Hidradenitis suppurativa

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GENERAL DISCUSSION AND FUTURE PERSPECTIVE

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HS is evolving from a relative orphan disease towards being a renewed scientific topic. Originally, HS was regarded as a disease of the apocrine sweat glands, hence the term "hidradenitis suppurativa". In 1952 it was dr. Brunsting who proposed, for the first time, that the initial inflammation occurrs in the hair follicle with apocrine involvement occurring only upon extension of the inflammatory process to deeper skin layers.[1] Ever since, several histological studies found that follicular hyperkeratization followed by occlusion are central pathogenic events, rather than involvement of the sweat glands.[2-5] Since no specific pathogenic bacteria have been identified in HS, it has been suggested that aberrant immunity rather than bacteria are responsible for the inflammatory reaction in HS[6-8] The suspected association of HS with other immune mediated diseases further supports a key role of aberrant immunity in its pathogenesis.[9]

Many questions remain regarding the pathogenesis of HS and therefore the identification of appropriate treatment targets is challenging. Although a wide arsenal of systemic agents and surgical interventions have been described for HS, many patients are refractory to treatment. Consequently, HS is a frustrating disease for both patients and clinicians. In this thesis we aimed at gaining more insight in the pathogenesis of HS and explored both current and new treatment options, including systemic and surgical therapies. The following chapter will discuss the main findings of this thesis, combined with perspectives for future research.

To further investigate the histopathogenesis of HS we studied the morphology of the basement membrane within the folliculopilosebaceous unit (FPSU), as shown in **chapter 2**. This was done by performing periodic-acid Schiff (PAS) and immunofluorescence (IF) staining for the main BMZ glycoproteins of perilesional HS skin and comparing this to skin of healthy volunteers. We demonstrated relative upregulation of integrin $\alpha 6\beta 4$ at the sebaceous glands of HS patients. Integrin $\alpha 6\beta 4$ is an important signaling molecule and its upregulation has also been described in pulmonary tissue upon infection with pathogenic micro-organisms.[10,11] It has been hypothesized that integrins function as pattern recognition receptors (PRP's) in lungs that upon interaction with bacteria induce cellular responses that activate the innate immune system and inflammation.[10] This may also be true for upregulated integrins in HS. Although bacterial cultures from HS lesions are often negative or only show commensal bacteria, DNA-based analyses have revealed that the skin's microbiome is much wider than previously assumed.[12] An aberrant composition of the skin's microbiome has been linked to several other inflammatory mediated skin diseases, including psoriasis and atopic dermatitis.[13] Interestingly, a relative deficiency of antimicrobial peptides (AMP's) was demonstrated in HS lesions that may promote bacterial propagation.[14] Pathogenic changes in the skin's microbiome may result

in chronic exposure of keratinocytes and Langerhans cells to pathogen-associated molecular patterns (PAMPs), resulting in upregulation of PRPs and therefore also integrins.[15] Several clinical and histological findings further support a role for the microbiome in HS, namely: 1) the microbiome composition in body folds differs from other areas, explaining the predilection of HS lesions at these locations[12] 2) life style factors (smoking and diet) associated with HS as well as hormonal factors influence the composition of the microbiome[12] 3) the composition of the microbiome is largely determined by inherited factors,[12] explaining the familial occurrence of HS 4) sebaceous glands have shown to be diminished in volume and number in clinically uninvolved skin of HS patients.[16] The latter may promote increased infundibular friction due to dimished sebum secretion but may also result in a diminished production of AMP's by sebocytes, further promoting bacterial colonization. Whether changes in the microbiome have a causal effect in skin diseases or occur secondarily to an altered skin biology remains elusive.[13] It would be interesting to investigate whether there are differences between sebocyte derived AMP's in HS patients and controls to determine the possible role of sebaceous glands in bacterial colonization. Genome based techniques, like 16S RNA gene clone sequencing, may be applied to study the microbiome in HS. In case an important role of the microbiome in HS is identified, restoring its composition would be an interesting future therapeutic strategy.
An important finding was that we could not confirm the presence of the so called "PAS-gaps" at the sebofollicular junction (SFJ) in HS skin as described by Danby *et al*.[17] Neither did we find diminished expression of specific glycoproteins (including type XVII collagen, type VII collagen, laminin-332, and integrin $\alpha 6\beta 4$) in the BMZ. Therefore we have strong doubts about the primary importance of hair follicle fragility in the HS pathogenesis.
There is increasing evidence that HS is an immune mediated disease as the dysregulated immune system plays a critical role in both the development and progression of HS. Therefore, immunosuppressive agents have been recognized as a cornerstone treatment. In HS overproduction of cytokines from both the innate (including IL-1β and TNF-α) and adaptive immune system (including IL-10, IL-12, IL-17 and IL-23) has been observed in skin tissue.[14,18-20] Additionally, diminished expression of IL-22 has been demonstrated, probably resulting from increased IL-10 production. This may contribute to the relative deficiency of AMP's in HS skin.[14] As the predominant cytokines driving the inflammatory reaction in HS have yet to be determined and consensus is lacking, the choice for a specific immunosuppresive agent is mainly based on the clinicians' experience.

HS is associated with genetic predisposition. Heterozygote mutations in the γ-secretase genes have been identified in familial HS with an autosomal dominant inheritance pattern.[21,22] In

our clinic we encountered five HS patients who were also diagnosed with Down syndrome (DS), suggesting that an association between these two conditions is possible. In **chapter 3** we formulated a hypothesis that may link Down syndrome (DS) to HS via functional deficiency of γ-secretase. Dysfunctional or deficient γ-secretase may induce HS by impairing Notch signaling pathways. Notch signaling controls epidermal cell proliferation and differentiation and is therefore involved in hair follicle and sebaceous gland maintenance.[21] Furthermore, it may promote epidermal cyst formation and impaired production of sebaceous glands, both important characteristics of HS. Whether DS and HS are truly associated requires investigation in a larger group of DS patients. Further work should focus on the role of γ-secretase in the skin and the hair follicle specifically. Furthermore, gene mapping techniques, including next-generation sequencing, may be applied to identify relevant mutations in genes encoding for other proteins in the γ-secretase complex or proteins involved in the Notch signaling pathway.

Chapter 4 describes the gene expression profile of HS affected tissue. Here we show significant upregulation of genes involved in pathways comprising amongst others leucocyte migration, inflammatory and immune responses as well as atherosclerosis signaling in lesional HS skin compared to clinically uninvolved skin. These results have yet to be confirmed with for instance quantitative real-time polymerase chain reaction (PCR) analyses and on protein level. No differences were observed in whole blood mRNA expression between HS patients and healthy subjects. This implicates that activated cells in HS reside in affected tissue, probably because leucocytes migrate from the circulation into skin tissue by an as yet unknown trigger. Inflammation in HS is thus restricted to the skin of specific anatomical areas and therefore it may be considered as a localized rather than a generalized skin disease. However, an increased frequency of metabolic syndrome has been shown in HS, implicating that it actually may be a systemic disease.[23] It remains unclear whether these metabolic changes occur secondary to the chronic inflammation and adverse lifestyle habits associated with HS or whether primary metabolic alterations trigger HS. Either way, systemic immunosuppressive and anti-inflammatory agents are important for preventing progression of HS to other skin regions. Future studies should also establish the role of topically applied agents like topical resorcine 15% cream,[24] topical antibiotics and zinc glucoanate,[25,26] especially in disease phenotypes where only one or two anatomical areas are involved.

Chapter 5 describes a systematic review on the effectiveness of systemic immunosuppressive agents and retinoids in HS. Here we show that the quality of performed studies, to date,

is generally poor, making it difficult to make any therapeutic recommendations. The best clinical results were found for the TNF-α-inhibitors adalimumab and infliximab, confirming the importance of TNF-α in HS. Additionally, promising results were identified for the retinoid acitretin as it was effective in 95% of the 22 described patients. Isotretinoin showed a poor response. This may be explained by the reduction in sebaceous glands in HS, as sebaceous glands are important targets of isotretinoin.[27] Although colchicine is a potent IL-1β inhibitor and suppressor of neutrophils,[28] it was not effective in HS. In conclusion, better studies are needed to determine the effect of other immunosuppressive agents showing promising results in small studies, like dapsone and cyclosporine.

As mentioned above, IL-12 and IL-23 have been found to be abundantly expressed by macrophages in lesional HS skin.[19] Additionally, Th-17 cell upregulation has been observed in HS tissue.[19] Similar to psoriasis, Th-17 cells are known to produce IL-17 and IL-21 which can lead to proinflammatory cytokine (IL-1β, IL-6 and TNF-α) production by keratinocytes, resulting in neutrophil, macrophage and lymphocyte attraction.[29] Targeting the IL-23/Th17 axis with the biologic agent ustekinumab could therefore be effective in treating HS.

In **chapter 6** we show in an open label study, for the first time, that ustekinumab can be applied in HS, as the majority of patients showed improvement. The results were not inferior to the results found for adalimumab in a large randomized controlled trial,[30] nor compared to the IL-1 receptor antagonist anakinra in a small open label study.[31] As inflammatory marker levels are higher in HS compared to psoriasis (where ustekinimab is an approved treatment option[18]) we would recommend to adjust the standard psoriasis schedule by increasing administration frequency and dosage. Eventually, clinical trials are warranted to compare IL-12/IL-23 inhibition to the current gold standard of TNF-α-inhibition in HS. Furthermore, it is important to investigate in larger studies whether certain clinical characteristics are predictive for a response to ustekinumab, including HS phenotype (for instance wide spread Hurley II disease versus localized Hurley III disease), life style factors (smoking and obesity) and co-morbidities.

Besides treatment effect, **chapter 6** also describes protein expression in serum of patients at baseline and during the study. At baseline, a significant difference was detected between healthy controls and HS patients in the expression of 54 serum proteins. These proteins are probably produced by activated cells present in skin tissue. Further investigation of these protein levels could possibly lead to putative biomarkers for diagnostic purposes or monitoring disease progression. Although we did not discover a specific biomarker reflecting the therapeutic effect of ustekinumab, low levels of enzyme leukotriene A4-hydrolase (LTA4H) in patients combined with relatively mild clinical disease severity may be prognostic for a good effect of ustekinumab.

Surgery is an important part of HS Hurley stage II/III treatment as systemic therapy alone is never sufficient for heal previously formed sinus tracts and scarring. These sinus tracts provide access for bacteria to invade the dermis, leading to repetitive inflammation and further extension of the disease. Several surgical - as well as closure techniques have been previously proposed for HS.[9] General anesthesia is preferred when large areas require surgical intervention or in cases where patients refuse local anesthetics.

In **chapter 7** we describe a new surgical technique to approach moderate to severe HS Hurley stage II/III: the skin tissue-sparing excision with electrosurgical peeling (STEEP) procedure. By performing tangential excisions and exploring the wound beds with a probe, all lesional tissue can be removed with minimal excision of healthy skin. This can lead to a shorter healing time and less post-surgical complications, (e.g contractures), compared to the traditional wide excision. The wounds are healed by secondary intention allowing continuous wound drainage and therefore the risk of post-operative inflammation is diminished. In **chapter 8** long term results (median follow-up time of 43 months) of the STEEP procedure and deroofing under general anesthesia were retrospectively studied. Here we show that natural progression of the disease outside the surgical scar but within the operated anatomical region occurred in 34% of patients. True relapses due to irradical surgery were seen in 29% of patients. However, in a subsequent prospective case series of 27 patients with a follow up time of 12 months by our group, relapses were observed in only one patient (Janse *et al*.; unpublished data). Due to the relatively high percentage with natural progression of the disease, future studies should focus on proper planning of surgery, whether the use of perioperative immunosupressiva improves surgical outcomes (time to wond closure, relapse rate, natural progression of the disease) and whether tissue saving procedures are superior to wide excision regarding these surgical outcomes. Also, ultrasound imaging prior to the operation may help the surgeon in estimating the extensiveness of fistulas and therefore assist in the choice for a specific technique.[32]

Finally, as many closure techniques have been proposed in HS, studies should be performed to investigate the type of indications where wound closure with grafts or flaps should be preferred over secondary intention healing. The choice will mainly depend on experience of the surgeon, the location of the disease, the size of the wound and patient's ability to take care of an open wound.

CONCLUSIONS AND FINAL CONSIDERATIONS

The multifactorial pathogenesis of HS remains elusive, complicating the development of new treatment strategies. Follicular occlusion may be caused by a primary keratinization disorder or may result from an overactive innate immune system, with a possible causative or secondary role of the skin's microbiome. The contribution of hormones, genetics and environmental factors requires further investigation. Consensus should be reached on what the treatment goal should be in HS and on what outcome measures must be applied in future clinical trials focusing on topical, systemic and surgical treatments. The ultimate goal would be to develop individualized treatment strategies for HS.

REFERENCES

1. Brunsting HA. Hidradenitis and other variants of acne. *AMA Arch Derm Syphilol* 1952; **65**:303-15.

2. Jemec GB, Hansen U. Histology of hidradenitis suppurativa. *J Am Acad Dermatol* 1996; **34**:994-99.

3. Attanoos RL, Appleton MA, Douglas-Jones AG. The pathogenesis of hidradenitis suppurativa: a closer look at apocrine and apoeccrine glands. *Br J Dermatol* 1995; **133**:254-58.

4. Sellheyer K, Krahl D. "Hidradenitis suppurativa" is acne inversa! An appeal to (finally) abandon a misnomer. *Int J Dermatol* 2005; **44**:535-40.

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

6. Lapins J, Jarstrand C, Emtestam L. Coagulase-negative staphylococci are the most common bacteria found in cultures from the deep portions of hidradenitis suppurativa lesions, as obtained by carbon dioxide laser surgery. *Br J Dermatol* 1999; **140**:90-95.

7. Sartorius K, Killasli H, Oprica C, et al. Bacteriology of hidradenitis suppurativa exacerbations and deep tissue cultures obtained during carbon dioxide laser treatment. *Br J Dermatol* 2012; **166**:879-83.

8. Matusiak L, Bieniek A, Szepietowski JC. Bacteriology of Hidradenitis Suppurativa - Which Antibiotics are the Treatment of Choice? *Acta Derm Venereol* 2014; **6**:699-702 .

9. 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; **4**:619-44.

10. 10. Ulanova M, Gravelle S, Barnes R. The role of epithelial integrin receptors in recognition of pulmonary pathogens. *J Innate Immun* 2009; **1**:4-17.

11. Gravelle S, Barnes R, Hawdon N, et al. Up-regulation of integrin expression in lung adenocarcinoma cells caused by bacterial infection: in vitro study. *Innate Immun* 2010; **16**:14-26.

12. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011; **9**:244-53.

13. Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. *Nat Rev Genet* 2012; **13**:260-70.

14. 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.

15. Zeeuwen PL, Boekhorst J, van den Bogaard EH, et al. Microbiome dynamics of human epidermis following skin barrier disruption. *Genome Biol* 2012; **13**:R101-2012-13-11-r101.

16. Kamp S, Fiehn AM, Stenderup K, et al. Hidradenitis suppurativa: a disease of the absent sebaceous gland? Sebaceous gland number and volume are significantly reduced in uninvolved hair follicles from patients with hidradenitis suppurativa. *Br J Dermatol* 2011; **164**:1017-22.

17. Danby FW, Jemec GB, Marsch WC, von Laffert M. Preliminary findings suggest hidradenitis suppurativa may be due to defective follicular support. Br J Dermatol 2013; **168**:1034-39.

18. 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**:1292-98.

19. Schlapbach C, Hanni T, Yawalkar N, Hunger RE. Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**:790-98.

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. Pink AE, Simpson MA, Desai N, et al. gamma-Secretase mutations in hidradenitis suppurativa: new insights into disease pathogenesis. *J Invest Dermatol* 2013; **133**:601-07.

22. Chen S, Mattei P, You J, et al. gamma-Secretase Mutation in an African American Family With Hidradenitis Suppurativa. *JAMA Dermatol* 2015; **151**:688-70.

23. Sabat R, Chanwangpong A, Schneider-Burrus S, et al. Increased prevalence of metabolic syndrome in patients with acne inversa. *PLoS One* 2012; **7**:e31810.

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

25. Brocard A, Dreno B. Innate immunity: a crucial target for zinc in the treatment of inflammatory dermatosis. *J Eur Acad Dermatol Venereol* 2011; **25**:1146-52.

26. Brocard A, Knol AC, Khammari A, Dreno B. Hidradenitis suppurativa and zinc: a new therapeutic approach. A pilot study. Dermatology 2007; **214**:325-27.

27. Rigopoulos D, Larios G, Katsambas AD. The role of isotretinoin in acne therapy: why not as first-line therapy? facts and controversies. *Clin Dermatol* 2010; **28**:24-30.

28. Martinon F, Petrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 2006; **440**:237-41.

29. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med* 2009; **361**:496-509.

30. 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.

31. Leslie KS, Tripathi SV, Nguyen TV, et al. An open-label study of anakinra for the treatment of moderate to severe hidradenitis suppurativa. *J Am Acad Dermatol* 2014; **70**:243-51.

32. Wortsman X, Moreno C, Soto R, et al. Ultrasound in-depth characterization and staging of hidradenitis suppurativa. *Dermatol Surg* 2013; **39**:1835-42.