approach of last observation carried forward. Safety data were analysed by descriptive statistics for all patients who received at least 1 dose of the study drug. Statistical analyses were conducted with SPSS Statistics 24.0 (IBM Corporation, Armonk, NY). A 2-sided *p*-value less than 0.05 was considered significant.

RESULTS

Patients

Enrolment occurred between February and August 2017, and a 16-week follow-up was completed in December 2017. In total, 21 patients were randomised, of whom 20 received at least 1 dose of study medication and were included in the intention-to-treat population. Of these, 18 (90%) completed the week 16 follow-up visit (Figure 1). Of the 15 patients receiving apremilast, 2 (13%) discontinued the study: 1 patient discontinued between week 4 and week 8 because of personal circumstances (travel distance in combination with divorced parents), and the other discontinued at week 8 on account of adverse effects. None of the 5 patients in the placebo arm dropped out. The baseline characteristics for the intervention groups are depicted in Table 1.

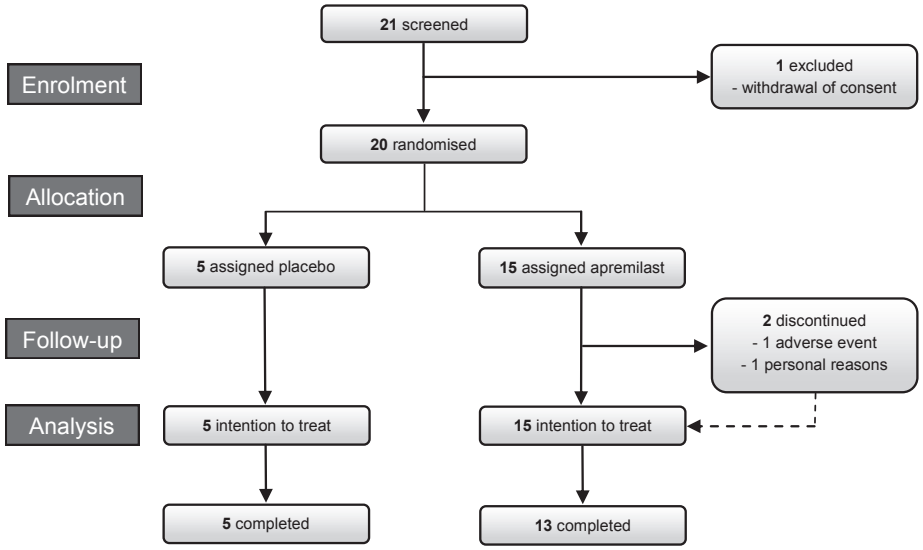

Figure 1. Flow chart of patient enrolment and the study procedure.

Table 1. Baseline characteristics.

Characteristic	Apremilast (n = 15)	Placebo (n = 5)
Mean age ± SD, y	35.7 ± 13.0	33.4 ± 8.2
Women, n (%)	12 (80)	5 (100)
Race, white, n (%)	15 (100)	4 (80)
Mean history of HS ± SD, y	21.6 ± 13.0	16.0 ± 7.1
Prior HS antibiotics treatment, n (%)	13 (87)	4 (80)
Prior HS biologics treatment, n (%)	4 (27)	2 (40)
Mean BMI ± SD, kg/m^2	32.7 ± 6.7	32.8 ± 5.3
Current smokers, n (%)	11 (73)	5 (100)
Mean current daily cigarette consumption ± SD	17.7 ± 8.2	19.6 ± 11.4
Mean total lesion count ± SD †	6.9 ± 2.3	7.2 ± 2.6
Mean AN count ± SD ‡	6.1 ± 1.7	5.8 ± 2.4
Mean DLQI ± SD, (0-30)	14.6 ± 7.6	11.8 ± 5.9
Mean NRS for pain ± SD, (0-10)	6.4 ± 2.4	5.8 ± 2.2
Mean NRS for itch ± SD, (0-10)	5.0 ± 2.9	6.8 ± 3.0
Mean NRS for disease burden ± SD, (0-10)	6.2 ± 2.1	7.0 ± 3.0

Values as mean ± standard deviation or number (percentage). † Total number of abscesses, draining sinuses/tunnels, and inflammatory nodules. ‡ Total number of abscesses and inflammatory nodules. AN: abscess and inflammatory nodule. BMI: body mass index. DLQI: Dermatology Life Quality Index. HS: hidradenitis suppurativa. NRS: numeric rating scale. SD: standard deviation.

Primary efficacy outcome

Of the 15 patients allocated to treatment with apremilast, 8 (53.3%) achieved a positive HiSCR at week 16 compared with none of the patients in the placebo group (p = 0.055) (Figure 2). The last observation was carried forward for the dropout after week 4 (negative HiSCR) and the dropout at week 8 (positive HiSCR). At week 4, 10 of 15 patients receiving apremilast (66.7%) achieved a positive HiSCR versus none of the patients in the placebo group (p = 0.033). A decrease in the proportion of patients treated with apremilast who achieved an HiSCR was observed from week 2 (an HiSCR in 11 of 15 patients) with a stabilisation between week 12 and 16 (a HiSCR in 8 of 15 patients). A characteristic example of the clinical response of a patient treated with apremilast is provided in Figure 3.

Secondary efficacy outcomes

Over 16 weeks of treatment, the patients receiving apremilast showed a significantly lower AN count (mean difference, –2.6; 95% confidence interval, –6.0 to –0.9; p = 0.011) (Figure 4) and significantly lower NRS scores for pain (mean difference, –2.7; 95% confidence interval, –4.5 to –0.9; p = 0.009) (Figure 5), itch (mean difference, –2.8; 95% confidence interval, –5.0 to –0.6; p = 0.015), and disease burden (mean difference,

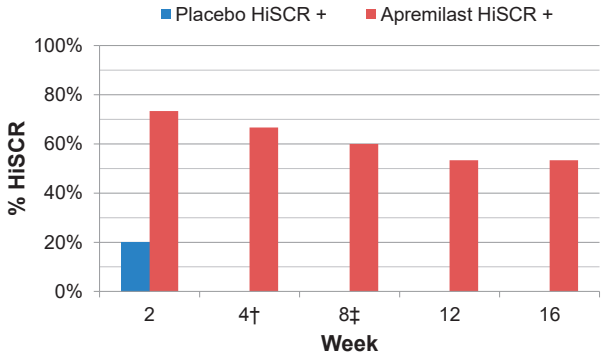

Figure 2. Percentage of patients in both treatment groups reaching the hidradenitis suppurativa clinical response (HiSCR) relative to baseline during the treatment period. Fisher's exact test was performed at week 4 (p = 0.033) and at week 16 (p = 0.055). † Last observation carried forward for 1 HiSCR non-achiever as a result of dropping out. ‡ Last observation carried forward for 1 HiSCR achiever as a result of dropping out.

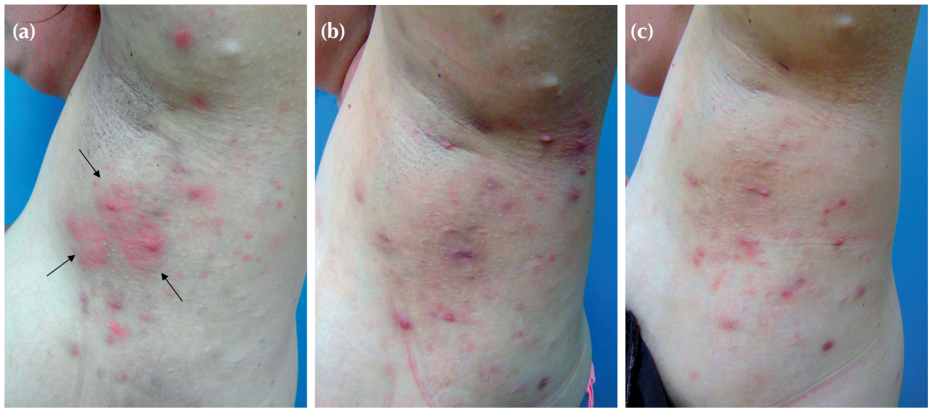

Figure 3. Example of a patient in the apremilast treatment group: baseline **(a)**, at week 4 (early response) **(b)**, and at week 16 (end of study) **(c)**. Arrows indicate index lesions (i.e. inflammatory nodules that were larger than 10 mm in diameter and painful by palpation at baseline. Note that all the deep-seated lesions have resolved. Erythematous lesions seen at week 16 are scars and superficial folliculitis.

−1.8; 95% confidence interval, −3.7 to −0.01; p = 0.049) than did patients in the placebo group. The total lesion count demonstrated a trend toward significant improvement in the apremilast group (mean difference, −2.6; 95% confidence interval, −5.5 to 0.2; p = 0.064). There were no differences in DLQI score over time between the treatment groups (mean difference, −3.4; 95% confidence interval, −9.0 to 2.3; p = 0.230).

The mean changes from baseline by treatment group for the secondary clinical outcomes at week 4 (early response) and week 16 (end of study) are summarised in Table 2. In the apremilast group the treatment outcomes observed at week 16 did

not further improve as compared with those at week 4 except for itch. In the placebo group all outcomes except the NRS for disease burden deteriorated at the end of study relative to week 4.

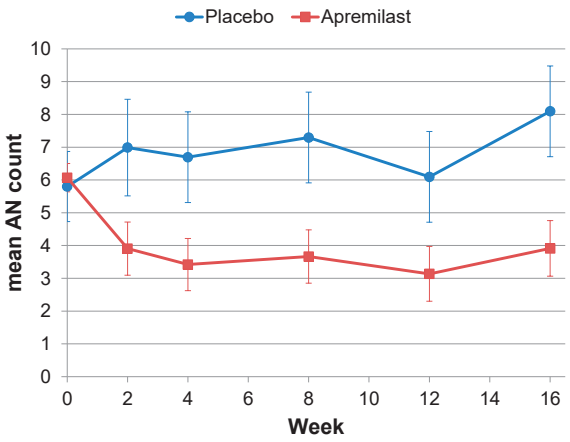

Figure 4. Mean estimated abscess and inflammatory nodule (AN) count from baseline until week 16 according to linear mixed effects modelling (analysis of covariance). Mean estimated difference for apremilast vs placebo: –2.6; 95% confidence interval, –6.0 to –0.9; $p = 0.011$. Bars display standard errors of the mean. AN count: total number of abscesses and inflammatory nodules.

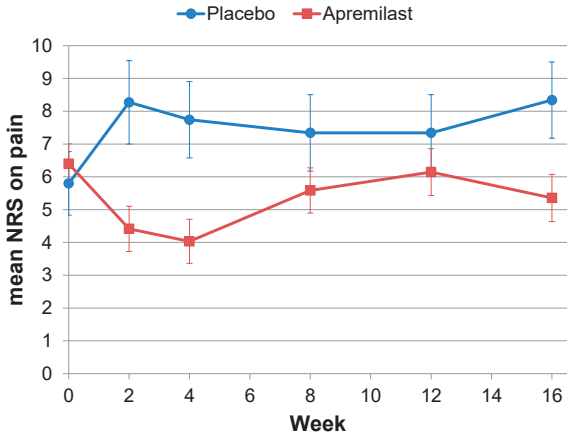

Figure 5. Mean estimated level of pain from baseline until week 16 according to linear mixed effects modelling (analysis of covariance). Mean estimated difference for apremilast vs placebo: –2.7; 95% confidence interval, –4.5 to –0.9; $p = 0.009$. Bars display standard errors of the mean. NRS: numeric rating scale.

Table 2. Mean estimated change from baseline for treatment outcomes at week 4 and week 16.

Characteristic	Apremilast* (n = 15)	Placebo* (n = 5)
Week 4		
Total lesion count†	−2.8 (−4.6 to −1.1)	−0.4 (−3.5 to 2.6)
AN count‡	−2.7 (−4.3 to −1.1)	+0.6 (−2.2 to 3.4)
DLQI (0-30)	−3.8 (−7.2 to −0.4)	+0.8 (−5.1 to 6.7)
NRS for pain (0-10)	−2.1 (−3.5 to −0.8)	+1.6 (−0.7 to 3.9)
NRS for itch (0-10)	−0.7 (−2.0 to 0.6)	+2.0 (−0.3 to 4.3)
NRS for disease burden (0-10)	−1.8 (−3.0, −0.6)	+1.1 (−1.0 to 3.2)
Week 16		
Total lesion count†	−2.0 (−3.9 to −0.2)	+1.4 (−1.7 to 4.4)
AN count‡	−2.2 (−3.9 to −0.5)	+2.0 (−0.8 to 4.8)
DLQI (0-30)	−2.3 (−5.9 to 1.3)	+4.2 (−1.7 to 10.1)
NRS for pain (0-10)	−0.8 (−2.2 to 0.6)	+2.2 (−0.1 to 4.5)
NRS for itch (0-10)	−1.1 (−2.5 to 0.3)	+2.4 (0.1 to 4.7)
NRS for disease burden (0-10)	−1.0 (−2.3 to 0.3)	+0.9 (−1.2 to 3.0)

* Mean estimated change from baseline at week 4 and week 16 for with corresponding 95% confidence intervals for outcome values according to linear mixed effects modelling (analysis of covariance). † Total number of abscesses, draining sinuses/tunnels, and inflammatory nodules. ‡ Total number of abscesses and inflammatory nodules. DLQI: Dermatology Life Quality Index. NRS: numeric rating scale.

Safety and tolerability

In total, 38 AEs were reported in the apremilast group versus 11 AEs in the placebo group (p = 0.514). An overview of the AEs is shown in Table 3. Most of the AEs were mild-to-moderate, and none was classified as a serious AE. One patient in the apremilast group discontinued study participation because of unbearable muscle and joint pain that occurred a few days after the first dose of study medication. A poststudy exploratory genetic analysis of cytochrome P450 family 3 subfamily A member 4 gene (*CYP3A4*) and cytochrome P450 family 1 subfamily A member 2 (*CYP1A2*) polymorphisms, which are important for the metabolism of apremilast, showed normal expression in this patient, implying that differential metabolism could not explain the AE. In all, 6 patients in the apremilast group versus 2 patients in the placebo group had ongoing AEs at the end of the study. The ongoing AEs in the patients receiving apremilast after 16 weeks were diarrhoea (n = 2), headache, nonspecific rash, increased fatigue, and a depressed feeling. At telephone follow-up 8 weeks after the end of study, these events were resolved without sequelae. There were no clinically significant changes in laboratory parameters.

Table 3. Adverse events.

Characteristic	Apremilast (n = 15) n (%) E*	Placebo (n = 5) n (%) E*
Headache	7 (47) 8	1 (20) 2
Diarrhoea	7 (47) 7	1 (20) 1
Nausea	4 (36) 4	1 (20) 1
Common cold	4 (36) 4	1 (20) 1
Vomiting	2 (13) 2	0 (0) 0
Nonspecific rash	2 (13) 2	0 (0) 0
Excessive joint and muscle pain	1 (7) 1^	0 (0) 0
Back pain	1 (7) 1	0 (0) 0
Increased fatigue	1 (7) 1	0 (0) 0
Depressed feeling	1 (7) 1	0 (0) 0
Pyelonephritis	1 (7) 1	0 (0) 0
Mycosis of the vulva	1 (7) 1	0 (0) 0
Increased itch	1 (7) 1	0 (0) 0
Sore throat	1 (7) 1	0 (0) 0
Elevation in serum ALT level†	1 (7) 1	0 (0) 0
Decrease in serum haemoglobin level‡	1 (7) 1	1 (20) 1
Nonspecific gastrointestinal symptoms	1 (7) 1	1 (20) 1
Dry mouth	0 (0) 0	1 (20) 1
Hair loss	0 (0) 0	1 (20) 1
Self-reported fever of unknown origin	0 (0) 0	1 (20) 1
Flare of herpes labialis	0 (0) 0	1 (20) 1

* Number of patients with at least one adverse event (n), percentage of patients with at least one adverse event (%), and number of adverse events (E). ALT: Alanine Transaminase. † Self-limiting elevation of 2-3 times the upper limit. ‡ Below lower limit according to local laboratory threshold. ^ Reason for dropout.

DISCUSSION

The main finding from this study was a clinically meaningful improvement of moderate HS after treatment with apremilast at a dose of 30 mg twice per day for 16 weeks. Apremilast significantly decreased disease activity as measured by the AN count, and 53.3% of patients receiving apremilast achieved the HiSCR in comparison with none of the patients receiving placebo. In addition, apremilast for 16 weeks was well tolerated by patients with moderate HS.

The response to treatment in this study was evaluated primarily by the HiSCR.[15] This validated efficacy end point has previously been used in 4 randomised controlled trials in HS.[5,16,21] The proportion of HiSCR responders in our study is within the range of HiSCR achievers in these previous studies: consecutively, 54.5% (adalimumab every week during 16 weeks of treatment [N = 147, of whom 43 received placebo]),

41.8% ([PIONEER I] adalimumab every week during 12 weeks of treatment (N = 307, of whom 154 received placebo]), 58.9% ([PIONEER II] adalimumab every week during 12 of weeks treatment [N = 326, of whom 163 received placebo]), and 60.0% (the monoclonal antibody MABp1 during 12 weeks of treatment [N = 20, of whom 10 received placebo]). Of note, in these trials patients with moderate-to-severe HS were enrolled and 3 of 4 studies were larger. Although a 1-to-1 comparison is inappropriate, the aforementioned results demonstrate that our findings are in line with the currently reported response rates.

Interestingly, the time to clinical response was much faster than that with apremilast in psoriasis, in which case an increasing proportion of patients achieving a 75% improvement in baseline Psoriasis Area Severity Index score has been observed up to 52 weeks after start of treatment.[22] This might potentially be explained by differences in underlying pharmacodynamic patterns, as HS auto-inflammatory pathology is initiated by an aberrant innate immune response followed by participation of the adaptive immune system that involves a broader array of cell types than in psoriasis pathophysiology.

Patients' assessment of HS-related pain and itch fluctuated during treatment, possibly reflecting the natural course of the disease, as the AN count does not take into account the size or severity of individual lesions. The DLQI score improved in the apremilast-treated patients but exhibited even larger variability during the study. An explanation might be the small number of patients studied and the fact that quality of life could have been affected by other (personal) circumstances in addition to the HS disease activity.

Treatment with apremilast at a dose of 30 mg twice daily was generally well tolerated, with the majority of the AEs rated mild-to-moderate. The safety profile of apremilast observed in this study is consistent with previous findings from larger trials in patients with psoriasis and psoriatic arthritis.[23,24] However, the number of patients included in our study was small, and the current safety results may not be representative for a larger population.

The evidence-based literature available on nonbiologic therapies in HS is limited and largely restricted to retrospective studies or expert opinion.[4] Therefore, recommendations for optimal medical management of HS are challenging. Although the efficacy of apremilast in HS must still be confirmed in larger studies, we believe that apremilast could be considered as a new treatment option for moderate HS after failure of conventional treatments, such as the combination of clindamycin and rifampicin. Currently, adalimumab is the first-choice agent in moderate-to-severe HS after failure of conventional treatments.[5] However, there may be advantages of apremilast over oral antibiotics and biologic therapies. It is well established that continuous antibiotic treatment can induce bacterial resistance, limiting its use for

chronic purposes, whereas subcutaneous administration and relatively frequent laboratory monitoring can pose an additional burden for patients who are treated with adalimumab. Another problem with the use of tumour necrosis factor-α antagonists such as adalimumab and infliximab is the risk of formation of antidrug antibodies, with neutralisation of the therapeutic effect over time.[25]

The major strength of this study is its randomised, double-blind, placebo-controlled design. In addition, the risk of inter-rater variability for the physician-reported outcomes was minimised by using only 2 clinical assessors. Limitations of our study are the small number of included patients, the study's relatively short duration, and the lack of data beyond the end of treatment. Missing HiSCR data were handled by using last observation carried forward in 2 patients taking apremilast, which potentially increased the effect of attrition bias. Furthermore, the AEs headache, diarrhoea, nausea, and vomiting were common in the apremilast group, which could have partially unblinded the observers.

In conclusion, the results from this study indicate that oral apremilast is a promising treatment option with good short-term safety and tolerability and that it could be a valuable addition to the armamentarium for the treatment of HS. Studies with larger populations and longer follow-up are needed to further elucidate the efficacy and safety profile of apremilast in HS.

REFERENCES

1 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

2 Vossen A, Schoenmakers A, van Straalen KR *et al*. Assessing Pruritus in Hidradenitis Suppurativa: A Cross-Sectional Study. *Am J Clin Dermatol* 2017; **18**: 687-95.

3 Kimball AB, Kerdel F, Adams D *et al*. Adalimumab for the treatment of moderate to severe Hidradenitis suppurativa: a parallel randomized trial. *Ann Intern Med* 2012; **157**: 846-55.

4 Zouboulis CC, Desai N, Emtestam L *et al*. European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619-44.

5 Kimball AB, Okun MM, Williams DA *et al*. Two Phase 3 Trials of Adalimumab for Hidradenitis Suppurativa. *N Engl J Med* 2016; **375**: 422-34.

6 Theut Riis P, Thorlacius LR, Jemec GB. Investigational drugs in clinical trials for Hidradenitis Suppurativa. *Expert Opin Investig Drugs* 2018; **27**: 43-53.

7 Weber P, Seyed Jafari SM, Yawalkar N *et al*. Apremilast in the treatment of moderate to severe hidradenitis suppurativa: A case series of 9 patients. *J Am Acad Dermatol* 2017; **76**: 1189-91.

8 Papp K, Reich K, Leonardi CL *et al*. Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, in patients with moderate to severe plaque psoriasis: Results of a phase III, randomized, controlled trial (Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis [ESTEEM] 1). *J Am Acad Dermatol* 2015; **73**: 37-49.

9 Bissonnette R, Pariser DM, Wasel NR *et al*. Apremilast, an oral phosphodiesterase-4 inhibitor, in the treatment of palmoplantar psoriasis: Results of a pooled analysis from phase II PSOR-005 and phase III Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis (ESTEEM) clinical trials in patients with moderate to severe psoriasis. *J Am Acad Dermatol* 2016; **75**: 99-105.

10 Edwards CJ, Blanco FJ, Crowley J *et al*. Apremilast, an oral phosphodiesterase 4 inhibitor, in patients with psoriatic arthritis and current skin involvement: a phase III, randomised, controlled trial (PALACE 3). *Ann Rheum Dis* 2016; **75**: 1065-73.

11 Schafer PH, Parton A, Gandhi AK *et al*. Apremilast, a cAMP phosphodiesterase-4 inhibitor, demonstrates anti-inflammatory activity in vitro and in a model of psoriasis. *Br J Pharmacol* 2010; **159**: 842-55.

12 Claveau D, Chen SL, O'Keefe S *et al*. Preferential inhibition of T helper 1, but not T helper 2, cytokines in vitro by L-826,141 [4-[2-(3,4-Bisdifluromethoxyphenyl)-2-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan- 2-yl)-phenyl]-ethyl]3-methylpyridine-1-oxide], a potent and selective phosphodiesterase 4 inhibitor. *J Pharmacol Exp Ther* 2004; **310**: 752-60.

13 Eigler A, Siegmund B, Emmerich U *et al*. Anti-inflammatory activities of cAMP-elevating agents: enhancement of IL-10 synthesis and concurrent suppression of TNF production. *J Leukoc Biol* 1998; **63**: 101-7.

14 Sbidian E, Chaimani A, Garcia-Doval I *et al*. Systemic pharmacological treatments for chronic plaque psoriasis: a network meta-analysis. *Cochrane Database Syst Rev* 2017; **12**: CD011535.

15 Kimball AB, Jemec GB, Yang M *et al*. Assessing the validity, responsiveness and meaningfulness of the Hidradenitis Suppurativa Clinical Response (HiSCR) as the clinical endpoint for hidradenitis suppurativa treatment. *Br J Dermatol* 2014; **171**: 1434-42.

16 Kimball AB, Sobell JM, Zouboulis CC *et al*. HiSCR (Hidradenitis Suppurativa Clinical Response): a novel clinical endpoint to evaluate therapeutic outcomes in patients with hidradenitis suppurativa from the placebo-controlled portion of a phase 2 adalimumab study. *J Eur Acad Dermatol Venereol* 2016; **30**: 989-94.

17 Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)—a simple practical measure for routine clinical use. *Clin Exp Dermatol* 1994; **19**: 210-6.

18 Bacchetti P, Deeks SG, McCune JM. Breaking free of sample size dogma to perform innovative translational research. *Sci Transl Med* 2011; **3**: 87ps24.

19 Miller I, Lynggaard CD, Lophaven S *et al*. A double-blind placebo-controlled randomized trial of adalimumab in the treatment of hidradenitis suppurativa. *Br J Dermatol* 2011; **165**: 391-8.

20 van der Zee HH, Laman JD, de Ruiter L *et al*. Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

21 Kanni T, Argyropoulou M, Spyridopoulos T *et al*. MABp1 Targeting IL-1alpha for Moderate to Severe Hidradenitis Suppurativa Not Eligible for Adalimumab: A Randomized Study. *J Invest Dermatol* 2018; **138**: 795-801.

22 Reich K, Gooderham M, Bewley A *et al*. Safety and efficacy of apremilast through 104 weeks in patients with moderate to severe psoriasis who continued on apremilast or switched from etanercept treatment: findings from the LIBERATE study. *J Eur Acad Dermatol Venereol* 2018; **32**: 397-402.

23 Papp K, Cather JC, Rosoph L *et al*. Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial. *Lancet* 2012; **380**: 738-46.

24 Schett G, Wollenhaupt J, Papp K *et al*. Oral apremilast in the treatment of active psoriatic arthritis: results of a multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2012; **64**: 3156-67.

25 Vincent FB, Morand EF, Murphy K *et al*. Antidrug antibodies (ADAb) to tumour necrosis factor (TNF)-specific neutralising agents in chronic inflammatory diseases: a real issue, a clinical perspective. *Ann Rheum Dis* 2013; **72**: 165-78.

Chapter 5.2

Apremilast for moderate hidradenitis suppurativa: no significant change in lesional skin inflammatory biomarkers

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ABSTRACT

Background

Treatment with apremilast has demonstrated clinically meaningful improvement in moderate hidradenitis suppurativa (HS).

Objective

To evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily (n = 15) for 16 weeks compared with placebo (n = 5).

Methods

At baseline 5-mm punch biopsies were obtained from an index lesion (HSL) and non-lesional skin (HSN) as control in the same anatomical area. Subsequent HSL samples were taken as close as possible to the previously biopsied site at week 4 and week 16. After sampling, biopsies were split; one half was processed for *in vivo* mRNA analysis using real-time quantitative PCR; the other half was cultured for *ex vivo* protein analysis using a proximity extension assay (Olink). Linear mixed effects models were calculated to compare the levels of inflammatory markers in HSL skin between apremilast and placebo over time.

Results

At baseline, 17 proteins with a fold change >2 in HSL versus HSN skin were identified in 20 patients. The top 5 were IL-17A (5), S100A12, CST5, IL-12/23p40, and CD6 (1) with respective fold changes ranging from 6.6 to 1638 (FDR < 0.044). Linear mixed effects models for 75 assays were calculated. Protein levels of S100A12 decreased during treatment in the apremilast group compared with the placebo group (p = 0.014, FDR = 0.186). None of the 14 genes exhibited significant changes in expression over time. However, an evident downward trend in relative mRNA expression of IL-17A and IL-17F was demonstrated in patients receiving apremilast.

Conclusion

We did not detect statistically significant changes in inflammatory markers in lesional skin of HS patients receiving apremilast compared with placebo, despite clinical improvement in the apremilast group. Nonetheless, S100A12 and IL-17A were significantly elevated in HS lesional skin and showed a decrease in response to apremilast. The translational model in clinical trials involving HS clearly needs further improvement.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, recurrent, auto-inflammatory skin disorder with limited effective treatment options.[1] Recently, treatment with apremilast for 16 weeks has demonstrated clinically meaningful improvement in moderate HS. In this study (NCT03238469; EudraCT 2016-000859-27) twenty patients with moderate HS (HS-PGA score of 3) were randomised in a 3 : 1 ratio to receive blinded treatment of apremilast 30 mg twice daily or placebo, respectively. Demographic and disease characteristics were similar between treatment arms. The mean age of the study population was 35 years (range 21-64), most patients were female (17/20) and current smokers (16/20), the mean (± SD) BMI was 32.7 ± 6.2, and the mean abscess and inflammatory nodule (AN) count was 6.0 ± 1.8. The objective of this translational study was to evaluate the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg twice daily for 16 weeks compared with placebo.

MATERIALS AND METHODS

Ethical statement

This investigator-initiated trial was approved by the medical ethics committee of the Erasmus University Medical Center Rotterdam, The Netherlands (NL.57003.078.16). All patients provided written informed consent for study participation and use of their skin biopsies.

Biopsy procedure

At baseline, two 5-mm punch biopsies were taken: one was obtained from an erythematous, indurated, inflamed lesion, i.e. the index lesion (HSL), and one from the normal-appearing, unaffected non-lesional skin (HSN) in the same anatomical area (Figure 1). Subsequent HSL skin samples were taken as close as possible to the previously biopsied index site at week 4 (early response) and week 16 (end of study). After sampling, biopsies were split whereby one half was processed for mRNA analysis of the biopsy, representing the *in vivo* gene expression, and the other half was cultured for *ex vivo* protein analysis. The supernatants and the biopsies in 250 µL lysis buffer containing 1% ß-mercaptoethanol were transferred to a polypropylene tube and stored at –20°C until further analysis.

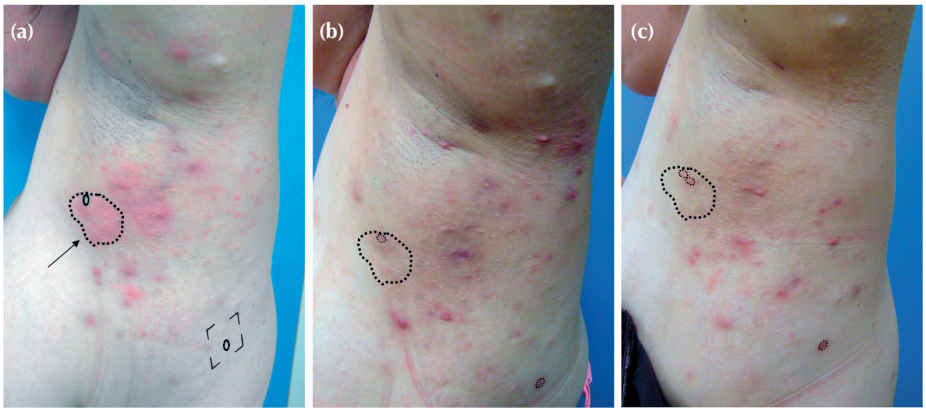

Figure 1. Biopsy procedure in a patient with a good clinical response to apremilast. Note that all deep-seated lesions have resolved. Erythematous lesions seen at week 16 are scars and superficial folliculitis. **(a)** Baseline. A 5-mm punch biopsy was obtained from an erythematous, indurated, inflamed lesion (arrow pointing to index lesion). An additional 5-mm punch biopsy of the non-lesional skin was taken from the same anatomical area to serve as control (circle within the square). **(b)** Week 4 and **(c)** Week 16. Subsequent skin samples were taken as close as possible to the previously biopsied index site (small circles within dashed line).

Ex vivo skin culture and protein quantification

After sampling, half of the 5-mm biopsy was cultured for 24h at 37°C in an atmosphere of 5% CO2 and 98% humidity in a transwell system (Netwell; Costar, Cambridge, MA) as previously described.[2,3] In short, samples were placed in a 12-well plate with the epidermis exposed to the air and the dermis immersed in 1 mL Iscove's modified Dulbecco's medium (Gibco, Paisley, U.K.) containing 0.5% human AB serum, penicillin (100 U/mL) and streptomycin (100 U/mL). A broad spectrum of 92 inflammatory markers was measured in the supernatant using the Olink Proseek Multiplex Inflammation panel (Olink Proteomics, Uppsala, Sweden).

In vivo gene expression

Total RNA was isolated using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MA), treated with 0.1U/µL DNAse (Invitrogen), and cDNA was synthesised with SuperScript II reverse transcriptase, random hexamer primers (Invitrogen) and oligo(dT)15 (Promega). Primers and probes were designed and chosen using ProbeFinder Software and the Universal Probe Library (Roche Applied Science, Indianapolis, IN). *ABL1* was used as housekeeping gene. Real-time quantitative PCR for 14 genes (*IFN-γ*, *TNF-α*, *IL-1ß*, *IL-6*, *IL-8*, *IL-10*, *IL-23p19*, *IL-12/23p40*, *IL-17A*, *IL-17F*, *IL-31*, *CXCL-10*, *CCL-5*, and *MPO*) was performed with the ViiA7 System and using the QuantStudio Real-Time PCR Software version 1.3 (Applied Biosystems, Waltham, MA).

Statistical analysis

Protein data are presented as normalised protein expression (NPX), which are log^2-transformed arbitrary units. Relative mRNA expression levels for week 4 and week 16 were calculated using the $2^{-\Delta\Delta CT}$ method using a baseline value of 1 for each individual sample.[4] First, differentially expressed proteins at baseline were explored by calculating a fold change with the median NPX of HSL skin divided by the median NPX of HSN skin. If the fold change was more than 2, a Wilcoxon signed rank test was subsequently used to pairwise compare protein levels between HSL and HSN skin. Second, linear mixed effects models and associated ANOVA for repeated measures were calculated to compare inflammatory protein and mRNA expression levels between apremilast and placebo over time. Fixed effects were treatment, time and treatment*time. Third, the Spearman's rho was used to assess translational linkage, i.e. correlation between the levels of inflammation and the AN count. A two-sided *p*-value below 0.05 was considered significant. *P*-values were adjusted for multiple testing within each test using the Benjamini-Hochberg approach. Statistical analyses were conducted in R Statistical Software version 3.5 (R Foundation for Statistical Computing, Vienna, Austria) using the lmerTest package.

RESULTS

At baseline, 17 proteins with a fold change >2 were identified (Table 1). The top-5 were IL-17A (5), S100A12, CST5, IL-12p40, and CD6 (1) with respective fold changes ranging from 6.6 to 1638 (FDR < 0.044). Out of 92 Olink assays, 75 were included in the linear mixed effects models. The removed assays had less than 25% detectability for among others TNF-α and IFN-γ. Only the protein levels of S100A12 decreased over time in the apremilast versus placebo group. However, this was not statistically significant after correction for multiple testing ($p = 0.014$, FDR = 0.186; Figure 2).

None of the 14 genes analysed by linear mixed effects models yielded significant differences. Only the relative mRNA expression of *IL-17A* and *IL-17F* demonstrated an evident but nonsignificant downward trend in patients receiving apremilast versus placebo ($p > 0.05$; Figure 3). In addition, there was no correlation between S100A12 protein levels or *IL-17A/F* mRNA expression and the favourable clinical response in the apremilast group as measured by the AN count (data not shown).

Table 1. Proteins with a fold change >2 in HSL versus HSN skin at baseline.

#	Protein	Fold change HSL/HSN	NPX HSL N = 20 median	NPX HSL N = 20 IQR	NPX HSN N = 20 median	NPX HSN N = 20 IQR	Unadj. *p*-value	Adj. *p*-value FDR	Missing data %
1	CD6	1638	3.5	2.0 - 4.1	0.002	0.0 - 0.2	3.4 e-07	0.018	None
2	IL-12p40	38	0.9	0.5 - 1.8	0.024	0.0 - 0.1	7.2 e-06	0.038	7.5
3	CST5	23	0.9	0.6 - 1.1	0.041	0.0 - 0.1	6.1 e-07	0.024	1.1
4	S100A12	11	4.5	3.1 - 6.3	0.4	0.4 - 0.8	2.7 e-05	0.041	20.0
5	IL-17A	6.6	3.1	1.8 - 3.8	0.5	0.5 - 0.6	2.9 e-05	0.044	26.3
6	TNFSF14	4.5	3.4	2.5 - 4.1	0.8	0.4 - 1.0	1.0 e-06	0.029	6.3
7	AXIN1	4.4	1.9	1.7 - 2.1	0.4	0.4 - 0.5	8.8 e-07	0.026	8.8
8	β-NGF	3.2	1.1	0.6 - 1.4	0.3	0.3 - 0.6	5.8 e-04	0.047	15.0
9	TNFSF11	3.1	2.2	1.2 - 3.3	0.7	0.7 - 0.7	4.6 e-07	0.021	28.8
10	CD244	3.0	2.7	2.3 - 3.1	0.9	0.9 - 0.9	1.1 e-07	0.012	23.8
11	CD5	3.0	7.3	5.9 - 7.6	2.5	2.2 - 2.6	1.3 e-08	0.003	None
12	TNFRSF9	2.9	6.4	5.2 - 7.6	2.2	1.8 - 2.7	1.3 e-08	0.006	1.3
13	OSM	2.9	6.4	6.0 - 7.8	2.2	1.3 - 3.4	4.0 e-06	0.035	3.8
14	SIRT2	2.8	5.0	4.5 - 5.4	1.8	1.5 - 1.9	1.9 e-07	0.015	5.0
15	GDNF	2.7	2.9	2.0 - 3.8	1.1	0.8 - 1.2	1.6 e-06	0.032	5.0
16	IL-18R1	2.3	4.2	3.6 - 5.2	1.8	1.7 - 2.0	1.3 e-08	0.009	None
17	ST1A1	2.1	1.5	0.8 - 1.8	0.7	0.5 - 1.2	1.3 e-02	0.050	10.0

Missing data: samples with NPX-values below background level throughout all samples, i.e. HSN and HSL baseline, HSL week 4 and HSL week 16. Unadj. *p*-value: unadjusted *p*-value by Wilcoxon signed rank, NPX HSL vs. NPX HSN. Adj. *p*-value (FDR): adjusted *p*-value Benjamini Hochberg test, NPX HSL vs. NPX HSN. FDR: false discovery rate. HSL: HS lesional. HSN: HS nonlesional. IQR: interquartile range. NPX: normalised protein expression, a high NPX value corresponds with a high protein concentration.

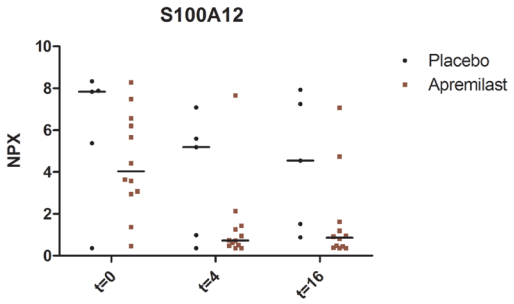

Figure 2. Protein levels of S100A12 in patients receiving apremilast (n = 15) and placebo (n = 5), linear mixed effect model: $p = 0.014$, FDR = 0.186. Relative expression: expression on t = 4 and t = 16 relative to baseline using the $2^{-}\Delta\Delta^{CT}$ method with a baseline value of 1 for each individual sample. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8. NPX, Normalized Protein eXpression; t, week.

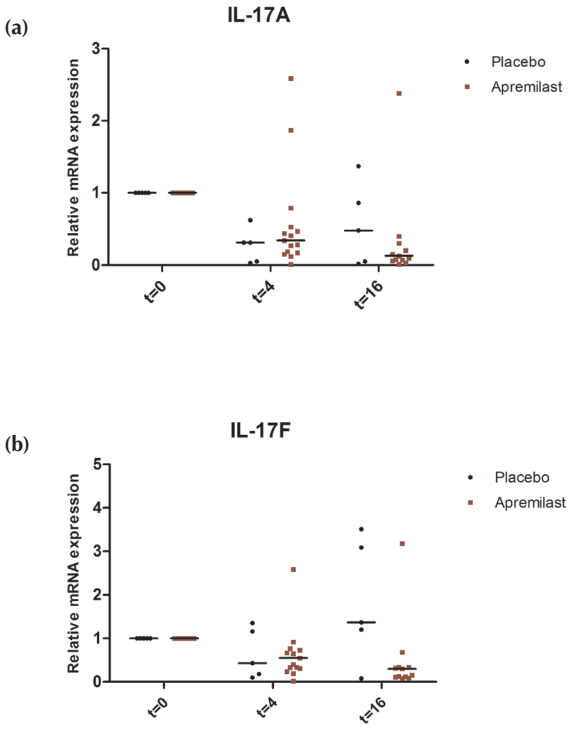

Figure 3. Relative mRNA expression levels of IL-17A and IL-17F in patients receiving apremilast (n = 15) and placebo (n = 5), linear mixed effect model: $p > 0.05$. **(a)** IL-17A. At t = 16 in the apremilast group: one data point is out of the y-axis range. **(b)** IL-17F. At both t = 4 and t = 16 in the apremilast group one data point is out of the y-axis range. Data at week 16 for two patients receiving apremilast are missing as these two patients discontinued after respectively week 4 and week 8. IL, interleukin; t, week.

DISCUSSION

This translational study aimed to identify inflammatory skin markers in response to apremilast 30 mg twice daily for 16 weeks in patients with moderate HS, which showed positive clinical results in a previously published study. Only S100A12 protein levels and *IL-17A* and *IL-17F* mRNA expression showed a clear but nonsignificant decrease in patients receiving apremilast compared with placebo.

S100A12 (calgranulin C, EN-RAGE) is a pro-inflammatory, calcium-binding antimicrobial protein that interacts with the receptor for advanced glycation end products (RAGE) and is overexpressed in various inflammatory skin diseases, e.g. psoriasis.[5,6] Previously, S100A8 and S100A9 (calprotectin or calgranulin A/B) have been demonstrated to be elevated in the serum and lesional skin of HS patients.[7,8] It is known

that IL-17 is a potent inducer of S100A8 and S100A9 in keratinocytes.[9,10] The other way around, S100A12 stimulates T cells to produce IL-17A.[11] Thus, elevated levels of IL-17A and S100A12 in HSL skin at baseline and the responsiveness of these inflammatory markers to apremilast treatment is not surprising. Moreover, apremilast's impact on the Th17 pathway was recently demonstrated by the significant reduction of IL-17A and IL-17F protein levels in the plasma of psoriasis patients.[12]

The nonsignificant findings using a broad panel of inflammatory markers may be explained by the regression to the mean phenomenon, particularly at the individual level.[13] The highly inflammatory index lesions could have spontaneously improved over time in the context of the fluctuating nature of the disease, especially in the placebo group. The effect of regression to the mean increases with larger measurement variability.[13] Expanding the number of biopsies from index lesions, representing several degrees of inflammation, would theoretically be a solution. However, taking more biopsies in the same patients would not be feasible for ethical reasons.

Another explanation for the decreased levels of inflammation over time in both groups could be that the inflammatory infiltrate of the index lesion was gradually reduced by successively taking biopsies from the same nodule. Moreover, the substrate for the foreign body inflammation such as hair fragments and keratin fibres, may have also been (partially) removed by the biopsy procedure.[14] Furthermore, this exploratory study might be underpowered which may also have resulted in nonsignificant results.

Previously, two prospective uncontrolled studies investigating infliximab and ustekinumab in HS were also unable to link translational data to the clinical response.[15,16] However, in these studies only inflammatory markers in serum were investigated. Strengths of our study include the placebo-controlled trial design, the use of skin (target organ) instead of serum or plasma, and the standardised biopsy procedure of an index lesion. A limitation is the small study population.

In conclusion, this translational study investigating apremilast in moderate HS did not detect statistically significant changes in important inflammatory markers in lesional skin compared to placebo over 16 weeks of treatment, despite positive clinical findings. Nonetheless, related S100A12 and IL-17A were significantly elevated in HSL skin and showed a decline only in the apremilast group. In addition, our findings highlight the challenge of assessing pharmacodynamics in the skin in a highly fluctuating inflammatory disease. A better translational model in clinical trials involving HS has yet to be developed.

REFERENCES

1 Zouboulis CC, Desai N, Emtestam L *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619-44.

2 Wei L, Debets R, Hegmans JJ *et al.* IL-1 beta and IFN-gamma induce the regenerative epidermal phenotype of psoriasis in the transwell skin organ culture system. IFN-gamma up-regulates the expression of keratin 17 and keratinocyte transglutaminase via endogenous IL-1 production. *J Pathol* 1999; **187**: 358-64.

3 Companjen AR, van der Wel LI, Wei L *et al.* A modified ex vivo skin organ culture system for functional studies. *Arch Dermatol Res* 2001; **293**: 184-90.

4 Rao X, Huang X, Zhou Z *et al.* An improvement of the 2^(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath* 2013; **3**: 71-85.

5 Eckert RL, Broome AM, Ruse M *et al.* S100 proteins in the epidermis. *J Invest Dermatol* 2004; **123**: 23-33.

6 Wilsmann-Theis D, Wagenpfeil J, Holzinger D *et al.* Among the S100 proteins, S100A12 is the most significant marker for psoriasis disease activity. *J Eur Acad Dermatol Venereol* 2016; **30**: 1165-70.

7 Lima AL, Karl I, Giner T *et al.* Keratinocytes and neutrophils are important sources of proinflammatory molecules in hidradenitis suppurativa. *Br J Dermatol* 2016; **174**: 514-21.

8 Wieland CW, Vogl T, Ordelman A *et al.* Myeloid marker S100A8/A9 and lymphocyte marker, soluble interleukin 2 receptor: biomarkers of hidradenitis suppurativa disease activity? *Br J Dermatol* 2013; **168**: 1252-8.

9 Cho KA, Suh JW, Lee KH *et al.* IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1beta by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *Int Immunol* 2012; **24**: 147-58.

10 Bai B, Yamamoto K, Sato H *et al.* Complex regulation of S100A8 by IL-17, dexamethasone, IL-4 and IL-13 in HaCat cells (human keratinocyte cell line). *J Dermatol Sci* 2007; **47**: 259-62.

11 Kessel C, Lippitz K, Weinhage T *et al.* Proinflammatory Cytokine Environments Can Drive Interleukin-17 Overexpression by gamma/delta T Cells in Systemic Juvenile Idiopathic Arthritis. *Arthritis Rheumatol* 2017; **69**: 1480-94.

12 Garcet S, Nograles K, Correa da Rosa J *et al.* Synergistic cytokine effects as apremilast response predictors in patients with psoriasis. *J Allergy Clin Immunol* 2018.

13 Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *Int J Epidemiol* 2005; **34**: 215-20.

14 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

15 Blok JL, Li K, Brodmerkel C *et al.* Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; **174**: 839-46.

16 Montaudie H, Seitz-Polski B, Cornille A *et al.* Interleukin 6 and high-sensitivity C-reactive protein are potential predictive markers of response to infliximab in hidradenitis suppurativa. *J Am Acad Dermatol* 2017; **76**: 156-8.

Chapter 6.1

Laser hair removal alters the disease course in mild hidradenitis suppurativa

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To the Editor:

Hidradenitis suppurativa (HS) is a chronic, recurrent inflammatory skin disorder of the hair follicle and is characterised by painful, deep-seated inflammatory nodules and abscesses.[1] The disease is difficult to treat, and current medical therapies focus on reducing inflammation rather than preventing flare-ups or sustaining remission.[2] In HS, occlusion of the hair follicle, caused by infundibular keratosis and hyperplasia of the infundibular epithelium, is considered to be the primary event.[3,4] We hypothesised that targeting the hair follicle with laser hair removal would ameliorate the disease. The aim of this case series was to evaluate the effect of hair removal using a long-pulsed 1064-nm neodymium-doped yttrium aluminum garnet (Nd:YAG) laser on the course of the disease in patients with mild HS.

The laser treatments were performed between July 2011 and December 2016 in the Dermatology department of the Erasmus University Medical Center (Rotterdam, The Netherlands). A questionnaire was sent to assess patient-reported outcomes. The primary outcome was the number of HS flares per month, and secondary outcomes were disease severity on a numeric rating scale (NRS) ranging from 0 (no suffering) to 10 (extreme/unbearable suffering), overall treatment satisfaction (NRS: 0 very dissatisfied; 10 very satisfied), and decrease of hair growth in the treated area (quartile grading scale). Descriptive statistics were used to report the outcomes with the mean (± SD) or number (%). For comparison of the number of monthly flares and the disease severity before and after treatment, the Fisher's exact test and the Wilcoxon signed rank test were used, respectively. A two-sided *p*-value of 0.05 was considered significant. Statistical analysis was performed using IBM SPSS Statistics Version 21.0 (Armonk, NY: IBM Corp).

Twenty-seven HS patients with the follicular sub-phenotype and mild disease (HS Physician Global Assessment score of 2, Hurley stage I-A) who had undergone hair removal using the Nd:YAG laser were identified from our records. Two patients were excluded as they had received suboptimal therapy of less than three treatments. The questionnaire was sent to the remaining 25 patients: two patients refused participation and eight patients did not respond to the invitation, resulting in 15 completed questionnaires. Ten men and five women, aged 34.1 ± 10.1 years with a body mass index of 25.1 ± 3.7 were included in the analysis. Twelve patients (80%) were current or ex-smokers, and eleven patients (73%) had skin types of II–III on the Fitzpatrick scale. Patients were most often treated in the inguinal/genital area and received approximately ten treatments on average. The laser settings that were used are shown in Table 1. Three patients received stable systemic co-medication for HS prior to (≥ 28 days) and during the course of the laser hair removal (clindamycin 300 mg twice

Table 1. Characteristics of treatment by laser hair removal.

Type of laser	**1064 nm Nd:YAG^**
Range of settings Nd:YAG	30-60 J/cm^2
	spot size 7-12 mm
	20-40 ms
	2 passes†
Areas, n	
Axillary	5
Inguinal/genital	10
Gluteal/perianal	6
Other*	3
Mean number of treatments ± SD	9.8 ± 9.4
Mean interval ± SD, weeks	5.6 ± 0.1
Mean follow-up ± SD, months	14.9 ± 14.1

^ One patient received 8 treatments with Nd:YAG followed by 11 treatments with Intense Pulsed Light (IPL). † Twelve patients received 2 passes, one patient received mostly 2 passes, and two patients received 1 pass. * Neck, thorax, thigh. SD: standard deviation.

daily and rifampicin 600 mg once daily, minocycline 100 mg once daily, and acitretin 25 mg once daily). None of these treatments statistically affected the study outcomes.

Nd:YAG depilation resulted in a decrease in the number of monthly flares (p = 0.019) (Table 2). In addition, the mean HS disease severity after depilation was significantly lower than before therapy, NRS 6.4 ± 2.8 versus NRS 3.6 ± 3.5 (p = 0.010) respectively. The majority of patients reported a 51-75% decrease of hair growth after treatment. Overall treatment satisfaction was rated with a NRS score of 6.7 ± 2.4, and two-thirds of the patients would recommend laser-assisted hair removal to other HS patients.

Three studies have previously demonstrated improvement of HS after 1064 nm Nd:YAG treatment. These studies focused mainly on targeting the inflammatory lesions (inflammatory nodules, abscesses, and sinuses).[5-7] Treatment involved two to three pulses on the lesions, and the anatomic area also a single pass. We used a different approach by destroying the hair follicles with the intention of altering the course of the disease. Because of this difference in approach, our HS patients were less severely affected than those in previous studies (Hurley II and III disease). Moreover, in the previous studies patients received fewer treatments (maximum four sessions), and the follow-up period was relatively short (maximum two months).[5-7] One study investigated the efficacy of intense pulsed light (IPL) in Hurley stage II and III HS patients; this showed significant improvement in the modified Sartorius score after a follow-up at twelve months.[8]

Table 2. Patient-reported outcomes on the area(s) treated by Nd:YAG (N = 15).

	Before treatment*	**After treatment**	***p*-value**
Flares per month, n			0.019 ^
<1	4	8	
1	2	0	
2	1	3	
3	3	3	
>3 / continuous inflammation	5	1	
Mean NRS for HS disease severity ± SD, (0-10)	6.4 ± 2.8	3.6 ± 3.5	0.010 †
Decrease of hair growth after Nd:YAG			
0-25%		1 (7%)	
26-50%		5 (33%)	
51-75%		7 (47%)	
75-100%		2 (13%)	
Mean NRS for pain due to Nd:YAG ± SD, (0-10)		5.4 ± 2.6	
Mean NRS for overall treatment satisfaction ± SD, (0-10)		6.7 ± 2.4	
Recommendation of laser depilation to other HS patients, n (%)			
yes		10 (67%)	
doubt		4 (26%)	
no		1 (7%)	

NRS: numeric rating scale (0-10). HS: hidradenitis suppurativa. SD: standard deviation * Average number of flares before treatment based on period 6 months prior to treatment. ^ Fisher's exact test. † Wilcoxon signed rank test.

A major strength of our study is that we focused, to the best of our knowledge, for the first time on disease prevention and used the number of flares as an outcome measure. Another strength is the relatively long follow-up period of more than one year. However, this survey-based case series is likely to be underpowered, and the findings we obtained could be biased due to natural fluctuation of the disease course. Other limitations are a possible recall bias and the absence of physician-reported outcomes such as counts of abscesses and nodules.

In conclusion, our results suggest that laser hair removal could be a novel therapeutic approach to prevent disease progression or ameliorate the disease, especially in HS patients with the follicular sub-phenotype. We believe that prospective randomised controlled trials are warranted to confirm the mechanism of action and long-term efficacy of laser hair removal in mild HS.

REFERENCES

1 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

2 Zouboulis CC, Bechara FG, Fritz K *et al.* [S1 guideline for the treatment of hidradenitis suppurativa / acne inversa (ICD-10 Ziffer: L73.2). *J Dtsch Dermatol Ges* 2012; **10 Suppl 5**: S1-31.

3 von Laffert M, Stadie V, Wohlrab J *et al.* Hidradenitis suppurativa/acne inversa: bilocated epithelial hyperplasia with very different sequelae. *Br J Dermatol* 2011; **164**: 367-71.

4 Yu CC, Cook MG. Hidradenitis suppurativa: a disease of follicular epithelium, rather than apocrine glands. *Br J Dermatol* 1990; **122**: 763-9.

5 Tierney E, Mahmoud BH, Hexsel C *et al.* Randomized control trial for the treatment of hidradenitis suppurativa with a neodymium-doped yttrium aluminium garnet laser. *Dermatol Surg* 2009; **35**: 1188-98.

6 Mahmoud BH, Tierney E, Hexsel CL *et al.* Prospective controlled clinical and histopathologic study of hidradenitis suppurativa treated with the long-pulsed neodymium:yttrium-aluminium-garnet laser. *J Am Acad Dermatol* 2010; **62**: 637-45.

7 Xu LY, Wright DR, Mahmoud BH *et al.* Histopathologic study of hidradenitis suppurativa following long-pulsed 1064-nm Nd:YAG laser treatment. *Arch Dermatol* 2011; **147**: 21-8.

8 Highton L, Chan WY, Khwaja N *et al.* Treatment of hidradenitis suppurativa with intense pulsed light: a prospective study. *Plast Reconstr Surg* 2011; **128**: 459-65.

Chapter 6.2

Aggravation of mild axillary hidradenitis suppurativa by microwave ablation: results of a randomised intrapatient-controlled trial

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J Am Acad Dermatol – in press

To the Editor:

Hidradenitis suppurativa (HS) is a common chronic, recurrent, autoinflammatory skin disease of the hair follicle with limited treatment options.[1] No definitive treatment exists for this debilitating entity. MiraDry (Miramar Labs Incorporated, Santa Clara, CA) is a microwave device targeting the eccrine and apocrine sweat glands as well as hair follicles through thermolysis in the dermal-hypodermal junction.[2,3] We hypothesised that this noninvasive ablative technique could potentially improve the clinical symptoms of HS by reducing the number of hair follicles (primary action) and the destruction of the inflammatory cell infiltrate (secondary action) in HS lesions. We, therefore, evaluated the efficacy and safety of miraDry treatment for mild axillary HS in a randomised intrapatient-controlled trial. Ethical approval was given by the IRB of the Erasmus University Medical Center (MEC-2017-390).

We aimed to include 20 HS patients for random allocation to a single miraDry treatment (5.8 GHz, energy level 5, manufacturer-recommended settings) of 1 axilla under tumescent anaesthesia. Patients were required to have a total of 3-5 abscesses or nodules per axilla with ≤1 abscess or draining sinus. Additional inclusion and exclusion criteria are available at https://www.clinicaltrials.gov (identifier NCT03238469). The primary outcome was a left-right comparison of the axillary areas using the Hidradenitis Suppurativa Clinical Response (HiSCR). Secondary outcomes included a numeric rating scale on pain per axilla, treatment satisfaction, and a hair follicle count. Two independent blinded observers performed lesion counts at baseline and 3 months after the procedure.

Only 9 of 20 HS patients were tested; negative clinical outcomes during the recruitment period made it pertinent for us to do an interim analysis, resulting in the decision to discontinue the study. One of the randomised patients did not tolerate the miraDry treatment due to extreme pain during the procedure, despite the use of several local anaesthetics. Of the 8 patients who concluded the miraDry treatment (all women, median age 31.5, interquartile range [IQR] 28.0-39.0 years), 7 completed the 3-month follow-up; 1 patient dropped out because of worsening of HS symptoms in the axilla treated by miraDry. Two patients achieved the HiSCR in the miraDry-treated axilla, and 2 patients achieved the HiSCR in the comparator axilla ($p = 1.00$) (Table 1). In total, 5 of 8 patients showed worsening of their disease after miraDry treatment, with an increase in the abscess and nodule and sinus count (Figure 1). Patients suffered from active lesions for a median (IQR) of 43.0 (4.0-90.0) days in the miraDry-treated axilla versus a median of 5.5 (2.0-26.0) days in the contralateral axilla ($p = 0.14$). After 3 months, the median numeric rating scale score for pain in the miraDry-treated axilla was 7.0 (2.0-8.0) versus 0 (0-5.0) for the untreated axilla

(p = 0.07). One patient developed cellulitis of the upper arm after miraDry treatment, requiring antibiotic treatment, which was classified as a severe adverse event.

We observed that the number of hair follicles after 3 months was numerically lower in the miraDry-treated axilla, median 4.0 (3.0-5.0)/cm^2, a 50.9% decrease from baseline, compared with the untreated counterpart, median 8.5 (6.0-10.0)/cm^2, a 2.0% decline from baseline (p = 0.07). Because the miraDry device targets the dermal zone rather than a particular structure, its nonselectivity might have resulted in the poor study outcomes. Accordingly, we argue that the microwave energy is able to rupture pre-existing and subclinical or microscopic HS precursor lesions (cysts), subsequently resulting in an intense inflammatory response beyond the initially visible lesions.

Although the intervention was completed in only 8 patients, our findings indicate that microwave ablation using the miraDry device has no apparent clinical benefit and could even be harmful in patients with mild HS. Commercial miraDry clinics in the Netherlands also observed a few cases of flaring of the disease in HS patients (personal communication: Dr A. Roopram and Dr W. Venema - May 2018). Taken together, we question the utility of microwave ablative therapy in patients with HS in clinical practice.

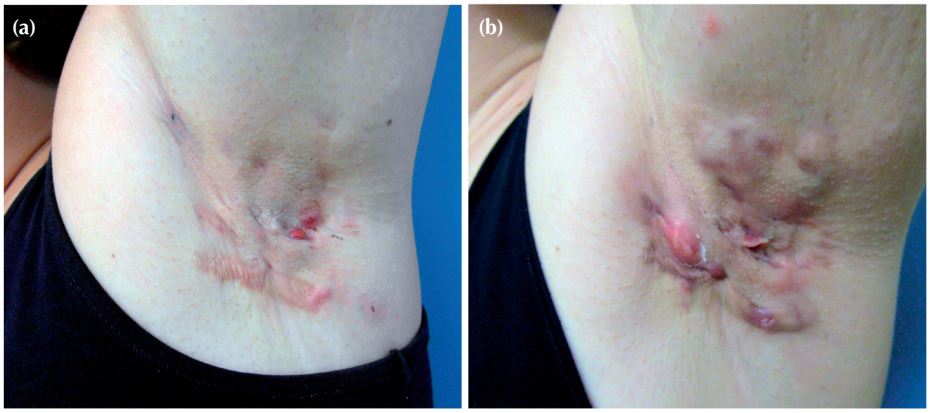


Figure 1. Baseline condition **(a)** and 3-month response **(b)** to miraDry treatment in the left axilla of patient 5 with hidradenitis suppurativa.

Table 1. Hidradenitis suppurativa patient demographics and clinical responses to miraDry treatment.

	Age, y		BMI, kg/m^2		Skintype, Fitzpatrick	HiSCR control axilla	HiSCR miraDry axilla	AN count miraDry		Sinus count miraDry		Hair follicle count miraDry		NRS pain miraDry		Recommendation miraDry	
No.		Sex		Smoking		3 mo	3 mo	0 mo	3 mo	0 mo	3 mo	0 mo	3 mo	0 mo	3 mo	at 3 mo	Adverse events
1	41	F	30.1	Yes	5	+	–	3	4	0	1	14	MI	6	7	No	Edema, erythema
2	28	F	28.1	Yes	5	–	–	4	D	0	D	9	D	9	D	D	Edema
3	28	F	40.5	Yes	2	–	+	3	0	0	0	12	3	6	2	Yes	Edema
4	53	F	31.3	Yes	5	–	+	5	0	0	0	15	9	5	0	Yes	Edema
5	28	F	32.5	No	1	+	–	3	4	0	2	6	4	1	8	No	Edema, erythema, cellulitis
6	29	F	31.9	Yes	4	–	–	4	3	0	1	8	2	6	7	Doubt	Edema, mobility impairment
7	37	F	40.9	Yes	1	–	–	3	3	0	0	4	4	7	6	Doubt	Edema
8	34	F	32.4	Yes	4	–	–	3	3	0	2	10	5	6	8	No	Edema, erythema

The control axilla was treated with topical clindamycin 10 mg/g BID, if necessary. miraDry (Miramar Labs Incorporated, Santa Clara, CA) axilla was treated by microwave ablation. Sinus count included draining sinus, fistula, and tunnel. Hair follicle count was the average number of hair-containing follicles in 3 fields of 1 cm^{-2} assessed by dermoscopy. A miraDry recommendation was obtained by asking patient "Would you recommend the miraDry treatment to other HS patients? – yes, no, doubt." Adverse events were all self-limiting; cellulitis was treated by flucloxacalin 500 mg QID for 10 days. AN: abscess and nodule. BMI: body mass index. D: discontinued because of worsening disease activity in the miraDry-treated axilla. HiSCR: Hidradenitis Suppurativa Clinical Response. MI: missing information. NRS: numeric rating scale for HS-related local pain.

REFERENCES

1 Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. *JAMA* 2017; **318**: 2019-32.

2 Glaser DA, Coleman WP, 3rd, Fan LK *et al*. A randomized, blinded clinical evaluation of a novel microwave device for treating axillary hyperhidrosis: the dermatologic reduction in underarm perspiration study. *Dermatol Surg* 2012; **38**: 185-91.

3 Brauer JA, Neckman JP, Zelickson B *et al*. A Prospective Study of Axillary Hair Reduction in Patients Treated With Microwave Technology. *Dermatol Surg* 2017; **43**: 558-65.

Chapter 7

General discussion

Parts of the discussion are based on:

Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model

Frontiers in Immunology – accepted for publication

CONCLUSIONS AND GENERAL DISCUSSION

Hidradenitis suppurativa (HS) is a chronic, recurrent skin disease harbouring a complex pathogenesis that is not yet fully understood. By identifying the latest publications on the pathophysiology of HS, our systematic review has collated substantial evidence that HS is an immune-mediated inflammatory disease (IMID) with both endogenous and exogenous factors contributing to the onset and progression of the disease.

Firstly, genetic factors play a key role in causing HS. Mutations in a range of genes, including *NCSTN* mutations in the γ-secretase complex and *PSTPIP1* mutations, are directly associated with auto-inflammatory disease.[1-4] However, the majority of HS cases appear to be non-familial, suggesting the existence of separate subsets and the need for stratification within patients diagnosed with HS.[5] Secondly, the simultaneous activation of multiple inflammatory pathways (inflammasome, Th1/Th17, toll-like receptor signalling) result in the upregulation of cytokines and chemokines, including TNF-α and a range of interleukins, which are connected to auto-inflammatory mechanisms in the pathogenesis of HS.[6-8] Thirdly, there is an alteration in the local microbiome of normal-appearing versus lesional skin.[9-12] Data also suggest that bacterial aggregates are associated with inflammation of chronic HS lesions, and it is proposed that they most likely occur as a secondary event, possibly due to predisposing local anatomical changes such as sinus tracts (tunnels), keratinous detritus and dilated hair follicles.[9] Finally, enhancement of HS risk and severity occurs via a range of physiological and environmental factors like smoking, obesity and mechanical friction.[13-16]

As there are multiple gaps in HS knowledge, the aim of this thesis was not restricted to one single facet of the disease. Instead, we took a broader approach and focused on both clinical and translational aspects of HS. Four key themes have emerged from this thesis. First, pruritus or itch is a frequent and bothersome symptom in patients with HS. We detected several pathophysiological substrates that could explain the occurrence of HS-related pruritus. Second, the overexpression of chemokines and cytokines in HS lesional skin reflects a chronic, activated local inflammatory milieu, indicating the need for effective anti-inflammatory HS therapies. Third, the potency and efficacy of novel anti-inflammatory agents for HS were demonstrated in respectively laboratory and clinical trial settings. Fourth, two treatment strategies (primarily) targeting the hair follicle were evaluated. This chapter closes with future perspectives on HS research.

Importance of the symptom itch including possible pathophysiological substrates

Key symptoms of HS are acute and chronic pain, discomfort, and a purulent, foul-smelling discharge, which overall contribute to a decreased quality of life.[17,18] Previous clinical studies have mainly focused on these well-known symptoms, while less is known about pruritus.[17,19,20] Therefore, we determined the prevalence and explored the characteristics of pruritus in a cohort of HS patients. In **Chapter 2** we found a high prevalence rate of 57% in 211 HS patients, who reported a mean (± SD) NRS score (range 0-10) of 6.1 ± 2.0 in the past 7 days. The majority (81%) of patients ranked the severity of pruritus as moderate-to-severe on a five-point Likert scale. Moreover, sleep and ADL were negatively impacted by pruritus in more than half of the patients. The mean modified 5-D itch score of 13.7 ± 3.6 in 52 HS patients is comparable with the 5-D score in 51 patients with an inflammatory skin condition like burn wounds (13.5 ± 3.2).[21]

The prevalence rate we found in HS was similar in a Spanish cohort of 191 HS patients (59%), and higher than a Polish cohort of 103 HS patients (42%).[22,23] However, Matusiak *et al*. did not report the VAS/NRS cut-off point for determination of the prevalence rate in their HS patients (N = 103).[22] In comparison with other dermatologic conditions, the rate in our cohort was similar to that in patients with psoriasis (49-90%),[24,25] although in the past psoriasis was considered a non-pruritic disease. The rate of pruritus in HS patients is lower than patients suffering burn injuries (67-93%)[26,27] or chronic idiopathic urticaria (79%).[28]

In our study, the degree of pruritus was positively correlated with pain intensity, number of HS-affected areas, and active smoking. In addition, we found a significant correlation between the intensity of pruritus and DLQI scores (N = 422, r = 0.47, p = 2.0 e-24, unpublished data) in a subpopulation of the HiScreen Registry, consisting of HS patients from the department of Dermatology in the Erasmus MC and DermaHaven. A similar correlation (r = 0.48) was found in the study of Matusiak *et al* (N = 103).[22] Furthermore, pruritus scores were responsive to anti-inflammatory treatment, as shown by the significant different trends of NRS scores for patients receiving apremilast versus patients receiving placebo (**Chapter 5.1**).

The occurrence of pruritus from HS lesions could be explained by inflammatory cell infiltration, especially the influx of eosinophils and the presence of neurogenic inflammation (**Chapter 2**). Moreover, protein levels of β-nerve growth factor were significantly elevated in lesional skin versus non-lesional skin in the same anatomical area of 20 HS patients (**Chapter 5.2**). Nerve growth factor is known to increase neuropeptide levels. These upregulated neuropeptides, such as substance P and calcitonin gene-related peptide, are associated with neurogenic inflammation and the hypersensitive perception of pruritus.[29,30]

Another explanation for the report of pruritus by HS patients is the presence of tryptase-positive mast cells, which were found to be increased in all stages of the disease including perilesional skin.[31] In **Chapter 3** we found overexpression of CCL-26 (eotaxin-3) in the circulation and lesional skin of HS patients. Additionally, increased serum levels of IgE have been reported in patients with HS.[32] The latter two findings in combination with a dense infiltration of mast cells in HS could trigger degranulation of these cells, releasing histamine and other mediators, such as proteases, which causes pruritus. Levels of IL-2, CCL-11, and CCL-17 (TARC), all inflammatory markers that are associated with pruritus, were not elevated in HS plasma and lesional skin (**Chapter 3**). However, the cause of pruritus in HS is probably multifactorial. Other possible mechanisms leading to itch are a small fibre neuropathy as a result of scar formation,[33] irritant contact dermatitis due to maceration or purulent discharge of HS lesions, and alteration in the signalling of mammalian target of rapamycin (mTOR).[34,35]

Overexpression of chemokines and cytokines reflects chronic inflammation

An aberrant immune response, characterised by the overexpression of several markers of inflammation, is an important element of the HS pathophysiology. Unravelling the role of cytokines and chemokines in disease initiation and progression is essential for the clinical and therapeutic stratification of HS. In **Chapter 3** we showed that the *in vivo* protein levels of IL-12/23p40, IL-16, IL-17A, CXCL-8, CXCL-10, CCL-4, and CCL-26 were significantly higher in HS patients compared with healthy controls. A limitation was the small sample size, which did not allow for subgroup analysis by Hurley stage disease severity. Interestingly, there was no significant correlation between protein levels in patient plasma and lesional skin. The question is whether pathway and drug target discovery in plasma or serum is useful in HS. In another study (**Chapter 5.2**), *ex vivo* protein levels of multiple inflammatory markers including IL-12/23p40, IL-17A and S100A12 (calgranulin C) were significantly elevated in lesional skin versus non-lesional skin in 20 patients suffering moderate HS.

Our results obtained in lesional skin confirm previous findings demonstrating overexpression of IL-17 pathway-associated cytokines and chemokines such as IL-17A, IL-12/23 and CXCL-8 in HS.[36-38] In the context of a strong upregulation of S100A12, CXCL-8, IL-17A and IL-23, we hypothesise that IL-16, CCL-4 and CXCL-10 may participate in the recruitment of leucocyte subsets, especially neutrophils, eosinophils, monocytes and dendritic cells, into the inflamed HS skin.[39,40] The significance of neutrophils in the HS pathogenesis is highlighted by the (very) high levels of CXCL-8 that can be cleaved by neutrophil elastase to activate Th17 cells to produce bioactive IL-17.[41] Furthermore, it has been demonstrated that activated neutrophils induce che-

motaxis of Th17 cells by a reciprocal cross-talk.[42] Some previously published results that showed upregulation of TNF-α, IL-1ß and IL-10 in (peri)lesional HS skin could not be reproduced.[38,43] This can be explained by the different methodologies used. In our study (**Chapter 3**), biopsies were homogenised for *in situ* analysis, while van der Zee *et al.* and Kelly *et al.* cultured the skin specimens for 24 and 3 hours respectively, and measured cytokines in the supernatant.[38,43]

The evidence presented above supports HS as a chronic inflammatory skin disorder associated with alterations in predominantly the innate immune system. An informal literature review was additionally conducted to consider our (**Chapter 3** and **Chapter 5.2**) and previous findings in relation to the immunopathogenesis of other established IMIDs such as Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriasis and psoriatic arthritis, pyoderma gangrenosum, and Behçet's disease. Although these IMIDs are characterised by different pathogeneses, they also share common immunological mechanisms and activated inflammatory pathways (Table 1). The most striking similarity among these diseases is that of aberrations in the innate immune response. Several cytokines are systemically-raised in many of these IMIDs, particularly those implicated in the Th1 and Th17 responses, including TNF-α, IFN-γ, IL-12/23, IL-17, IL-1β and other cytokines of the IL-1 family such as IL-36.[44-48] Several of the pro-inflammatory cytokines have also been shown to be upregulated in HS (e.g. IFN-γ[6], IL-2, TNF-α[7,46] and TNF-β[6]), and are produced by Th1 cells, implicating the Th1 pathway in the pathogenesis of HS. Understanding the distinct and shared immunologic characteristics of IMIDs will aid the development of effective treatments to target the pathogenic mechanisms involved and to modify the disease course.

Novel anti-inflammatory treatments in laboratory and clinical settings

Most evidence to guide management decisions for HS is based on small cohort studies, case reports, and expert opinion.[49] This is illustrated by the low number (only eight) of randomised controlled trials that have investigated the efficacy of anti-inflammatory agents in HS. First-line treatments encompass oral antibiotics with anti-inflammatory properties, mainly tetracyclines and the combination of clindamycin and rifampicin.[19] Second-line therapies include anti-TNF-α biologics such as adalimumab, which is the only registered drug for moderate-to-severe HS with clinically relevant improvements.[50] Other biologic therapies such as secukinumab (anti-IL-17A) and ustekinumab (anti-IL-12/23p40) have not widely been studied in patients with HS or are in the early stages of clinical development.[51] Moreover, very little is known about small molecule drugs that modulate the production of pro- and anti-inflammatory cytokines in HS. Taken together, there is limited high-quality evidence on HS treatment indicating a significant need for novel efficacious anti-inflammatory therapies.

Table 1. Key cytokines of established immune mediated inflammatory diseases in relation to hidradenitis suppurativa.

Disease	Disease overview	Key^ cytokine profile	References
HS	Inflammatory skin disease with genetic, immunological, and environmental background	Th1, Th17 IL-1β, 6, CXCL/IL-8,12, 17, 23, IFN-γ, TNF-α	Banerjee *et al.*, 2017; Boer, 2017; Calderon-Castrat *et al.*, 2016; Di Caprio *et al.*, 2017; Hessam *et al.*, 2015; Jimenez-Gallo *et al.*, 2017; Kromann *et al.*, 2014; Marzano *et al.*, 2017; Marzano *et al.*, 2013; Nomura *et al.*, 2013; Thomi *et al.*, 2017; Xiao *et al.*, 2016; Zhang *et al.*, 2013
CD	Imbalance between gut microbiome and host immune system with genetic background	Th1, Th17 IL-1β, 6, 12, 17, 23, IFN-γ, TNF-α	Hugot *et al.*, 2001; Ogura *et al.*, 2001; Park *et al.*, 2017
UC	Imbalance between gut microbiome and host immune system with genetic background	Th2, Th17 IL-1β, 6, 12, 13, 17, 23 TNF-α	Park *et al.*, 2017
AS	Imbalance between gut microbiome and host immune system with genetic background	Th17 IL-6, 17, 22, 23, 26, IFN-γ, TNF-α	Brown, 2017; Gooren *et al.*, 2000; Jethwa and Bowness, 2016; Li and Brown, 2017; O'Rielly *et al.*, 2016; Sparks and Costenbader, 2014; Videm *et al.*, 2014
Psoriasis	Inflammatory skin disease with genetic and immunological background	Th1, Th17 IL-2, 17, 22, 23, 26, TNF-α, IFN-γ	El-Boghdady *et al.*, 2017; Ho *et al.*, 2005; Love *et al.*, 2012; Nguyen *et al.*, 2018; Ogawa *et al.*, 2017; Strange *et al.*, 2010
PsA	Inflammatory arthritis associated with psoriasis with genetic, immunological, and environmental background	Th1, Th17 IL-17, 23, TNF-α	
PG	Inflammatory, ulcerating, neutrophilic skin disease with genetic, immunological, and environmental background	IL-1β, 17, TNF-α	Al Ghazal *et al.*, 2012; Marzano *et al.*, 2017; Shavit *et al.*, 2017; Thomsen and Sorensen, 2010
Behçet's disease	Multi-systemic, inflammatory, vasculitis with genetic, immunological, and environmental background	Th1, Th17 IL-6, 11, 17, 21, 22, 26, TNF-α, Chitinase3-like1, gp130/sIL -6Rb, sTNF-R1, sTNF-R2	Hemminki *et al.*, 2012; Lopalco *et al.*, 2017; Scherrer *et al.*, 2017; Thomsen and Sorensen, 2010

^ Data summarise key cytokines of these diseases but many other genes, cells types and mediators are involved in the pathogenesis. AS: ankylosing spondylitis. CD: Crohn's disease. HS: hidradenitis suppurativa. PG: pyoderma gangrenosum; PsA, psoriasis and psoriatic arthritis; UC, ulcerative colitis.

In an *ex vivo* disease model, we evaluated the anti-inflammatory effects of currently available biologics targeting TNF-α, IL-17A, IL-12/23p40, and CD20. Adalimumab, infliximab, secukinumab, ustekinumab and rituximab in addition to prednisolone significantly inhibited a selection of pro-inflammatory cytokines and antimicrobial peptides in HS lesional skin (**Chapter 4**). Furthermore, adalimumab, infliximab and prednisolone reduced the levels of a broader mix of individual cytokines than secukinumab, ustekinumab, and rituximab. These findings correspond with the observed efficacy of both TNF-α inhibitors, and systemic and intralesional corticosteroids in the treatment of HS patients in daily practice.[50,52,53] Moreover, the inter-patient variability in the response to the biologics could explain why some HS patients are successfully treated while others show a lesser response. Interestingly, in our *ex vivo* assay, secukinumab did not inhibit protein concentration of respectively IL-17 in the same way as adalimumab and infliximab reduced that of TNF-α. Unfortunately, IL-12p40 protein, an important indicator of the IL-17 pathway, fell below the level of detection in the multiplex assay. However, the lower mRNA expression of the AMPs, IL-6 and CXCL-8 can be considered as the indirect result of blocking the bioactivity of IL-17A and IL-12/23p40 by secukinumab and ustekinumab, respectively. Rituximab was the only biologic without a significant inhibitory effect on individual inflammatory mRNA and protein levels. This is not surprising as B cell blockade in inflammatory diseases acts via inhibition of antibody production, antigen presentation and indirectly via cytokine reduction.[54]

In conclusion, our *ex vivo* skin culture system represents an adequate model for studies in search of novel candidate drugs for the treatment of HS, and to personalise the treatment in specific patients. Future *ex vivo* studies could focus on dose-response relationships, combinations of monoclonal antibodies or bi/trifunctional antibodies, and biologics in combination with low dose prednisolone.

In a clinical setting, we studied the efficacy and tolerability of apremilast at a dosage of 30 mg twice daily in patients with moderate HS. A clinical response as measured by the HiSCR50 was met in 8 of 15 (53.3%) patients in the apremilast group and none of 5 patients (0%) in the placebo group at week 16 (**Chapter 5.1**). This response rate is within the range of the proportion of HiSCR50 achievers reported in four studies which have investigated biologics in patients suffering moderate-to-severe HS; 41.8% to 60.0%.[50,55,56] In addition, patients treated with apremilast showed a significantly lower abscess and nodule count, and levels of pain and pruritus over 16 weeks compared with placebo-treated patients. Although the DLQI improved in the apremilast-treated patients, its trend over time was not significantly different between the treatment arms, possibly because of the large variability observed. Furthermore, treatment with apremilast was generally well tolerated, similar to the safety data from larger trials in patients with psoriasis and psoriatic arthritis.[57,58]

We believe that apremilast is a valuable option after failure of conventional treatments such as the combination of clindamycin and rifampicin. Moreover, apremilast may have the following potential advantages over both antibiotics and biologics. A drawback of recurrent or long-term antibiotic treatment is the risk of inducing bacterial resistance. A problem with the use of TNF-α antagonists such as adalimumab and infliximab is the risk of anti-drug antibody formation with neutralisation of the therapeutic effect over time.[59]

In **Chapter 5.2** we aimed to detect changes in the expression of important inflammatory markers in lesional skin between the two treatment arms. Although we did not observe significant differences, related S100A12 and IL-17A were significantly elevated in lesional skin and showed a decline only in the apremilast group. Moreover, in psoriasis, the significant reduction of IL-17A and IL-17F plasma protein levels after apremilast treatment highlights its impact on the Th17 pathway.[60] Our nonsignificant results could be explained by several determinants. First, the regression to the mean phenomenon should be considered as a possible cause as the highly inflammatory index lesions could have spontaneously improved over time in the context of the fluctuating nature of the disease, especially in the placebo group. Second, the inflammatory infiltrate and substrates for the foreign body inflammation were gradually reduced by successively taking biopsies from the same nodule. Third, the small and possibly underpowered study population could have resulted in the nonsignificant findings. Previously, two open label studies investigating respectively infliximab and ustekinumab in HS patients neither found a change in inflammatory protein serum levels after treatment, nor could link translational data to the clinical response.[61,62] In conclusion, assessing pharmacodynamics (in the skin) in a highly fluctuating inflammatory disease remains challenging. A better translational model in clinical trials involving HS has yet to be developed.

Modifying the disease course by targeting the hair follicle yielded ambiguous results

Current treatment strategies primarily focus on treating the consequences rather than preventing flare-ups or sustaining remission. Because follicular occlusion is considered to be the primary event in the pathogenesis of HS, we hypothesised that targeting the hair follicle would ameliorate the disease. Therefore, two non-invasive techniques that could potentially improve the symptoms and clinical course of HS were separately studied: hair reduction using the 1064-nm neodymium-doped yttrium aluminium garnet (Nd:YAG) laser and microwave ablation (miraDry).

Three previous studies that used the Nd:YAG laser in HS primarily focused on targeting the inflammatory lesions rather than destroying the hair follicles.[63-65] We retrospectively evaluated the effect of Nd:YAG depilation of a whole anatomic area

in 15 patients with Hurley stage I and HS-PGA 2. Because of the different approach in using the Nd:YAG laser, our HS patients were less severely affected than those in previous studies (Hurley II and III disease severity). Treatment with Nd:YAG resulted in a significant decrease in the number of patient-reported monthly flares after a follow-up period of on average more than one year (**Chapter 6.1**). In addition, mean HS disease severity after depilation as measured by a NRS was significantly lower in comparison with before therapy. Two-thirds of the patients would recommend Nd:YAG depilation to other HS patients. These results suggest that laser hair removal could be a novel therapeutic approach to prevent disease progression or ameliorate the disease, especially in HS patients with the follicular sub-phenotype.[66] However, our findings could be biased due to natural fluctuation of the disease course as there was no control group. Other study limitations are a possible recall bias and the absence of physician-reported outcomes such as an abscess and nodule count. Prospective randomised controlled trials are warranted to confirm the mechanism of action and long-term efficacy of laser hair removal in mild HS.

Recently, non-invasive microwave ablation (MWA) using the miraDry device have demonstrated promising results for permanent reduction of axillary hairs.[67] There may be advantages of MWA over light- and laser-based methods. MWA requires only 1 or 2 sessions to achieve 70% reduction of hair growth,[67] while light- and laser-based methods often require 6 to 10 sessions to realise such results. Moreover, outcomes of MWA are independent of skin type and hair colour.[67]

We hypothesised that MWA could potentially improve the symptoms of HS by reducing the number of hair follicles (primary action) and the destruction of the inflammatory cell infiltrate (secondary action) in HS lesions. Therefore, in **Chapter 6.2**, we evaluated the efficacy and safety of MWA for mild axillary HS in a randomised intrapatient-controlled trial. Only 9 of 20 planned HS patients were included because the study was prematurely terminated due to negative clinical outcomes. In total, 8 patients completed the three month follow-up, of which 5 showed worsening of their disease after microwave ablative therapy. Commercial miraDry clinics in The Netherlands also observed a few cases of flaring of the disease in HS patients (personal communication).

As the miraDry device targets a zone rather than a particular structure, its non-selectivity might have resulted in the poor study outcomes. Accordingly, we argue that the microwave energy is able to rupture pre-existing and subclinical or microscopic HS precursor lesions (cysts), subsequently resulting in an intense inflammatory response beyond the initially visible lesions. In addition, the development of HS-like lesions such as abscesses and nodules are a relatively frequent complication of miraDry treatment in otherwise healthy subjects with axillary hyperhidrosis.[68] We used miraDry energy level 5 (manufacturer's recommended setting) corresponding

with an energy delivery time of 3.0 seconds. Although the delivery time in level 1 (lowest setting) is only 0.6 seconds shorter than level 5 (highest setting), the effect on hair reduction using the lowest setting has never been reported. In conclusion, our findings indicate that MWA using the miraDry device has no apparent clinical benefit and may even be harmful in patients with mild axillary HS. Therefore, we question the utility of microwave ablative therapy in patients with HS in clinical practice.

FUTURE PERSPECTIVES

Pathomechanisms

Despite the rising number of publications on HS in the recent years, there are still many questions to be addressed. Therefore, further research in various arenas is warranted to ultimately improve the management and treatment of patients with HS and related syndromic conditions. Large gaps remain in the understanding of the pathogenesis of HS. Genetic research should aim to add more detail to the proposed mechanism by which loss of function of NCSTN or of other γ-secretase proteins causes familial HS, and to better stratify patients with HS. Immunologic studies should focus on molecular drivers of tissue inflammation and injury in HS and the relationship between the HS cytokine profile and disease activity. Next generation sequencing methods will help unravelling the genome, transcriptome, and proteome of HS patients. Furthermore, microbiome research is needed to better characterise the disruption to the microbial ecosystem and to elucidate whether the disruption causes the disease or whether the disease causes the dysbiosis. High-throughput metagenomic methods can make this work possible. Finally, it will be important to focus research on the interaction of environmental factors and immunogenetic factors.

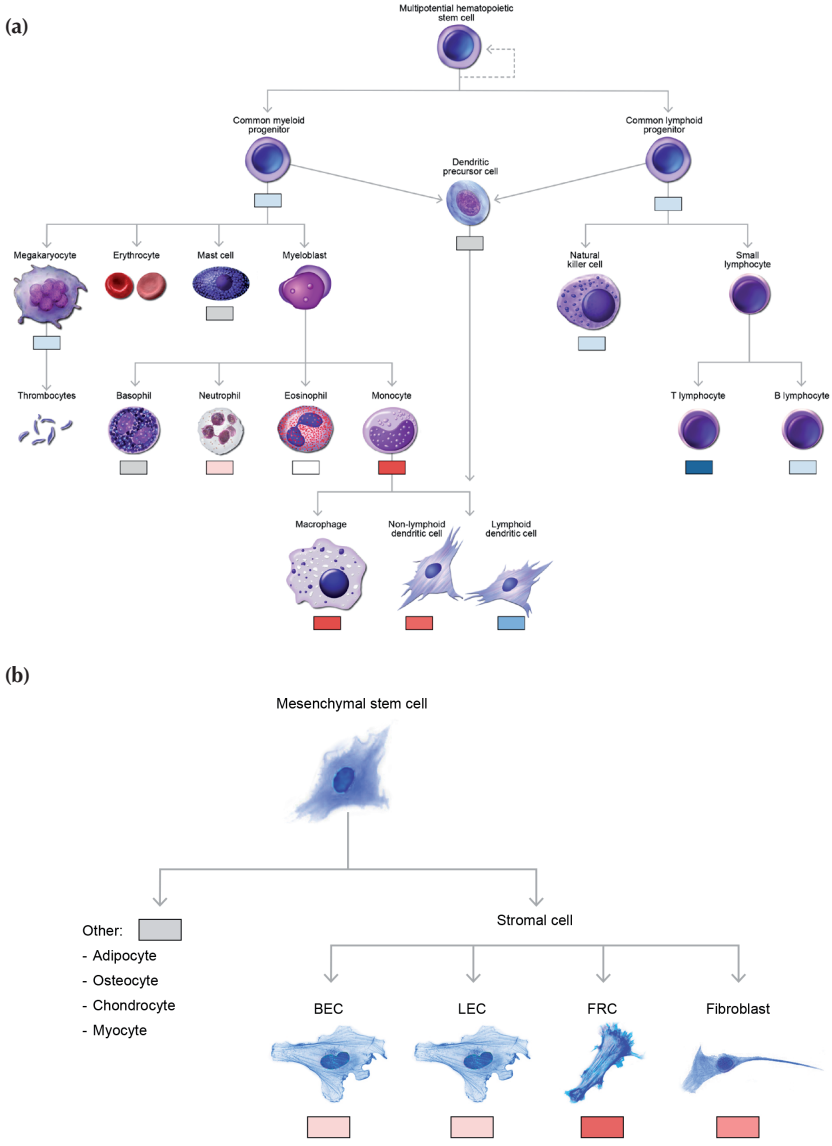
Immunogenetic research in progress

In 2017, we reported a novel *NCSTN* mutation in a three-generation Dutch family with HS.[69] In HS, 83% (30/36) of the previously reported sequence variants in the γ-secretase complex are scattered throughout the extracellular domain of the *NCSTN* gene without particular hotspots,[70] indicating a critical role for *NCSTN* in the stability of the γ-secretase complex.[71] Because the nicastrin protein exhibits multiple conserved residues and is for 88% (63/72 amino acids) homologous to the murine counterpart, we used the microarray dataset of the Immunological Genome Project (ImmGen) to perform a thorough dissection of the expression and function of *NCSTN* in the immune system. In short, the expression data of the *NCSTN* gene were normalised as part of the ImmGen pipeline by Robust Multichip Average as described by Jojic *et al*.[72]

Gene Expression Omnibus data were subsequently log^2-transformed and signatures for 16 cell lineages (both hematopoietic and mesenchymal) were calculated.

Wildtype *NCSTN* appeared to be upregulated in myeloid cells like monocytes and macrophages, and mesenchymal cells such as fibroblastic reticular cells and fibroblasts (Figure 1). We hypothesise that mutated *NCSTN* variants could affect the function of these cell lineages, ultimately leading to an aberrant immune response, especially in the skin. In addition, *ARNT*, *PPARδ* and *CAPNS1* were identified in the 25 highest co-expressed genes with *NCSTN* (Figure 2). The *ARNT* gene encodes the aryl hydrocarbon receptor nuclear translocator protein. The aryl hydrocarbon receptor (AhR) is involved in the induction of several enzymes that participate in xenobiotic metabolism, including dioxin and polycyclic aromatic hydrocarbons which are present in cigarette smoke.[73] *PPARδ* facilitates AhR signalling, enhances fatty acid catabolism, and induces keratinocyte differentiation.[74,75] Calpain-like proteases process the precursor form of IL-1α into the biologically active mature form, an important pro-inflammatory cytokine in epithelial and myeloid cells.[76,77]

In summary, the associated immunobiological functions of *NCSTN*, *ARNT* and *PPARδ* link genetics to the environment, which are smoking, the metabolic syndrome, and the skin microbiome.[78] Because we observed a positive association between pack years of smoking and disease severity in the three affected family members, we are investigating the role of AhR ligands and its relation to bacterial products in patients with both familial and common HS in a laboratory setting.



Legend

	Z-score	
Count per cell line	≥ 0	≤ 0
No activity		
0[a]		
1 to 3[b]		
4 to 6		
7 to 9		
10 to 12		
13 to 15		
16+		
No ImmGen reference		

[a] 0 corresponds with ^{2}Log ≤0.5 or ≥-0.5
[b] ≥1 corresponds with ^{2}Log ≥0.5 or ≤-0.5

Figure 1. Wildtype *NCSTN* expression in hematopoietic and mesenchymal cell lineages. The colour intensity correlates with the degree of change. **(a)** Myeloid signature with upregulation in monocytes, macrophages, non-lymphoid dendritic cells, and neutrophils. **(b)** Upregulation of stromal cells and typically fibroblastic reticular cells and fibroblasts. BEC: blood endothelial cell. FRC: fibroblastic reticular cell. LEC: lymphatic endothelial cell.

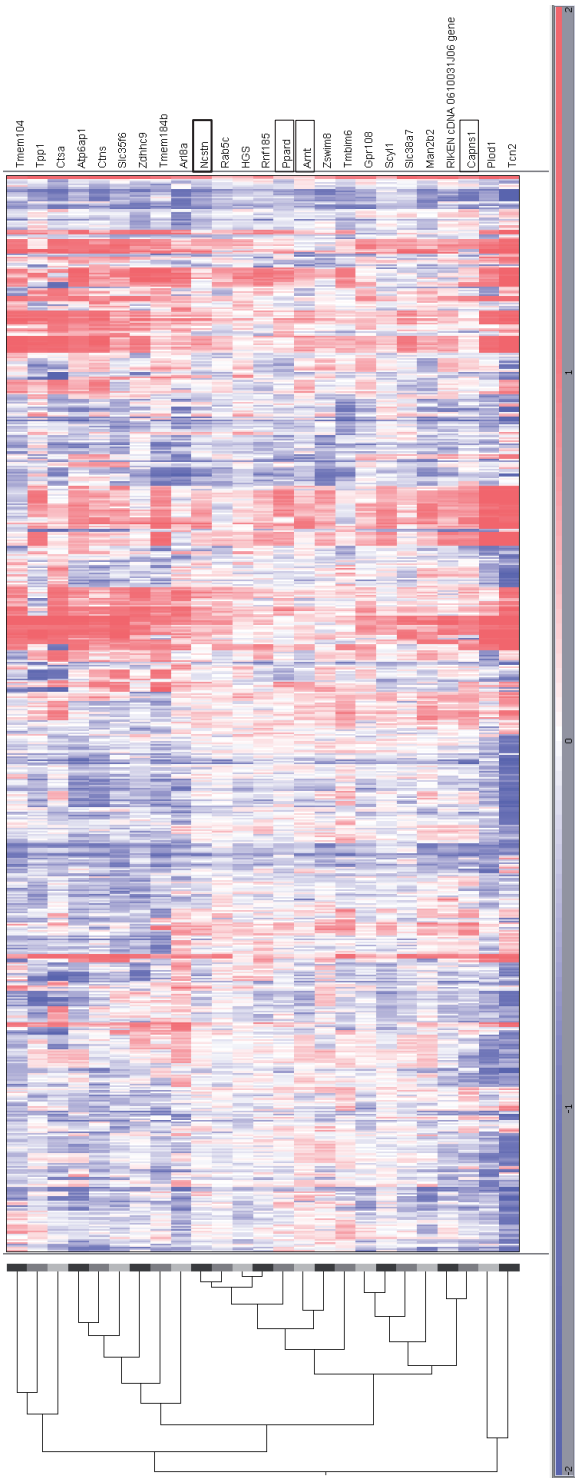


Figure 2. OmniViz Treescape showing the top 25 co-expressed genes related to *NCSTN*. Gene expression levels: red colour, up-regulated genes compared with the geometric mean; blue colour, down-regulated genes compared with the geometric mean. The colour intensity positively correlates with the degree of change. *ARNT*: aryl hydrocarbon receptor nuclear transporter. *CAPNS1*: calpain small subunit 1. *PPARD*: peroxisome proliferator-activated receptor delta.

REFERENCES

1 Zhang C, Wang L, Chen L *et al*. Two novel mutations of the NCSTN gene in Chinese familial acne inverse. *J Eur Acad Dermatol Venereol* 2013; **27**: 1571-4.

2 Zeeli T, Padalon-Brauch G, Ellenbogen E *et al*. Pyoderma gangrenosum, acne and ulcerative colitis in a patient with a novel mutation in the PSTPIP1 gene. *Clinical and Experimental Dermatology* 2015; **40**: 367-72.

3 Marcos T, Ruiz-Martin V, de la Puerta ML *et al*. Proline-serine-threonine phosphatase interacting protein 1 inhibition of T-cell receptor signaling depends on its SH3 domain. *The FEBS Journal* 2014; **281**: 3844-54.

4 Xiao X, He Y, Li C *et al*. Nicastrin mutations in familial acne inversa impact keratinocyte proliferation and differentiation through the Notch and phosphoinositide 3-kinase/AKT signalling pathways. *Br J Dermatol* 2016; **174**: 522-32.

5 Nomura Y, Nomura T, Sakai K *et al*. A novel splice site mutation in NCSTN underlies a Japanese family with hidradenitis suppurativa. *Br J Dermatol* 2013; **168**: 206-9.

6 Banerjee A, McNish S, Shanmugam VK. Interferon-gamma (IFN-gamma) is Elevated in Wound Exudate from Hidradenitis Suppurativa. *Immunological investigations* 2017; **46**: 149-58.

7 Mozeika E, Pilmane M, Nurnberg BM *et al*. Tumour necrosis factor-alpha and matrix metalloproteinase-2 are expressed strongly in hidradenitis suppurativa. *Acta Derm Venereol* 2013; **93**: 301-4.

8 Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L *et al*. The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa. *Mediators Inflamm* 2017; **2017**: 2450401.

9 Ring HC, Bay L, Nilsson M *et al*. Bacterial biofilm in chronic lesions of hidradenitis suppurativa. *Br J Dermatol* 2017; **176**: 993-1000.

10 Ring HC, Thorsen J, Saunte DM *et al*. The Follicular Skin Microbiome in Patients With Hidradenitis Suppurativa and Healthy Controls. *JAMA dermatology* 2017; **153**: 897-905.

11 Guet-Revillet H, Coignard-Biehler H, Jais JP *et al*. Bacterial pathogens associated with hidradenitis suppurativa, France. *Emerg Infect Dis* 2014; **20**: 1990-8.

12 Guet-Revillet H, Jais JP, Ungeheuer MN *et al*. The Microbiological Landscape of Anaerobic Infections in Hidradenitis Suppurativa: A Prospective Metagenomic Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**: 282-91.

13 Kromann CB, Deckers IE, Esmann S *et al*. Risk factors, clinical course and long-term prognosis in hidradenitis suppurativa: a cross-sectional study. *Br J Dermatol* 2014; **171**: 819-24.

14 Hessam S, Sand M, Gambichler T *et al*. Correlation of inflammatory serum markers with disease severity in patients with hidradenitis suppurativa (HS). *Journal of the American Academy of Dermatology* 2015; **73**: 998-1005.

15 Boer J. Should Hidradenitis Suppurativa Be Included in Dermatoses Showing Koebnerization? Is It Friction or Fiction? *Dermatology* 2017; **233**: 47-52.

16 Vossen A, van der Zee HH, Onderdijk AJ *et al.* Hidradenitis suppurativa is not associated with the metabolic syndrome based on body type: A cross-sectional study. *The Journal of Dermatology* 2017; **44**: 154–9.

17 Dufour DN, Emtestam L, Jemec GB. Hidradenitis suppurativa: a common and burdensome, yet under-recognised, inflammatory skin disease. *Postgrad Med J* 2014; **90**: 216-21.

18 Matusiak L, Bieniek A, Szepietowski JC. Hidradenitis suppurativa markedly decreases quality of life and professional activity. *J Am Acad Dermatol* 2010; **62**: 706-8.

19 Zouboulis CC, Desai N, Emtestam L *et al.* European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa. *J Eur Acad Dermatol Venereol* 2015; **29**: 619-44.

20 Jemec GB. Clinical practice. Hidradenitis suppurativa. *N Engl J Med* 2012; **366**: 158-64.

21 Elman S, Hynan LS, Gabriel V *et al.* The 5-D itch scale: a new measure of pruritus. *Br J Dermatol* 2010; **162**: 587-93.

22 Matusiak L, Szczech J, Kaaz K *et al.* Clinical Characteristics of Pruritus and Pain in Patients with Hidradenitis Suppurativa. *Acta Derm Venereol* 2018; **98**: 191-4.

23 Pascual JC, Alvarez P, Encabo B *et al.* Comment on: "Assessing Pruritus in Hidradenitis Suppurativa: A Cross-Sectional Study". *Am J Clin Dermatol* 2017; **18**: 705-6.

24 Remrod C, Sjostrom K, Svensson A. Pruritus in psoriasis: a study of personality traits, depression and anxiety. *Acta Derm Venereol* 2015; **95**: 439-43.

25 Kopyciok ME, Stander HF, Osada N *et al.* Prevalence and Characteristics of Pruritus: A One-Week Cross-sectional Study in a German Dermatology Practice. *Acta Derm Venereol* 2016; **96**: 50-5.

26 Van Loey NE, Bremer M, Faber AW *et al.* Itching following burns: epidemiology and predictors. *Br J Dermatol* 2008; **158**: 95-100.

27 Carrougher GJ, Martinez EM, McMullen KS *et al.* Pruritus in adult burn survivors: postburn prevalence and risk factors associated with increased intensity. *J Burn Care Res* 2013; **34**: 94-101.

28 Yosipovitch G, Ansari N, Goon A *et al.* Clinical characteristics of pruritus in chronic idiopathic urticaria. *Br J Dermatol* 2002; **147**: 32-6.

29 Verge VM, Richardson PM, Wiesenfeld-Hallin Z *et al.* Differential influence of nerve growth factor on neuropeptide expression in vivo: a novel role in peptide suppression in adult sensory neurons. *J Neurosci* 1995; **15**: 2081-96.

30 Steinhoff M, Stander S, Seeliger S *et al.* Modern aspects of cutaneous neurogenic inflammation. *Arch Dermatol* 2003; **139**: 1479-88.

31 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

32 Pascual JC, Garcia-Martinez FJ, Martorell A *et al.* Increased total serum IgE levels in moderate-to-severe hidradenitis suppurativa. *Br J Dermatol* 2016; **175**: 1101-2.

33 Chuquilin M, Alghalith Y, Fernandez KH. Neurocutaneous disease: Cutaneous neuroanatomy and mechanisms of itch and pain. *J Am Acad Dermatol* 2016; **74**: 197-212.

34 Monfrecola G, Balato A, Caiazzo G *et al.* Mammalian target of rapamycin, insulin resistance and hidradenitis suppurativa: a possible metabolic loop. *J Eur Acad Dermatol Venereol* 2016; **30**: 1631-3.

35 Essig K, Hu D, Guimaraes JC *et al.* Roquin Suppresses the PI3K-mTOR Signaling Pathway to Inhibit T Helper Cell Differentiation and Conversion of Treg to Tfr Cells. *Immunity* 2017; **47**: 1067-82.

36 van der Zee HH, Laman JD, de Ruiter L *et al.* Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

37 Schlapbach C, Hanni T, Yawalkar N *et al.* Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**: 790-8.

38 Kelly G, Hughes R, McGarry T *et al.* Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. *Br J Dermatol* 2015; **173**: 1431-9.

39 Wolpe SD, Davatelis G, Sherry B *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J Exp Med* 1988; **167**: 570-81.

40 Tecchio C, Cassatella MA. Neutrophil-derived chemokines on the road to immunity. *Semin Immunol* 2016; **28**: 119-28.

41 Souwer Y, Groot Kormelink T, Taanman-Kueter EW *et al.* Human TH17 cell development requires processing of dendritic cell-derived CXCL8 by neutrophil elastase. *J Allergy Clin Immunol* 2018; **141**: 2286-9.

42 Pelletier M, Maggi L, Micheletti A *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010; **115**: 335-43.

43 van der Zee HH, de Ruiter L, van den Broecke DG *et al.* Elevated levels of tumour necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-alpha and IL-1beta. *Br J Dermatol* 2011; **164**: 1292-8.

44 O'Rielly DD, Uddin M, Rahman P. Ankylosing spondylitis: beyond genome-wide association studies. *Current Opinion in Rheumatology* 2016; **28**: 337-45.

45 Ogawa E, Sato Y, Minagawa A *et al.* Pathogenesis of psoriasis and development of treatment. *The Journal of dermatology* 2017.

46 Marzano AV, Damiani G, Ceccherini I *et al.* Autoinflammation in pyoderma gangrenosum and its syndromic form (pyoderma gangrenosum, acne and suppurative hidradenitis). *Br J Dermatol* 2017; **176**: 1588-98.

47 Lopalco G, Lucherini OM, Lopalco A *et al.* Cytokine Signatures in Mucocutaneous and Ocular Behcet's Disease. *Frontiers in Immunology* 2017; **8**: 200.

48 Di Caprio R, Balato A, Caiazzo G *et al.* IL-36 cytokines are increased in acne and hidradenitis suppurativa. *Archives of Dermatological Research* 2017; **309**: 673-8.

49 Zouboulis CC, Bechara FG, Dickinson-Blok JL *et al.* Hidradenitis suppurativa/acne inversa: A practical framework for treatment optimization - systematic review and recommendations from the HS ALLIANCE working group. *J Eur Acad Dermatol Venereol* 2018.

50 Kimball AB, Okun MM, Williams DA *et al.* Two Phase 3 Trials of Adalimumab for Hidradenitis Suppurativa. *N Engl J Med* 2016; **375**: 422-34.

51 Theut Riis P, Thorlacius LR, Jemec GB. Investigational drugs in clinical trials for Hidradenitis Suppurativa. *Expert Opin Investig Drugs* 2018; **27**: 43-53.

52 Delage M, Samimi M, Atlan M *et al.* Efficacy of infliximab for hidradenitis suppurativa: assessment of clinical and biological inflammatory markers. *Acta Derm Venereol* 2011; **91**: 169-71.

53 Riis PT, Boer J, Prens EP *et al.* Intralesional triamcinolone for flares of hidradenitis suppurativa (HS): A case series. *J Am Acad Dermatol* 2016; **75**: 1151-5.

54 Dorner T, Kinnman N, Tak PP. Targeting B cells in immune-mediated inflammatory disease: a comprehensive review of mechanisms of action and identification of biomarkers. *Pharmacol Ther* 2010; **125**: 464-75.

55 Kanni T, Argyropoulou M, Spyridopoulos T *et al.* MABp1 Targeting IL-1alpha for Moderate to Severe Hidradenitis Suppurativa Not Eligible for Adalimumab: A Randomized Study. *J Invest Dermatol* 2018; **138**: 795-801.

56 Kimball AB, Sobell JM, Zouboulis CC *et al.* HiSCR (Hidradenitis Suppurativa Clinical Response): a novel clinical endpoint to evaluate therapeutic outcomes in patients with hidradenitis suppurativa from the placebo-controlled portion of a phase 2 adalimumab study. *J Eur Acad Dermatol Venereol* 2016; **30**: 989-94.

57 Papp K, Cather JC, Rosoph L *et al.* Efficacy of apremilast in the treatment of moderate to severe psoriasis: a randomised controlled trial. *Lancet* 2012; **380**: 738-46.

58 Schett G, Wollenhaupt J, Papp K *et al.* Oral apremilast in the treatment of active psoriatic arthritis: results of a multicenter, randomized, double-blind, placebo-controlled study. *Arthritis Rheum* 2012; **64**: 3156-67.

59 Vincent FB, Morand EF, Murphy K *et al.* Antidrug antibodies (ADAb) to tumour necrosis factor (TNF)-specific neutralising agents in chronic inflammatory diseases: a real issue, a clinical perspective. *Ann Rheum Dis* 2013; **72**: 165-78.

60 Garcet S, Nograles K, Correa da Rosa J *et al.* Synergistic cytokine effects as apremilast response predictors in patients with psoriasis. *J Allergy Clin Immunol* 2018.

61 Blok JL, Li K, Brodmerkel C *et al.* Ustekinumab in hidradenitis suppurativa: clinical results and a search for potential biomarkers in serum. *Br J Dermatol* 2016; **174**: 839-46.

62 Montaudie H, Seitz-Polski B, Cornille A *et al.* Interleukin 6 and high-sensitivity C-reactive protein are potential predictive markers of response to infliximab in hidradenitis suppurativa. *J Am Acad Dermatol* 2017; **76**: 156-8.

63 Tierney E, Mahmoud BH, Hexsel C *et al.* Randomized control trial for the treatment of hidradenitis suppurativa with a neodymium-doped yttrium aluminium garnet laser. *Dermatol Surg* 2009; **35**: 1188-98.

64 Mahmoud BH, Tierney E, Hexsel CL *et al.* Prospective controlled clinical and histopathologic study of hidradenitis suppurativa treated with the long-pulsed neodymium:yttrium-aluminium-garnet laser. *J Am Acad Dermatol* 2010; **62**: 637-45.

65 Xu LY, Wright DR, Mahmoud BH *et al.* Histopathologic study of hidradenitis suppurativa following long-pulsed 1064-nm Nd:YAG laser treatment. *Arch Dermatol* 2011; **147**: 21-8.

66 van der Zee HH, Jemec GB. New insights into the diagnosis of hidradenitis suppurativa: Clinical presentations and phenotypes. *J Am Acad Dermatol* 2015; **73**: S23-6.

67 Brauer JA, Neckman JP, Zelickson B *et al.* A Prospective Study of Axillary Hair Reduction in Patients Treated With Microwave Technology. *Dermatol Surg* 2017; **43**: 558-65.

68 Huang A, Lindgren A, Krempa M *et al.* Hidradenitis Suppurativa-Like Condition Occurring After Microwave Sweat Gland Ablation. *Dermatol Surg* 2018.

69 6th Conference of the EHSF 2017, European Hidradenitis Suppurativa Foundation e.V., 8-10 February, Copenhagen, Denmark. *Exp Dermatol* 2017; **26 Suppl 1**: 3-38.

70 Frew JW, Vekic DA, Woods J *et al.* A systematic review and critical evaluation of reported pathogenic sequence variants in hidradenitis suppurativa. *Br J Dermatol* 2017; **177**: 987-98.

71 Zhang YW, Luo WJ, Wang H *et al.* Nicastrin is critical for stability and trafficking but not association of other presenilin/gamma-secretase components. *J Biol Chem* 2005; **280**: 17020-6.

72 Jojic V, Shay T, Sylvia K *et al.* Identification of transcriptional regulators in the mouse immune system. *Nat Immunol* 2013; **14**: 633-43.

73 Quintana FJ. The aryl hydrocarbon receptor: a molecular pathway for the environmental control of the immune response. *Immunology* 2013; **138**: 183-9.

74 Borland MG, Krishnan P, Lee C *et al.* Modulation of aryl hydrocarbon receptor (AHR)-dependent signaling by peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) in keratinocytes. *Carcinogenesis* 2014; **35**: 1602-12.

75 Mao-Qiang M, Fowler AJ, Schmuth M *et al.* Peroxisome-proliferator-activated receptor (PPAR)-gamma activation stimulates keratinocyte differentiation. *J Invest Dermatol* 2004; **123**: 305-12.

76 Kavita U, Mizel SB. Differential sensitivity of interleukin-1 alpha and -beta precursor proteins to cleavage by calpain, a calcium-dependent protease. *J Biol Chem* 1995; **270**: 27758-65.

77 Gross O, Yazdi AS, Thomas CJ *et al.* Inflammasome activators induce interleukin-1alpha secretion via distinct pathways with differential requirement for the protease function of caspase-1. *Immunity* 2012; **36**: 388-400.

78 Vossen A, van der Zee HH, Prens EP. Sequence variants in hidradenitis suppurativa: in search of the pathogenic mechanisms. *Br J Dermatol* 2017; **177**: 895-6.

Chapter 8

Summary

SUMMARY

Chapter 1 is the general introduction and provides the main objectives of this thesis. Hidradenitis suppurativa (HS) is a chronic, debilitating, autoinflammatory skin disease with a prevalence rate of approximately 1% in Europe. HS usually presents in the adolescence with inflammatory nodules and abscesses, followed by sinus tract formation and scarring in predominantly the inverse body areas such as the axillae and groins. Key symptoms of HS include pain, discomfort, and a purulent, foul-smelling discharge. The physical and psychological consequences of the disease can profoundly reduce several aspects of patient's quality of life. Although the pathophysiology of HS is not fully understood, follicular occlusion is supposed to be the key triggering event. This is followed by dilatation and subsequent rupture of the hair follicle causing an intense immune response. Exogenous factors such as smoking, obesity, and mechanical friction in addition to genetic predisposition and alterations in the microbiome may contribute to the onset and progression of the disease. Rapidly evolving understanding of pathogenic mechanisms and clinical perspectives are needed to improve disease awareness, disease management, and ultimately improve patient outcomes. Because multiple facets of HS are not yet known, the outline of this thesis was not limited to only one aspect of the disease. We focused on clinical features and (immuno)pathogenic mechanisms as a rationale for the development of novel treatment strategies.

In **Chapter 2** we determined the prevalence, and explored the characteristics of pruritus in a well-defined cohort of patients with HS. The prevalence of HS-related itch (NRS score ≥3) in 211 patients was 57.3%. Patients with a pruritus NRS score ≥3 suffered more HS-affected body sites ($p < 0.001$), more often Hurley stage III disease severity ($p < 0.001$), and higher levels of HS-related pain ($p < 0.001$) compared with patients reporting a NRS score <3. In a subpopulation (n = 51), the most commonly reported characterisation of pruritus was moderate (54%) to severe (27%) itching sensations for less than 6 hours per day (56%), which had not changed in the previous 2 weeks (48%). Histological examination on 24 random HS skin samples showed that eosinophilic granulocytes were present in 25% (2/8) of the perilesional skin and 63% (10/16) of the lesional skin, while a perineural infiltrate was found in 25% (2/8) and 69% (11/16) of the perilesional and lesional skin, respectively. Our results suggest that pruritus is a frequent but underreported aspect of HS. Its moderate-to-severe intensity and consequential impact on sleep and activities of daily living have great potential to impair patients' quality of life. Therefore, standardised assessment of pruritus (e.g. using a NRS or VAS) in both daily practice and clinical research settings,

together with the DLQI and EQ-5D, may form a helpful additional patient-reported outcome measure to evaluate disease severity/activity and treatment outcome.

In **Chapter 3** we measured the *in vivo* protein levels of 30 important markers of inflammation, including Th1 and Th17 cytokines and chemokines, in the plasma and (lesional) skin of 20 HS patients and 10 healthy controls using a multiplex electrochemiluminescent immunoassay platform (Meso Scale Discovery). In the circulation of HS patients, CCL-26 (eotaxin-3) was significantly elevated and CXCL-10 significantly lowered compared with healthy controls. In lesional skin, protein levels of IL-16, IL-17A, CXCL-8, IL-12/23p40, CCL-4, and CXCL-10 were significantly higher than in controls. Additionally, immunohistochemistry demonstrated overexpression of CCL-4, CXCL-10, and CCL-26 in the HS infiltrate. Interestingly, there was no significant correlation between protein levels in patient plasma and lesional skin with correlation coefficients varying between −0.53 and +0.42. In conclusion, the cytokine and chemokine profile of HS patients, including newly identified IL-16, CCL-4, CXCL-10 and CCL-26, reflects the ongoing skin inflammation in HS. Moreover, the local and systemic upregulation of CCL-26 in HS patients can be linked to the high pruritus score in HS. Lastly, our results demonstrate that cytokine and chemokine levels in plasma give a limited reflection of the activated local cutaneous inflammatory milieu.

In **Chapter 4** we sought to quantify the anti-inflammatory potency of currently available biologics targeting TNF-α, IL-17A, IL-12/23p40, or CD20 in an *ex vivo* disease model. Real-time quantitative PCR and cytokine bead arrays were used to measure the inhibitory effects of adalimumab, infliximab, secukinumab, ustekinumab, and rituximab in addition to prednisolone (positive control) on cytokines and AMPs in HS lesional skin compared to healthy control skin. The relative mRNA expression of all tested cytokines and AMPs was significantly downregulated by all anti-inflammatory agents ($p < 0.0001$). The release of the important pro-inflammatory cytokines TNF-α, IFN-γ, IL-1ß, IL-6, and IL-17A was significantly inhibited by adalimumab, infliximab, ustekinumab, prednisolone (all $p < 0.0001$), and rituximab ($p = 0.0071$), but not by secukinumab ($p = 0.0663$). Moreover, adalimumab, infliximab and prednisolone reduced the levels of a broader mix of individual cytokines than secukinumab, ustekinumab, and rituximab. Our results suggests that TNF-α inhibitors and prednisolone are the most powerful inhibitors of pro-inflammatory cytokines and AMPs in HS lesional skin. Lastly, our *ex vivo* skin culture system represents an adequate model for studies in search of novel candidate drugs for the treatment of HS and to personalise the treatment in specific patients.

In **Chapter 5.1** we evaluated the efficacy and short-term safety of apremilast in patients with moderate HS. Apremilast is a small molecule drug that specifically inhibits phosphodiesterase 4, thereby modulating the expression of a variety of pro-inflammatory and anti-inflammatory mediators. A total of 20 patients with moderate HS were randomised in a 3 : 1 ratio to receive blinded treatment with apremilast, 30 mg twice daily, or placebo for 16 weeks. The Hidradenitis Suppurativa Clinical Response (HiSCR) was met in 8 of 15 patients in the apremilast group (53.3%) and none of 5 patients in the placebo group (0%) ($p = 0.055$) at week 16. Moreover, the apremilast-treated patients showed a significantly lower abscess and nodule count (mean difference, −2.6; 95% CI, −6.0 to −0.9; $p = 0.011$), NRS for pain (mean difference, −2.7; 95% CI, −4.5 to −0.9; $p = 0.009$), and itch (mean difference, −2.8; 95% CI, −5.0 to −0.6; $p = 0.015$) over 16 weeks compared with the placebo-treated patients. There was no significant difference in the DLQI over time between the two treatment arms (mean difference, −3.4; 95% CI, −9.0 to 2.3; $p = 0.230$). The most frequently reported adverse events in the apremilast-treated patients were mild-to-moderate headache and gastrointestinal symptoms, which did not lead to dropouts. Concluding, apremilast is a promising new treatment option for HS. Studies with larger populations and longer follow-up are needed to further elucidate the efficacy and safety profile of apremilast in HS.

In **Chapter 5.2** we assessed the change in expression of inflammatory markers in lesional skin of HS patients receiving apremilast 30 mg or placebo twice daily for 16 weeks. At baseline, 5-mm punch biopsies were obtained from an index lesion (HSL) and non-lesional skin (HSN) in the same anatomical area. Subsequent HSL samples were taken as close as possible to the previously biopsied site at week 4 and week 16. After sampling, biopsies were split; one half was processed for *in vivo* mRNA analysis using real-time quantitative PCR and the other half was cultured for *ex vivo* protein analysis using a proximity extension assay (Olink). At baseline, 17 proteins with a fold change >2 in HSL versus HSN skin were identified in 20 patients. The top-5 were IL-17A (5), S100A12, CST5, IL-12/23p40, CD6 (1) with fold changes ranging from 6.6 to 1638, respectively (FDR < 0.044). Protein levels of S100A12 decreased during treatment in the apremilast group compared with the placebo group ($p = 0.014$, FDR = 0.186). None of the 14 genes exhibited significant changes in expression over time, although an evident downward trend in relative mRNA expression of *IL-17A* and *IL-17F* was demonstrated in patients receiving apremilast. Our findings highlight the challenge of assessing pharmacodynamics in the skin in a highly fluctuating inflammatory disease.

In **Chapter 6.1** we evaluated the effect of hair removal using a long-pulsed 1064-nm Nd:YAG laser on the course of the disease in a case series of 15 patients with mild HS. A questionnaire was used to assess several patient-reported outcomes. Nd:YAG depilation resulted in a decrease in the number of monthly flares (p = 0.019). In addition, the mean (± SD) HS disease severity after depilation was significantly lower than before therapy, NRS 6.4 ± 2.8 versus NRS 3.6 ± 3.5 (p = 0.010), respectively. The majority of patients reported a 51-75% decrease of hair growth after treatment. Overall treatment satisfaction was rated with a NRS score of 6.7 ± 2.4, and two-thirds of the patients would recommend Nd:YAG depilation to other HS patients. Our results suggest that laser hair removal could be a novel therapeutic approach to prevent disease progression or ameliorate the disease, especially in HS patients with the follicular sub-phenotype. However, the findings we obtained could be biased due to natural fluctuation of the disease course. We believe that randomised controlled trials are warranted to confirm the mechanism of action and long-term efficacy of laser hair removal in mild HS.

In **Chapter 6.2** we evaluated the efficacy and safety of microwave ablative therapy using the miraDry device for mild axillary HS in a randomised intrapatient-controlled trial. Only 8 of 20 HS patients completed miraDry treatment; negative clinical outcomes during the recruitment period resulted in the decision to discontinue the study. Two patients achieved the HiSCR in the miraDry-treated axilla, and two patients achieved the HiSCR in the comparator axilla (p = 1.00). In total, 5 of 8 patients showed worsening of their disease after miraDry treatment. Moreover, the median (IQR) NRS score for pain in the miraDry-treated axilla was 7.0 (2.0-8.0) versus 0 (0-5.0) for the untreated axilla after 3 months (p = 0.07). The number of hair follicles after 3 months was numerically lower in the miraDry-treated axilla, median 4.0 (3.0-5.0)/cm^2, a 50.9% decrease from baseline, compared with the untreated counterpart, median 8.5 (6.0-10.0)/cm^2, a 2.0% decline from baseline (p = 0.07). We argue that the microwave energy is able to rupture pre-existing and subclinical or microscopic HS precursor lesion (cysts), subsequently resulting in an intense inflammatory response. Taken together, we question the utility of microwave ablative therapy in patients with HS in clinical practice.

In **Chapter 7** we provided a general overview of the main findings, discussed the clinical implications of these findings, and suggested directions for future research. Four key themes have emerged from this thesis. Firstly, pruritus or itch is a frequent and bothersome symptom in patients with HS. Several pathophysiological substrates could explain the occurrence of HS-related pruritus. Secondly, the overexpression of chemokines and cytokines in HS lesional skin reflects a chronic, activated local in-

flammatory milieu, indicating the need for effective anti-inflammatory HS therapies. Thirdly, the potency and efficacy of novel anti-inflammatory agents for HS were demonstrated in respectively laboratory and clinical trial settings. Fourthly, two treatment strategies (primarily) targeting the hair follicle yielded ambiguous results in mild HS. Further research in various arenas is needed to ultimately improve the management and treatment of patients with HS and related syndromic conditions.

Appendices

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 is de algemene inleiding en geeft de belangrijkste doelstellingen van dit proefschrift. Hidradenitis suppurativa (HS) is een chronische, invaliderende, auto-inflammatoire huidziekte met een prevalentie van ongeveer 1% in Europa. HS openbaart zich meestal in de adolescentie met inflammatoire nodi en abcessen, gevolgd door sinusvorming en littekens in voornamelijk de lichaamsplooien, zoals oksels en liezen. De belangrijkste symptomen van HS zijn pijn, ongemak en een etterige, riekende afscheiding. De fysieke en psychologische gevolgen van de ziekte kunnen verschillende facetten van de kwaliteit van leven van de patiënt ingrijpend verminderen. Hoewel de pathofysiologie van HS niet volledig bekend is, wordt verondersteld dat folliculaire occlusie een belangrijke activerende gebeurtenis in de pathogenese is. Na occlusie volgt dilatatie met daaropvolgende ruptuur van de haarfollikel, welke een intense immuunrespons veroorzaakt. Exogene factoren zoals roken, obesitas en mechanische wrijving kunnen naast genetische aanleg en veranderingen in het microbioom bijdragen aan het ontstaan en de progressie van de ziekte. Toenemend inzicht in pathogene mechanismen en klinische inzichten zijn nodig om het ziektebewustzijn, therapieën en uiteindelijk de behandelresultaten van patiënten met HS te verbeteren. Omdat meerdere aspecten van HS nog niet bekend zijn, is de hoofdlijn van dit proefschrift niet beperkt tot slechts één aspect van de ziekte. We hebben ons gericht op klinische kenmerken en (immuno)pathogene mechanismen als basis voor de ontwikkeling van nieuwe behandelstrategieën.

In **Hoofdstuk 2** hebben we de prevalentie van pruritus met bijbehorende kenmerken onderzocht in een goed gedefinieerd cohort HS patiënten. De prevalentie van HS-gerelateerde jeuk (NRS-score ≥3) bij 211 patiënten was 57.3%. Patiënten met een pruritus-NRS-score ≥3 hadden meer aangedane anatomische regionen ($p < 0.001$), vaker Hurley stadium III ziekte-ernst ($p < 0.001$) en hogere niveaus van HS-gerelateerde pijn ($p < 0.001$) vergeleken met patiënten die een NRS-score <3 rapporteerden. In een subpopulatie (n = 51) was de meest gerapporteerde typering van pruritus: matig (54%) tot ernstige (27%) jeukende sensaties gedurende minder dan 6 uur per dag (56%), wat niet veranderd was in de afgelopen 2 weken (48%). Histologisch onderzoek van 24 willekeurige HS huidbiopten toonde aan dat eosinofiele granulocyten aanwezig waren in 25% (2/8) van de perilaesionale huid en 63% (10/16) van de laesionale huid, terwijl een perineuraal infiltraat werd aangetroffen in respectievelijk 25% (2/8) en 69% (11/16) van de perilaesionale en laesionale huid. Onze resultaten suggereren dat pruritus een vaak voorkomend symptoom van HS is. De matige tot ernstige intensiteit van pruritus en de bijkomende invloed op slaap en dagelijkse activiteiten kunnen de kwaliteit van leven van patiënten met HS

verminderen. Het gestandaardiseerd uitvragen van jeuk (bijvoorbeeld met een NRS of VAS) kan naast de DLQI en EQ-5D een nuttige aanvullende patiënt-gerapporteerde uitkomstmaat zijn om de ziekte-ernst/activiteit te evalueren in de dagelijkse praktijk en in studieverband.

In **Hoofdstuk 3** hebben we de *in vivo* eiwitniveaus gemeten van 30 belangrijke inflammatoire markers, waaronder Th1- en Th17-cytokines en chemokines, in het plasma en de (laesionale) huid van 20 HS patiënten en 10 gezonde controles. Hiervoor maakten wij gebruik van een multiplex elektrochemiluminescent immunoassay-platform (Meso Scale Discovery). In het plasma van HS patiënten was CCL-26 (eotaxine-3) significant verhoogd en CXCL-10 significant lager dan in gezonde controles. In de HS laesionale huid waren de eiwitniveaus van IL-16, IL-17A, CXCL-8, IL-12/23p40, CCL-4 en CXCL-10 significant hoger dan in de controlehuid. Bovendien was er sterke immunohistochemische aankleuring voor CCL-4, CXCL-10 en CCL-26 in het HS infiltraat. Interessant genoeg was er geen significante correlatie tussen eiwitniveaus in het plasma en de laesionale huid van HS patiënten, waarbij de correlatiecoëfficiënten varieerden tussen −0.53 en +0.42. Samenvattend concluderen wij dat het geïdentificeerde cytokine- en chemokine-profiel, waaronder de nieuwe markers IL-16, CCL-4, CXCL-10 en CCL-26, de aanhoudende inflammatie in HS patiënten weerspiegelt. Bovendien kan de lokale en systemische verhoging van CCL-26 in patiënten met HS verklaard worden door de hoge pruritusscore in HS. Tenslotte tonen onze resultaten aan dat cytokine- en chemokinewaarden in plasma een beperkte reflectie geven van het geactiveerde lokale inflammatoire milieu.

In **Hoofdstuk 4** hebben we getracht de anti-inflammatoire potentie van de momenteel beschikbare biologics gericht tegen TNF-α, IL-17A, IL-12/23p40 of CD20 te kwantificeren in een *ex vivo* ziektemodel. Real-time kwantitatieve PCR en cytokine *bead-arrays* werden gebruikt voor het meten van het remmende effect van adalimumab, infliximab, secukinumab, ustekinumab, rituximab en prednison (positieve controle) op cytokines en AMP's in laesionale HS huid in vergelijking met gezonde controlehuid. De relatieve mRNA-expressie van alle geteste cytokinen en AMP's werd significant verlaagd door alle anti-inflammatoire middelen ($p < 0.0001$). De afgifte van de belangrijke pro-inflammatoire cytokinen TNF-α, IFN-γ, IL-1ß, IL-6, IL-17A werd significant geremd door adalimumab, infliximab, ustekinumab, prednison (allen $p < 0.0001$) en rituximab ($p = 0.0071$), maar niet door secukinumab ($p = 0.0663$). Daarnaast verminderden adalimumab, infliximab en prednison een bredere mix van individuele cytokines dan secukinumab, ustekinumab en rituximab. Onze resultaten suggereren dat anti-TNF-α biologics en prednison de krachtigste remmers zijn van pro-inflammatoire cytokines en AMP's in laesionale HS huid. Verder

vormt ons *ex vivo* huidkweeksysteem een adequaat model voor studies naar nieuwe kandidaat-geneesmiddelen voor HS en voor het personaliseren van een behandeling bij specifieke HS patiënten.

In **Hoofdstuk 5.1** zijn de effectiviteit en korte-termijn veiligheid van apremilast bij patiënten met matige HS onderzocht. Apremilast is een *small molecule* geneesmiddel dat specifiek fosfodiësterase-4 remt, waardoor de expressie van een verscheidenheid aan pro-inflammatoire en ontstekingsremmende mediatoren wordt gemoduleerd. In totaal werden 20 patiënten met matig HS gerandomiseerd in een 3 : 1 ratio voor een geblindeerde behandeling met apremilast 30 mg tweemaal daags of placebo gedurende 16 weken. De Hidradenitis Suppurativa Clinical Response (HiSCR) op week 16 werd bereikt bij 8 van de 15 (53.3%) patiënten in de apremilast-groep en geen van de 5 (0%) patiënten in de placebogroep ($p = 0.055$). Daarnaast verbeterden andere klinische scores significant gedurende 16 weken in de patiënten met apremilast ten op zichte van patiënten met placebo: abcessen en inflammatoire nodi (gemiddeld verschil, −2.6; 95% CI, −6.0 tot −0.9; $p = 0.011$), NRS voor pijn (gemiddeld verschil, −2.7; 95% CI, −4.5 tot −0.9; $p = 0.009$) en jeuk (gemiddeld verschil, −2.8; 95% CI, −5.0 tot −0.6; $p = 0.015$). Er was geen significant verschil in de DLQI tussen de twee behandelgroepen (gemiddeld verschil, −3.4; 95% CI, −9.0 tot 2.3; $p = 0.230$). De meest frequent gemelde bijwerkingen waren lichte tot matige hoofdpijn en gastro-intestinale symptomen, die niet tot drop-outs hebben geleid. Concluderend is apremilast een veelbelovende nieuwe behandeloptie voor HS. Studies met grotere populaties en met een langere follow-up zijn nodig om de effectiviteit en het veiligheidsprofiel van apremilast in HS verder te onderzoeken.

In **Hoofdstuk 5.2** beoordeelden we de verandering in de expressie van inflammatoire markers in de laesionale huid van HS patiënten die behandeld werden met tweemaal daags apremilast 30 mg of placebo. Op baseline werden 5 mm stansbiopten verkregen van een indexlaesie (HSL) en de niet-laesionale huid (HSN) in hetzelfde anatomische gebied. Daaropvolgende HSL biopten in week 4 en week 16 werden zo dicht mogelijk bij de eerder gebiopteerde locatie genomen. Elk huidbiopt werd gesplitst; de ene helft werd verwerkt voor *in vivo* mRNA-analyse met behulp van real-time kwantitatieve PCR en de andere helft werd gekweekt voor *ex vivo* eiwitanalyse met behulp van een *proximity extension assay* (Olink). Op baseline werden 17 eiwitten geïdentificeerd met een fold change >2 in HSL versus HSN huid bij 20 patiënten. De top-5 bestond uit IL-17A (5), S100A12, CST5, IL-12/23p40, CD6 (1) met een *fold change* variërend van respectievelijk 6.6 tot 1638 (FDR < 0.044). Het eiwitgehalte van S100A12 nam af in de apremilast-groep in vergelijking met de placebogroep ($p = 0.014$, FDR = 0.186). Geen van de 14 genen vertoonden significante veranderingen

in de tijd, hoewel een duidelijke neerwaartse trend in relatieve mRNA-expressie van *IL-17A* en *IL-17F* werd aangetoond bij patiënten die apremilast ontvingen. Onze bevindingen benadrukken de uitdaging om de farmacodynamiek te beoordelen in de huid van een ontstekingsziekte met een sterk fluctuerend natuurlijk beloop.

In **Hoofdstuk 6.1** evalueerden we het effect van ontharing met behulp van een *long-pulsed* 1064-nm Nd:YAG-laser op het beloop van de ziekte in een case serie van 15 patiënten met milde HS. Een vragenlijst werd gebruikt om verscheidene patiënt-gerapporteerde uitkomsten te beoordelen. Nd:YAG ontharing resulteerde in een afname van het aantal maandelijkse opvlammingen (p = 0.019). Daarnaast was de gemiddelde (± SD) HS ziekte-ernst na ontharing significant lager dan vóór de behandeling, respectievelijk NRS 6.4 ± 2.8 versus NRS 3.6 ± 3.5 (p = 0.010). De meerderheid van de patiënten rapporteerde een 51-75% afname van de haargroei na behandeling. De algehele tevredenheid over de behandeling werd beoordeeld met een NRS-score van 6.7 ± 2.4 en twee derde van de patiënten zou Nd:YAG ontharing aanbevelen aan andere HS patiënten. Onze resultaten suggereren dat laserontharing een nieuwe therapeutische benadering kan zijn om progressie van de ziekte te voorkómen of de symptomen te verminderen, vooral bij HS patiënten met het folliculaire subfenotype. De gevonden resultaten kunnen echter vertekend zijn door natuurlijke fluctuaties in het ziektebeloop. Gerandomiseerde onderzoeken zijn gerechtvaardigd om het werkingsmechanisme en de lange-termijn effectiviteit van laserontharing in milde HS te bevestigen.

In **Hoofdstuk 6.2** onderzochten we de werkzaamheid en veiligheid van ablatieve therapie met *microwaves*, gebruikmakend van het miraDry-apparaat, voor milde axillaire HS in een *randomised intrapatient-controlled trial*. Slechts 8 van de 20 HS patiënten werden (volledig) behandeld met de miraDry omdat negatieve klinische uitkomsten tijdens de wervingsperiode in de beslissing resulteerde om het onderzoek voortijdig te beëindigen. Twee patiënten bereikten de HiSCR in de miraDry-behandelde axilla en twee patiënten bereikten de HiSCR in de contralaterale axilla (p = 1.00). In totaal vertoonden 5 van de 8 patiënten verslechtering van hun ziekte na behandeling met miraDry. Bovendien was de mediane (IQR) NRS-score voor pijn in de met miraDry behandelde axilla 7.0 (2.0-8.0) versus 0 (0-5.0) in de onbehandelde axilla na 3 maanden (p = 0.07). Het aantal haarfollikels na 3 maanden was numeriek lager in de met miraDry behandelde axilla, mediaan 4.0 (3.0-5.0)/cm^2, een daling van 50.9% ten opzichte van baseline, vergeleken met de onbehandelde axilla, mediaan 8.5 (6.0-10.0)/cm^2, een daling van 2.0% ten opzichte van baseline (p = 0.07). Omdat de *microwave* energie bestaande en subklinische of microscopische HS voorloper-laesies (cysten)

kan activeren, is het de vraag of er in de dagelijkse praktijk een toegevoegde waarde is voor het gebruik van deze techniek bij patiënten met HS.

In **Hoofdstuk 7** hebben we een algemeen overzicht gegeven van de belangrijkste bevindingen, de klinische implicaties van deze bevindingen besproken, en suggesties voor toekomstig onderzoek gedaan. Er zijn vier kernthema's voortgekomen uit dit proefschrift. Ten eerste is gebleken dat jeuk een frequent voorkomend en hinderlijk symptoom is bij patiënten met HS. Hierbij identificeerden we verschillende pathofysiologische substraten die de HS-gerelateerde jeuk kunnen verklaren. Ten tweede detecteerden we verhoging van verscheidene pro-inflammatoire cytokinen en chemokinen in de HS laesionale huid, wat de ontwikkeling van effectieve anti-inflammatoire HS-therapieën rechtvaardigt. Ten derde konden we de potentie en effectiviteit van nieuwe anti-inflammatoire geneesmiddelen voor HS aantonen met behulp van respectievelijk laboratorium- en klinische studies. Ten vierde waren er ambigue resultaten voor twee behandelstrategieën die zich (primair) richten op destructie van de haarfollikels. Tot slot is meer onderzoek naar uiteenlopende aspecten van HS nodig om de behandelresultaten van patiënten met HS en gerelateerde syndromen te verbeteren.

ABBREVIATIONS

ADL	activities of daily living
AE	adverse event
AhR	aryl hydrocarbon receptor
AMP	antimicrobial peptide
AN	abscesse and nodule
BMI	body mass index
CCL	CC chemokine ligand
CD	cluster of differentiation
CI	confidence interval
CRP	C-reactive protein
CXCL	CXC chemokine ligand
DAMP	damage-associated molecular pattern
DLQI	Dermatology Life Quality Index
EDTA	ethylenediaminetetraacetic acid
EQ-5D	EuroQoL-5D, a standardised instrument for measuring generic health status
FDR	false discovery rate
GM-CSF	granulocyte-macrophage colony-stimulating factor
HBD	human beta defensin
HE	haematoxylin and eosin
HiSCR	Hidradenitis Suppurativa Clinical Response
HS	hidradenitis suppurativa
HS-PGA	Hidradenitis Suppurativa Physician's Global Assessment
IBD	inflammatory bowel disease
IFN	interferon
IL	interleukin
IMID	immune-mediated inflammatory disease
IQR	interquartile range
IRB	institutional review board
LLOQ	lowest limit of quantification
LPS	lipopolysaccharide
MMP	matrix metalloproteinase
MPO	myeloperoxidase
mRNA	messenger ribonucleic acid
MWA	microwave ablation
Nd:YAG	neodymium-doped yttrium aluminium garnet
NRS	numeric rating scale, ranging from 0 to 10
PAC	pyoderma, acne, colitis ulcerosa
PAMP	pathogen-associated molecular pattern
PASH	pyoderma gangrenosum, acne, suppurative hidradenitis
PCR	polymerase chain reaction
PG	pyoderma gangrenosum
PI3K	phosphoinositide 3-kinase
PROM	patient-reported outcome measure
SD	standard deviation
TGF	tissue growth factor
Th	T helper
TIMP	tissue inhibitor of metalloproteinase
TLR	toll like receptor
TNF	tumour necrosis factor
VAS	visual analogue scale
VEGF	vascular endothelial growth factor

PUBLICATIONS

In this thesis

A.R.J.V. Vossen, C.B. Ardon, H.H. van der Zee, E. Lubberts, E.P. Prens. The anti-inflammatory potency of biologics targeting TNF-α, IL-17A, IL-12/23 and CD20 in hidradenitis suppurativa: an ex vivo study
Submitted

A.R.J.V. Vossen, H.H. van der Zee, E.P. Prens. Hidradenitis suppurativa: a systematic review integrating inflammatory pathways into a cohesive pathogenic model
Frontiers in Immunology – accepted for publication

A.R.J.V. Vossen, H.H. van der Zee, L.C. Tsoi, X. Xing, M. Devalaraja, J.E. Gudjonsson, E.P. Prens. Novel cytokine and chemokine markers of hidradenitis suppurativa reflect chronic inflammation and itch
Allergy – accepted for publication

A.R.J.V. Vossen, H.H. van der Zee, N. Davelaar, A.M.C. Mus, M.B.A. van Doorn, E.P. Prens. Apremilast for moderate hidradenitis suppurativa: no significant change in lesional skin inflammatory biomarkers
J Eur Acad Dermatol Venereol – accepted for publication

C.C. Zouboulis, F.G. Bechara, J.L. Dickinson-Blok, W. Gulliver, B. Horváth, R. Hughes, A.B. Kimball, B. Kirby, A. Martorell, M. Podda, E.P. Prens, H.C. Ring, T. Tzellos, H.H. van der Zee, K.R. van Straalen, **A.R.J.V. Vossen**, G.B.E. Jemec. Hidradenitis suppurativa/acne inversa: a practical framework for treatment optimisation – systematic review and recommendations from the HS ALLIANCE working group
J Eur Acad Dermatol Venereol – in press

A.R.J.V. Vossen, M.B.A. van Doorn, H.H. van der Zee, E.P. Prens. Apremilast for moderate hidradenitis suppurativa: results of a randomised controlled trial
J Am Acad Dermatol – in press

A.R.J.V. Vossen, M.A.P.C. van Huijkelom, T.E.C. Nijsten, E.W.P. Bakker, H.H. van der Zee, M.B.A. van Doorn, E.P. Prens. Aggravation of mild axillary hidradenitis suppurativa by microwave ablation: results of a randomised intrapatient-controlled trial
J Am Acad Dermatol – in press

K.R. van Straalen, **A.R.J.V. Vossen**, E.P. Prens, H.H. van der Zee. Surgical approaches to hidradenitis suppurativa management
Dermatologic Surgery by Jonathan Kantor, New York: McGraw Hill, 2018, print

A.R.J.V. Vossen, H.H. van der Zee, M. Terian, M.B.A. van Doorn, E.P. Prens. Laser hair removal alters the disease course in mild hidradenitis suppurativa
J Dtsch Dermatol Ges 2018; 16: 901-3

A.R.J.V. Vossen, A. Schoenmakers, K.R. van Straalen, E.P. Prens, H.H. van der Zee. Assessing pruritus in hidradenitis suppurativa: a cross-sectional study
Am J Clin Dermatol 2017; 18: 687-95

Other publications

A.M.J.D. Vanlaerhoven, C.B. Ardon, K.R. van Straalen, **A.R.J.V. Vossen**, E.P. Prens, H.H. van der Zee. Hurley III hidradenitis suppurativa has an aggressive disease course
Dermatology – in press

K.R. van Straalen, T. Verhagen, B. Horváth, C.B. Ardon, **A.R.J.V. Vossen**, R. Driessen, J. Boer, A. Rondags, E.P. Prens, H.H. van der Zee. Poor inter-rater reliability of hidradenitis suppurativa phenotypes
J Am Acad Dermatol. 2018 Sep; 79: 577-578

A.R.J.V. Vossen, H.H. van der Zee, E.P. Prens. Sequence variants in hidradenitis suppurativa: in search of the pathogenic mechanisms
Br J Dermatol 2017; 177: 895-6

A.R.J.V. Vossen, K.R. van Straalen, H.H. van der Zee, E.P. Prens. Menses and pregnancy affect symptoms in hidradenitis suppurativa: a cross-sectional study
J Am Acad Dermatol 2017; 76: 155-6

A.R.J.V. Vossen, H.H. van der Zee, E.P. Prens. Accelerated wound healing after wide excisions in hidradenitis suppurativa using autologous split-thickness skin grafting and platelet-rich plasma
Int Wound J 2017; 14: 583-6

A.R.J.V. Vossen, H.H. van der Zee, A.J. Onderdijk, J. Boer, E.P. Prens. Hidradenitis suppurativa is not associated with the metabolic syndrome based on body type: a cross-sectional study
J Dermatol 2017; 44: 154-9

H.H. van der Zee, **A.R.J.V. Vossen**, E.P. Prens. Hidradenitis suppurativa: development of outcome measure instruments
Br J Dermatol 2016; 175: 242

A.R.J.V. Vossen, L.S.M. Boesten, P.D. Siersema, R.G.L. Nellen. Porphyria cutanea tarda – nut van aanvullende diagnostiek
Ned Tijdschr Geneeskd 2016; 160: A9166

PORTFOLIO

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	Year	Workload (Hours/ECTS)
1. PhD training		
General courses		
- MolMed: Biomedical English Writing and Communication	2017	2.0 ECTS
- Research Integrity, Erasmus MC	2017	0.3 ECTS
- Molmed: Basic Introduction Course on SPSS	2016	1.0 ECTS
- Good Clinical Practice: 'Basiscursus Regelgeving en Organisatie voor Klinische Onderzoekers' (BROK)	2016	1.0 ECTS
- Workshop EndNote, Erasmus MC	2016	4 hours
- Workshop Open Clinica, Erasmus MC	2015	8 hours
Specific courses		
- MolMed: 6th Course on Molecular Diagnostics	2017	1.0 ECTS
- MolMed: 15th Course on Advanced Immunology	2016	24 hours
- MolMed: 12th Course on SNPs and Human Diseases	2015	2.0 ECTS
Conferences and symposia		
- 7th conference of the European Hidradenitis Suppurativa Foundation (EHSF) in Rotterdam	2018	24 hours
- PhD weekend Dermatology 2016 in Antwerpen, 2017 in Den Bosch, 2018 in Breda	2016 - 2018	36 hours
- Skintermezzo meetings, department of Dermatology Erasmus MC	2015 - 2018	48 hours
- 6th conference of the EHSF in Copenhagen	2017	24 hours
- 5th conference of the EHSF in Berlin	2016	24 hours
Expert groups		
- Working group member on the 2017 and 2019 Dutch (NVDV) HS treatment guideline	2016 - present	3.0 ECTS
- Bibliographic fellow of the HS ALLIANCE working group on the international HS treatment guideline	2016 - 2017	3.0 ECTS
Organising committee		
- 7th conference of the EHSF in Rotterdam	2017 - 2018	5.0 ECTS
- PhD weekend Dermatology Erasmus MC in Den Bosch	2017	1.5 ECTS
Oral presentations		
- Skintermezzo – *Klinische les in HS*	2018	1.0 ECTS
- Laser Skin and Body Conference in Rotterdam – *Microwave ablation in mild HS*	2018	1.0 ECTS

	Year	Workload (Hours/ECTS)
- 7th conference of the EHSF in Rotterdam – *Anti-inflammatory impact of biologics in HS skin*	2018	1.0 ECTS
- Meeting Dermatology - Gastroenterology in Rotterdam – *Sinus or fistula?*	2017	0.5 ECTS
- 6th conference of the EHSF in Copenhagen – *1 A novel nicastrin mutation in a Dutch family with HS; 2 Pruritus in HS*	2017	2.0 ECTS
- Meeting HS ALLIANCE in Copenhagen – *HS surgery and integration with medical therapy*	2017	0.5 ECTS
- Meeting Hidradenitis Patiënten Vereniging in Utrecht – *Hidradenitis suppurativa*	2016	0.5 ECTS
- 5th conference of the EHSF in Berlin – *Waist to hip ratio in HS*	2016	1.0 ECTS
Other		
- Project leader of the HiScreen Registry & Biobank, Erasmus MC and DermaHaven	2016 - present	100 hours
- Research meetings: 'Methodology' and 'Journal Club', department of Dermatology of the Erasmus MC	2015 - 2018	50 hours
2. Teaching		
Lecturing		
- Minor Inflammunity for medical students	2018	0.5 ECTS
- Auto-immune diseases of the skin for medical interns	2018	1.0 ECTS
- National EIDON meetings on HS for dermatologists and (plastic) surgeons	2017 - 2018	2.0 ECTS
- International EIDON meetings on HS for dermatologists and (plastic) surgeons	2016 - 2018	1.0 ECTS
Tutoring		
- Tutor 'Kennismaking Beroeps Praktijk' – Geneeskunde jaar 1, Erasmus MC	2016 - 2017	10 hours
Supervising students		
- Master thesis of medical student Seth Dykstra	2017 - 2018	1.0 ECTS
- Research project of medical student Marinka Terian	2016 - 2017	0.5 ECTS
- Master thesis of medical student Annelien Schoenmakers	2016	1.0 ECTS
Occasional reviewer for		
- European Journal of the European Academy of Dermatology and Venereology	2018	4 hours
- Journal of Investigative Dermatology	2018	4 hours
- British Journal of Dermatology	2017-2018	8 hours
- Experimental Dermatology	2017	4 hours
- Journal of Dermatology	2016	4 hours

CURRICULUM VITAE

Allard Vossen werd op 20 februari 1989 geboren te Groningen. Niet veel later verhuisde hij met zijn ouders naar het Noord-Brabantse Halsteren alwaar hij zijn verdere jeugd doorbracht. In 2007 behaalde hij zijn gymnasium diploma aan het R.K. Gymnasium Juvenaat H. Hart te Bergen op Zoom. Hetzelfde jaar startte hij met het mr.drs.-programma (Economie en Recht) aan de Erasmus Universiteit Rotterdam nadat hij was uitgeloot voor de studie Geneeskunde. Een jaar later behaalde hij zijn propedeuse Economie en werd hij via decentrale selectie alsnog aangenomen om Geneeskunde te studeren. Het was tijdens zijn coschap Dermatologie in het Erasmus Universitair Medisch Centrum, onder begeleiding van dr. M.B.A. van Doorn, dat zijn interesse voor dit specialisme werd gewekt. Na het behalen van het artsexamen in 2014 heeft hij als arts-assistent niet in opleiding op de afdeling Heelkunde van het Reinier de Graaf Gasthuis te Delft gewerkt om klinische ervaring op te doen. Eind 2015 startte hij met zijn promotieonderzoek op de afdeling Dermatologie in het Erasmus Universitair Medisch Centrum onder begeleiding van promotor prof. dr. E.P. Prens en copromotor dr. H.H. van der Zee. Naast zijn promotieonderzoek werkte hij mee aan meerdere klinische trials en zette hij zich in voor de reguliere zorg van patiënten met hidradenitis suppurativa. Verder was hij medeorganisator van het European Hidradenitis Suppurativa Foundation (EHSF) congres te Rotterdam in 2018, en is hij werkgroeplid van de nationale (NVDV) en internationale (HS ALLIANCE) behandelrichtlijn voor hidradenitis suppurativa. In januari 2019 begint hij aan de specialisatie tot dermatoloog in het Erasmus Universitair Medisch Centrum te Rotterdam.

DANKWOORD

Dit proefschrift was niet tot stand gekomen zonder de hulp van velen. Graag wil ik een aantal hiervan in het bijzonder bedanken.

Prof. dr. E.P. Prens, beste Errol, ik kan onze eerste kennismaking in september 2015 op het laboratorium nog goed herinneren: jij enthousiast vertellende over translationele studies – ik nog klinisch werkende bij de Heelkunde met de ambitie om dermatoloog te worden. Na een week bedenktijd besloot ik de uitdaging van een promotietraject aan te gaan en daar heb ik geen moment spijt van gehad. Onze goede samenwerking is in mijn ogen het fundament geweest voor de totstandkoming van mijn proefschrift. Ik kon altijd bij je binnenlopen en de laatste maanden – sinds de verhuizing naar de nieuwbouw – kwam je minstens zo vaak langs op de onderzoekskamer. Ik bewonder je enorme drive, je creatieve klinische en wetenschappelijke visie, en het feit dat je in elk denkbare situatie de rust bewaart. Je bent een waardig leermeester en een inspirator voor velen.

Dr. H.H. van der Zee, beste Hessel, exact 7 jaar na jouw promotiedatum verdedig ik mijn proefschrift over dezelfde aandoening. Als mijn dagelijks begeleider ben ik je dankbaar voor de scherpe adviezen over tal van praktische onderzoeks-gerelateerde zaken. Met name je frisse kijk op de opbouw van een manuscript heb ik gewaardeerd. Bovendien hebben je routinebezoeken aan de onderzoekskamer op de woensdagochtend ("kan ik nog wat voor je nakijken?") er mede voor gezorgd dat ik in 3 jaar kon promoveren.

Dr. M.B.A. van Doorn, beste Martijn, onder jouw begeleiding, tijdens het coschap Dermatologie in het Erasmus MC, ben ik enthousiast geworden over dit specialisme. Bedankt dat je me de kans gaf om onderzoek op te starten, en – nog belangrijker – dat je me in contact bracht met Errol. Daarnaast ben ik je dankbaar voor je kritische blik op meerdere manuscripten.

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Kelsey, Christine, HS buddy's, onze samenwerking bij tal van gemeenschappelijke projecten is altijd vlekkeloos verlopen – van statistiek tot het uitvoeren van ex vivo

experimenten en qPCR, van het schrijven van artikelen en richtlijnen tot het organiseren van de EHSF2018 en EIDON. Bedankt voor jullie gezelligheid en hulp bij verscheidene klinische en wetenschappelijke activiteiten.

Mijn kamergenoten (Nd-212), Jill, Linde, Joanne, Christine, Kelsey, met jullie aanwezigheid was mijn promotietraject in alle fasen leuker en makkelijker. Een koffiemomentje, een inhoudelijk overleg, lunchen in het museumpark, de afleiding als iemand op de achtergrond met een patiënt aan het bellen was, de altijd beschikbare SPSS hulplijn van Jill of Kelsey – alles op de kamer zorgde ervoor dat ik beter kon presteren. Daarnaast bedankt dat jullie de hoofdstukken van mijn proefschrift hebben doorgelezen voor een opmaak- en spellingscheck.

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In 2007 begon mijn tijd in Rotterdam en inmiddels zijn er vele vriendschappen ontstaan. De mannen van JC Duke, Huize Pinkeltje, Leonidas H5, maten uit Rotterdam en Bergen op Zoom, de Suriname clan, met elkaar hebben we mooie herinneringen opgebouwd – dat er nog vele mogen volgen.

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Jeanne-Marie, in het afgelopen jaar is er echt wat moois ontstaan tussen ons – wat fijn dat ik jou aan mijn zijde heb. Dankjewel voor de liefde die je me geeft en dat je altijd voor me klaar staat.

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