Summary

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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.