HIDRADENITIS SUPPURATIVA
PATHOGENESIS & TREATMENT

HESSEL VAN DER ZEE

HIDRADENITIS SUPPURATIVA: PATHOGENESIS AND TREATMENT

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Hidradenitis Suppurativa: Pathogenesis and Treatment

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1

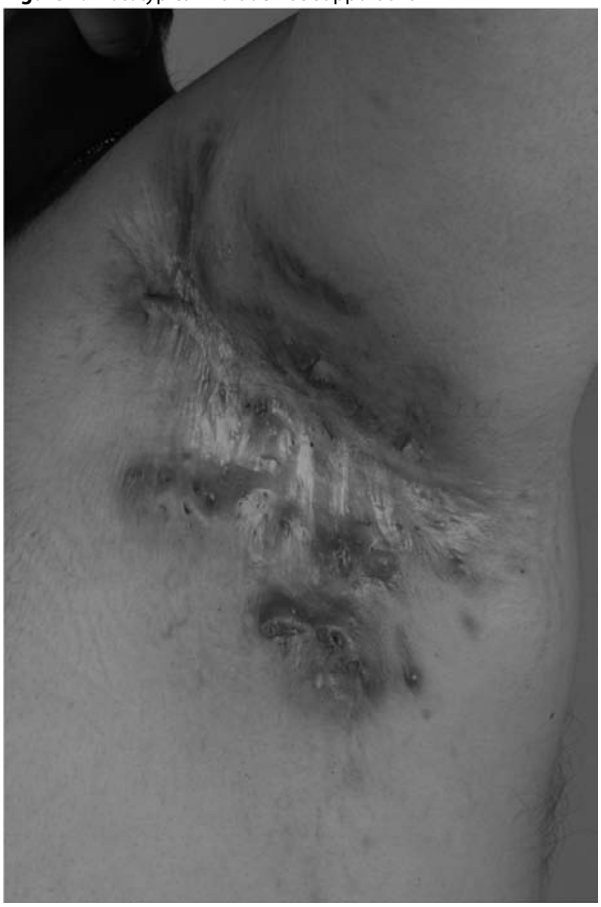
General introduction and aims

Parts of this chapter are in press in Eur J Pharmacology, and are submitted for publication.

GENERAL INTRODUCTION

Hidradenitis suppurativa (HS), also known as acne inversa, is an inflammatory, debilitating follicular skin disease with natural flare ups. It usually presents after puberty with painful, deep-seated, inflamed lesions in the inverse areas of the body, most commonly the axillary, inguinal and anogenital regions (Figure 1). HS is not a rare disease since estimated prevalence rates range between 1% and 4%[1,2] in European countries. HS prevalence rates in other parts of the world are unknown. The historic figure and founder of communism Karl Marx is thought to have been a HS sufferer[3]. Generally, HS develops almost always after puberty, usually in the second decade of life at a median age of 20 years[4]. Women are more frequently affected than men with a sex ratio of 3:1[1,4]. In a study of 302 HS patients the most frequently affected area was the inguinofemoral area in 90%, hereafter, the axillary region in 69%, perineal/perianal involvement in 37%, buttocks in 27% and the breasts in 18% of cases[4]. Due to its frequent

Figure 1. Prototypical hidradenitis suppurativa

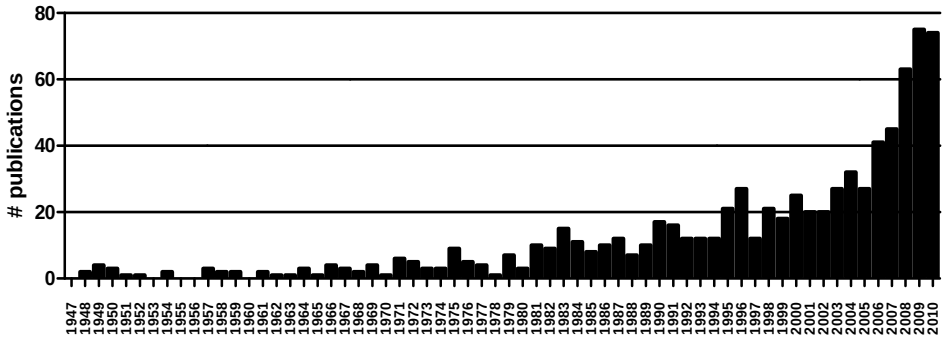


Axillary HS in a man (source Dept. Dermatology, Erasmus MC). (See also Color section, p. 193.)

occurrence, patients with HS are seen or being treated by a variety of medical specialists such as general practitioners, dermatologists, surgeons, plastic surgeons, gynaecologists and urologists. However, many physicians are not familiar with the disease, which is indirectly illustrated by the reported mean time till diagnosis of 7 years[5], the misconception that HS is a bacterial infection of the apocrine glands, and the assumption that HS is an ectopic variant of acne vulgaris. The latter has frequently resulted in fruitless treatment with isotretinoin. HS is associated with frequent and long-term sick leave[6], suggesting a substantial underestimated socio-economic impact. Furthermore the development of the life-threatening squamous cell carcinoma, triggered by persistent inflammation, has been reported in chronic HS lesions, especially on the buttocks of men[7].

Despite its common occurrence, its profound effect on quality of life and its socio-economic impact, HS is a scientifically relatively neglected disease. The limited research so far has yielded few novel insights into the pathomechanisms of the disease and hence little basis for rational improvement of therapy. Consequently, there is a large unmet medical need in patients with HS. Fortunately, in the last decade, HS has gradually gained more attention, and has become a more attractive topic of research, illustrated by the increasing numbers of scientific publications each year (Figure 2). This indicates that this devastating disease is finally receiving more attention.

Figure 2. Number of publications on HS is increasing



Number of hits in Pubmed (www.pubmed.org) per year using the search terms hidradenitis suppurativa, acne inversa, ectopic acne, Verneuil's disease, pyoderma fistulans significa, and fox den disease.

Historical perspective and nomenclature

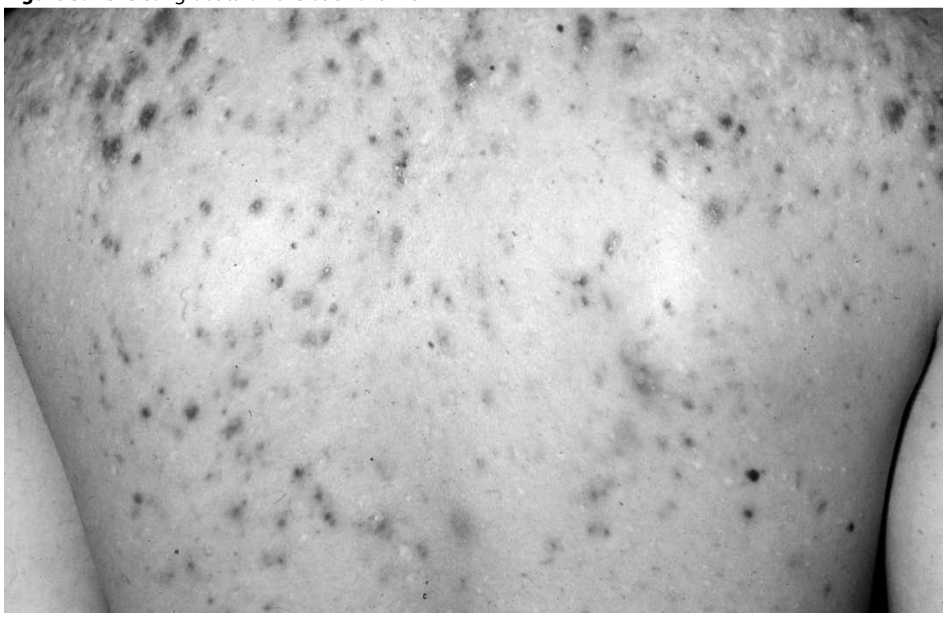
Literally translated, hidradenitis suppurativa means 'pussing sweat gland inflammation' and is derived from the Greek hidros, meaning sweat and aden meaning glands. Concepts on HS pathogenesis changed over time, but at very slow pace. In 1854, the French surgeon Verneuil associated the symptoms with inflammation of areas characterized by many sweat glands and named it HS[8]. In 1955, Shelly and Cahn experimentally obstructed the sweat glands in the axilla and saw HS like lesions develop, supporting the concept that obstructed sweat

Box 1. The follicular occlusion tetrad

Acne conglobata
Dissecting cellulitis of the scalp
Hidradenitis suppurativa
Pilonidal sinus

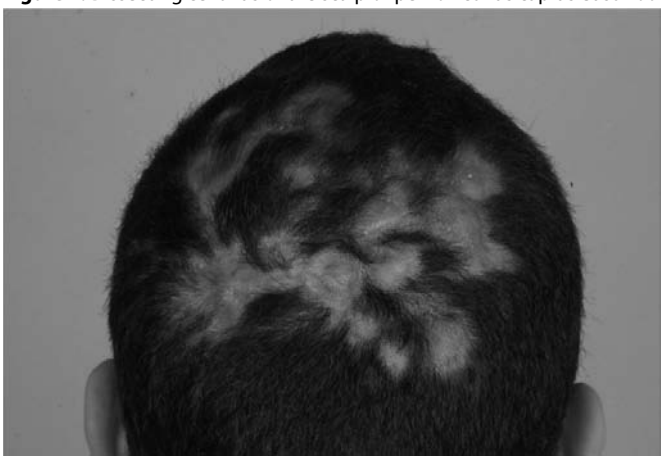
ducts result in HS[9]. With plugging as the primary pathogenic mechanism, HS was classified with three other follicular occlusion diseases in the follicular occlusion tetrad[10]. The follicular occlusion tetrad consists of acne conglobata, dissecting cellulitis of the scalp or perifolliculitis capitis et suffodiens, pilonidal sinus and hidradenitis suppurativa (Box 1). Acne conglobata is a form of acne vulgaris characterized by severe, eruptive nodulocystic acne without systemic manifestations (Figure 3). Dissecting cellulitis of the scalp is characterized by scarring alopecia with suppurating nodules and fistulas (Figure 4) and pilonidal sinus is characterized by inflammatory nodules and fistulas caused by ingrown hairs, usually in the gluteal cleft (Figure 5). It is unknown how often these diseases co-occur and what their pathogenic interrelations are. With plugging of the acrosyringium as the suspected primary event, Plewig and Steger introduced the name acne inversa in 1989[11]. But it was not until 1990 that Yu and Cook, in a small histological study, demonstrated that apocrinitis was present only in a minority of HS specimens and follicular occlusion was a far more constant histopathological feature[12]. Over

Figure 3. Acne conglobata on the back of a man



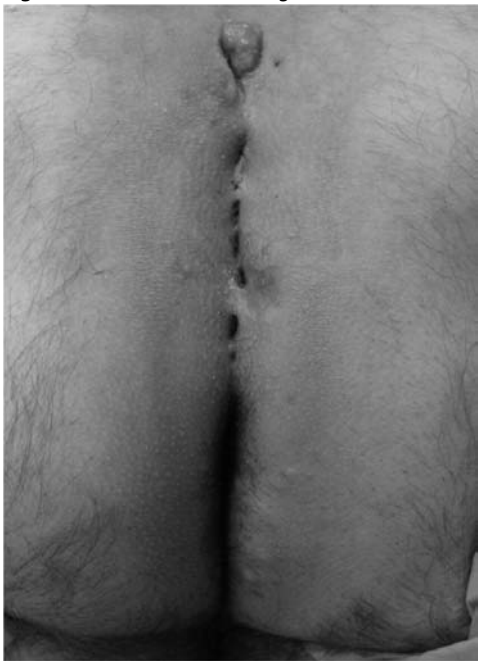
Acne conglobata is characterized by severe eruptive nodulocystic acne without systemic manifestations (source Dept. Dermatology, Erasmus MC). (See also Color section, p. 193.)

Figure 4. Dissecting cellulitis of the scalp or perifolliculitis capitis et suffodiens



Dissecting cellulitis of the scalp is characterized by scarring alopecia with suppurating nodules and fistulas (source Dept. Dermatology, Erasmus MC). (See also Color section, p. 194.)

Figure 5. Pilonidal sinus at the gluteal cleft



Pilonidal sinus characterized by inflammatory nodules and fistulas caused by ingrown hairs (source Dept. Dermatology, Erasmus MC). (See also Color section, p. 194.)

the years, several synonyms for the disease emerged, but none sufficiently covers the pathogenesis (Box 2). Synonyms used are: acne inversa (generally German Literature), ectopic acne (Dutch literature), Verneuils´s disease (French literature), pyoderma fistulans significa and fox

Box 2. Synonyms for hidradenitis suppurativa

Acne inversa
Ectopic acne
Verneuils disease
Pyoderma fistulans significa
Fox den disease

den disease, according to the resemblance of the fistula network with the underground maze of tunnels of fox holes. Today, HS experts agree HS is a misnomer, but since none of these synonyms sufficiently cover the pathogenesis, most experts prefer to use the term HS at the current time.

Disease severity classification

In 1989, a severity classification was first proposed by Hurley[13] (Box 3 and Figure 6). Fortunately the stage I disease is the most common (68% of patients), while stage II occurs in 28% of patients, and 4% of HS patients have stage III[4]. All HS patients initially start in stage I, but data on prognosis are limited and consequently it is unpredictable which patients progress to the more severe stage of the disease. Today, the Hurley classification is still useful for the classification in three severity groups but the classification has limitations. The Hurley classification is a not very quantitative one, consisting of only 3 stages and based on rather static disease characteristics such as scarring and fistulas. Hence, it is not suitable for monitoring the efficacy of interventions in clinical trials. A more detailed and dynamic HS severity score was created by Sartorius et al. and was later modified and validated[14-16] (Figure 7). The main parameter in the modified Sartorius score is the counting of individual nodules and fistulas. This modified Sartorius score is difficult to use and its usability is limited especially in severe cases in which lesions become confluent.

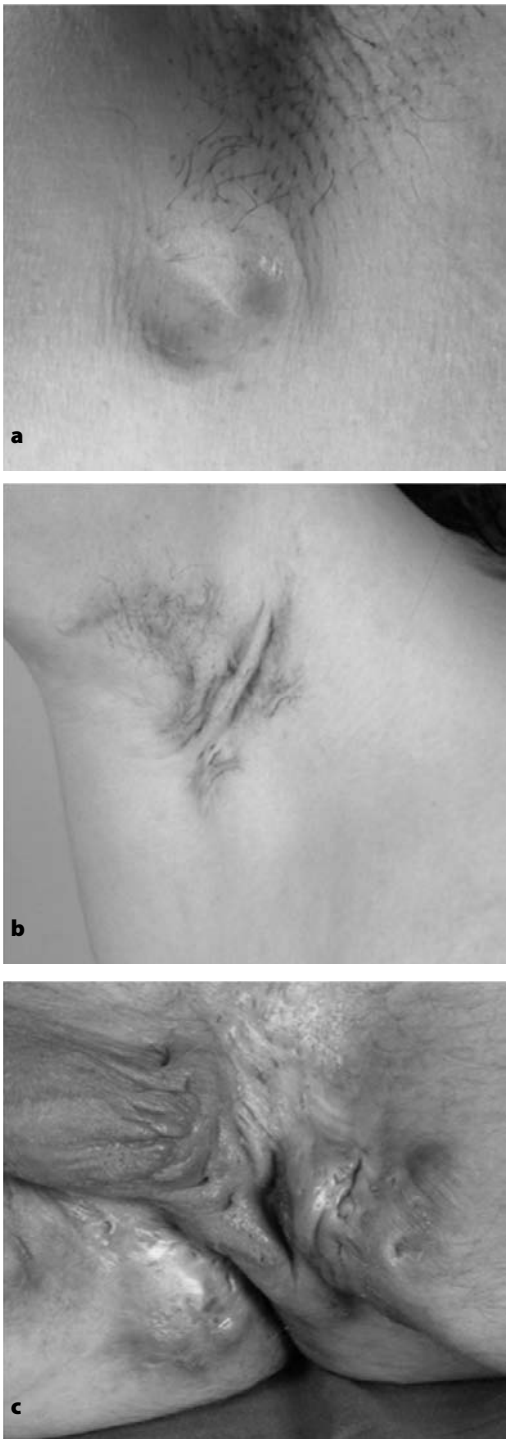
Box 3. HS severity staging according to Hurley[13]

Hurley stage	Clinical description
Stage I	Abscess formation, single or multiple, without sinus tract and cicatrization.
Stage II	Recurrent abscesses, with tract formation and cicatrization. Single or multiple, widely separated lesions.
Stage III	Diffuse or near diffuse involvement, or multiple interconnected tracts and abscesses across entire area.

Quality of life

HS can have a great impact on the patient's quality of life[6,17-21]. This reduction in quality of life is even more significant than in other recalcitrant dermatologic diseases such as severe psoriasis or hand eczema[21,22]. This decline in quality of life is easy to comprehend. First, the inflammatory lesions can be extremely painful leading to impairment of daily activities. Second, the purulent discharge can be foul-smelling, require multiple changes of clothing

Figure 6. Examples of HS by Hurley stage



a) Hurley stage I HS in the axilla of a woman
b) Hurley stage II HS in the axilla of a woman
c) Hurley stage III HS in the groin of a man

All images: source Dr. J. Boer, Deventer Hospital, The Netherlands. (See also Color section, p. 195.)

Figure 7. Modified Sartorius score[15]

Hidradenitis Suppurativa Score

Right Axilla .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Left Axilla .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Right Groin .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Left Groin .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Right Gluteal Region .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Left Gluteal Region .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Other Region .....
noduli & fistulae .....
longest distance .....
Hurley III no/yes .....
Σ

Total sum:

Patient Report
(not included in the score):

Number of boils during the latest month: .....

Soreness of most symptomatic lesion:
VAS (0–10) .....

Legend to Hidradenitis Suppurativa Score

Parameters	Points/parameter
1. Number of regions	
3 points per region	3
2. Number and severity of lesions	
noduli	1
fistulae	6
3. Longest distance between two relevant lesions	
< 5 cm	1
5–10 cm	3
> 10 cm	9
4. Lesions clearly separated by normal skin?	
yes	0
no (Hurley III)	9

during the day and in extreme cases the use of diapers or ladies bandages. Third, HS mainly occurs at private areas of the body. This influences sex life and induces fear of entering new relationships[22]. Separately and jointly, all these aspects result in embarrassment, shame and low self-esteem[22].

Pathogenic factors

The pathogenesis of HS is poorly known. Yet, it is generally accepted that the hair follicles are the primary origin of the pathological process rather than the apocrine glands. Genetic predisposition, cigarette smoking, shear forces, obesity, a deregulated immune response and hormonal abnormalities are all suspected risk factors[23]. In our own experience patients report psychosocial stress as an exacerbating factor; however its effect on HS severity has not been studied. These six putative factors are concisely discussed below.

Genetic predisposition

Genetic predisposition is considered an important HS disease factor. Up to 40% of HS patients know a family member with similar symptoms[24]. This percentage is probably an underestimation, since boils in the groin is not something to discuss at family meetings or birthday parties. Moreover, as symptoms of severe disease cannot be concealed or ignored, patients are more likely only to know family members with severe HS. Table 1 provides an overview on genetic

Table 1. Genetic studies in HS

Conclusion	Critical comment	Reference #
Autosomal dominant inheritance	Vague HS definition, short of expected 50% mendelian value	26
Autosomal dominant inheritance	Short of expected 50% mendelian value	27
Not linked to HLA -A, -B, or -DR	Limited population size n=42	28
Linked to D6S440-D6S442 LOD 2.83	Not published other than abstract	29
Linked to D19S911-D19S1170 LOD 3.66	Not published other than abstract	30
Linked to D1S248-D1S2711 LOD>3	Follicular occlusion tetrad rather than solitary HS	30
Not linked to 1p21.1-1q25.3 region		31
Not linked to P450 1A1 (CYP1A1) SNPs	Limited population size n=51	32
Not linked to NOD2 mutations	Limited population size n=10	33
Not linked to NOD2 mutations	Limited population size n=51	34
Linked to heterozygous point mutation (C119T)	One patient with follicular occlusion triad and keratitis-ichthyosis-deafness syndrome	35
Linked to PSENEN NCSTN mutations in γ-secretase signaling	Follicular occlusion tetrad phenotype rather than solitary HS	36
Linked to PSENEN NCSTN mutations in γ-secretase signaling	In 5 out 7 pedigrees no mutation was found	37
Linked to NCSTN mutations in γ-secretase signaling	Follicular occlusion tetrad rather than solitary HS	38

HS studies. In 1968, the first HS family history was described[25] followed by the first structured family investigation in 1985[26]. Despite uncertainties about the diagnosis, an autosomal dominant pattern of inheritance was postulated[26]. In 2000, these previously studied families were re-examined and again the results supported the concept of familial inheritance of HS with an autosomal dominant mode, despite a less than 50% mendelian value[27]. HS does not seem to be associated with HLA genotypes. In 1988, a significantly different frequency of HLA-A19 was found in HS patients using serological tissue-typing techniques but later, using genomic methods, Lapins et al. found no association with HLA in another HS population[28]. The first HS-associated loci were defined at 6q25.2 and chromosome 19, but these results were only reported in an abstract[29]. Another HS locus was claimed at 1p21.1-1q25.3 in a Chinese family comprising 10 patients across four generations[30]. However, this locus could not be confirmed in another family[31]. Since another HS pathogenic factor is smoking, Ludowsky et al. studied SNPs (single nucleotide polymorphisms) in the P450 1A1 (CYP1A1) gene[32]. This cytochrome metabolizes the xenobiotic compounds of tobacco. Overall, similar SNP were found between HS patients and healthy controls[32]. Two other studies found no correlation with HS and SNPs of the innate immunity pattern recognition receptor NOD2[33,34]. In one case, a possible genetic locus for the follicular occlusion tetrad, of which HS is part, was determined to be the heterozygous point mutation C119T[35]. However, this patient had comorbid keratitis-ichtyosis deafness syndrome[35]. More recently, a study investigated six large Chinese families with supposedly HS, inherited in an autosomal dominant mode. In this study mutations in γ-secretase genes were revealed[36]. However, these results should be interpreted with caution, because the phenotypes of the investigated patients did not represent classic HS, but rather the rare follicular occlusion tetrad. Therefore, the results of this study are representative for the follicular occlusion tetrad as a disease entity, but are not convincing for pure HS. Conversely, these defects in γ-secretase signalling were rapidly confirmed in a Caucasian family revealing a haploinsufficiency of the γ-secretase genes[37]. In another report demonstrating γ-secretase mutations in a large Chinese family, the provided clinical photographs again suggested the follicular occlusion tetrad rather than solitary HS[38]. Gamma-secretase is a transmembrane protease consisting of four essential protein subunits: one catalytic presenilin subunit and three cofactor subunits presenilin enhancer 2 (PEN2), nicastrin (NCT), and anterior pharynx defective 1 (APH1) mediates intramembranous cleavage of various type I membrane proteins, including amyloid precursor protein and Notch[36]. In mice the genetic inactivation of γ-secretase produces epidermal cysts and spontaneous squamous cell carcinomas (rare in HS) or basal cell carcinomas (not associated with HS). In humans, mutations in the two presenilin genes (PSEN1 and PSEN2), but not the other γ-secretase component genes, cause early-onset familial Alzheimer dementias and non-Alzheimer dementias[36]. Yet, the co-occurrence of dementias in HS has not been reported.

In conclusion, HS is probably a genetic heterogeneous disease of which rare clinical phenotypes can be inherited in an autosomal dominant mode.

Smoking

A striking HS behavioural risk factor is tobacco smoking. A matched-pair case control study showed that 88.9% of HS patients were active smokers compared to 46% in the control group; odds ratio (OR) 9.4; 95% confidence interval (CI) 3.7-23.7[39]. In a large French population survey more than 70% of self-assessed HS sufferers were smokers; OR 12.55; 95% CI 8.58-18.38[2]. Furthermore, in a cohort of 302 HS patients, 76% was a current smoker and 15% a former smoker[4]. HS smokers were more severely affected according to the modified Sartorius score than non-smokers[15]. As discussed above, Lukowsky et al. studied SNPs in the tobacco-compound metabolizing cytochrome P450 in HS patients[32]. No SNPs related to HS were detected[32]. Cigarette smoke contains more than 4,500 components in its gaseous and particulate phases[40] which all theoretically could contribute to HS pathogenesis.

Potential pathogenic effects of smoking in HS pathogenesis are discussed here briefly. (Table 2). First, cigarette smoke can promote follicular occlusion. Hana et al. were the first to study the effect of nicotine on a cultured epidermis equivalent[41]. They concluded that nicotine and the non-neuronal cholinergic system play a significant role in the pathogenesis of HS by promoting infundibular epithelial hyperplasia and thus follicular plugging[41]. Second, cigarette smoke affects a wide range of host defence mechanisms[42], such as increased chemotaxis of polymorphic neutrophils since smoking has been shown to induce expression of IL-8 in the lungs[43]. Smoking increases the secretion of tumor necrosis factor alpha (TNF-α) by human keratinocytes[44]. Recently in the blood of smoking psoriasis patients increased rates of Th17 cells were demonstrated compared to non-smoking psoriasis patients[45]. Moreover, it was demonstrated that tobacco smoking generated Th17 cells ex vivo[45]. Third, bacteria are thought by some to be responsible for aggravating HS, and nicotine has been shown to stimulate *Staphylococcus aureus* growth[46].

To understand how smoking might modulate pathogenic processes in HS, data on other evidently smoking associated diseases might provide useful information. Palmoplantar pustulosis (PPP), also known as localised pustular psoriasis is such a skin disease associated with smoking with an OR of 10.5 and CI 3.3-33.5[47]. PPP is characterized by groups of sterile pustules with thickened, scaly, red skin which easily develop painful cracks at the palms and soles. Receptors for nicotine, namely the alpha-7 nicotinic acetylcholine receptors, are

Table 2. Putative mechanisms of smoking in HS pathogenesis

Effect	Reference #
Promotion of follicular occlusion	41
Promotion of polymorphic neutrophil chemotaxis	43
Promotion of TNF-α secretion by keratinocytes	44
Promotion of Th17 cell development	45
Stimulation of *Staphylococcus aureus* growth	46

overexpressed in the eccrine sweat glands and ducts of PPP patients[48,49]. But the precise mechanism by which these receptors can modulate inflammation is not known.

Several studies have shown that cigarette smoke contain dioxins and dioxin-like chemicals[50]. The aryl hydrocarbon receptor (AhR), present in the cytoplasm of all immune cells, is triggered by dioxin and dioxin-like chemicals and has immunomodulating properties upon stimulation[51]. AhR activation results in expansion of Th17 cells as well as cytokine production and caused earlier and more severe disease in a mouse model for multiple sclerosis experimental autoimmune encephalomyelitis, (EAE)[51]. Recently, it was shown that abnormal AhR pathway activation promotes chronic inflammation in the mucosa of Crohn's disease patients[52]. Since HS is associated with smoking and probably with Crohn's disease[39,53] (Chapter 2.2), ligation of the AhR potentially ameliorates HS inflammation in a similar fashion. Although the evidence discussed above suggests that smoking plays a role in HS pathogenesis it cannot formally be excluded that conversely the smoking habit is the result of HS. It is well established that HS patients have a decreased quality of life[18] and that a low quality of life generally is associated with smoking habits[54], which suggests that HS patients more often smoke due to their poor quality of life.

In conclusion, despite an evident epidemiologic association between smoking and HS, it remains unclear if and how pathogenic mechanisms of smoking contribute to HS disease initiation and/or maintenance.

Mechanical friction

Based on clinical observations it has been postulated that mechanical friction is associated with HS[55]. Predisposed HS skin areas are sites of regular mechanical stress. This becomes particularly clear in the obese in which additional HS locations include the abdominal folds and the medial upper leg. In the latter case, HS is induced or promoted by rubbing of the legs during walking. Mechanical friction can also cause acne mechanica, which has been described as a distinct entity. Acne mechanica is considered to be a form of acne, usually inflammatory, that is exacerbated by repeated friction, inducing bending and folding of hairs/hair follicles back and forward inducing microtrauma followed by hyperkeratinisation and occlusion of the follicle, and finally by rupture of microcomedones[56].

Causes of friction resulting in acne mechanica are diverse. They include clothing (tight straps and belts), and occupational pressure (rubbing of back, buttocks in truck drivers). Acne mechanica also occurs at sites of mechanical stress due to leg stump prostheses[57]. Another friction related disorder is jeep disease[58]. During World War II, riding jeeps, trucks and tanks caused considerable morbidity in US army personnel. This jeep disease was characterized by abscesses, fistulas and pilonidal sinuses and cysts of the buttocks, and was presumably caused by frictional forces induced by long bumpy rides[58]. In 2010, Dufour et al. postulated a hypothesis on how mechanical stress could contribute to HS development. An infant developed hidradenitis-like lesions in an inguinal naevus comedonicus, when the child started

to move around, thereby increasing shear forces[59]. The authors proposed that the degree of strain on a hair follicle increases with its diameter, leading to follicle wall ruptures[59].

In summary, mechanical stress could promote HS in two ways. First, it may promote follicular occlusion similar to acne mechanica. Second, it may trigger rupture of the fragile dilated follicles.

Obesity

There is strong evidence that HS is associated with a high body mass index (BMI)[60-64]. Moreover it has been shown that a positive correlation exists between BMI and disease severity[4]. However, there are individual cases, in which the patient has a normal stature or even a low BMI evidencing that high BMI is not a requirement for HS. Clinically, in the obese, additional predilection sites for HS are the extended skinfolds, mainly the abdominal folds and the medial upper leg. The mechanisms by which a high BMI influences HS likely are complex since several features of obesity can affect HS pathogenesis (Table 3). First, the obese have more and larger skinfolds, especially the abdominal folds, in which skin on skin contact occurs, enhancing mechanical friction. Second, the higher than usual skin temperature and humid microclimate in skinfolds favour bacterial growth. Third, obesity brings along the concept of immunometabolism. Immunometabolism is the interplay between immunological and metabolic processes. Nowadays, adipose tissue is not only considered as an energy storage site, but additionally as an endocrine and an immune organ capable of producing adipokines, cytokines and chemokines[65]. Pro-inflammatory adipokines include TNF-α, IL-6, resistin, retinol binding protein 4 (RBP4), lipocalin 2, CCL2, IL-18, nicothiamide, nicotinamide phosphoribosyltransferase (NAMPT), and CXCL5. Anti-inflammatory adipokines include adiponectin and IL-10. In addition, production of the anti-inflammatory SFRP5 (secreted frizzled-related protein 5) decreases when fat mass increases. Moreover, obesity is causally linked to a low-grade, sub-acute, inflammatory state[65,66].

In conclusion, obesity might influence HS pathogenesis by creating a bacteria friendly environment, enhance shear forces and modulate immunity to a more pro-inflammatory state. Quite surprisingly, it is unknown whether or not HS activity wanes or disappears when patients lose weight.

Table 3. Putative pathogenic mechanisms of obesity in HS

Mechanism	Reference #
Larger and more skinfolds	
Enhanced mechanical friction	
Microclimate in skin folds favours bacterial growth	
Production of pro-inflammatory adipokines	65
Pro-infammatory state	65,66

Bacteria

Frequently, HS patients fear their affliction is contagious. Some assume it is the result of a persistent bacterial infection, contracted after visiting a swimming pool or sauna. The fear of being contagious can isolate patients from participating in social activities and is therefore a factor negatively affecting quality of life and self-esteem. There is no evidence that viral or fungal infections cause or contribute to HS pathogenesis. The role of bacteria in HS pathogenesis is far from clarified. At first sight the clinical aspects of HS do suggest infection. But results of bacterial cultures, despite the volume of discharge, are often negative or do only yield skin commensal microbiota[67,68]. In a retrospective study, axillary bacterial cultures from HS patients demonstrated that the most prevalent aerobic bacteria species were *Staphylococcus aureus, Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, the most frequent anaerobic bacteria were *Peptostreptococcus* species, *Prevotella* species, microaerophilic streptococci, *Fusobacterium* species, and *Bacteroides* species[67]. However, these bacteria were collected by epidermal swabs which were potentially contaminated with skin commensals. Later, this contamination factor was circumvented in a study aspirating pus from the deeper parts of HS lesions[68]. Using this method bacteria were cultured in only half of the samples[68]. The most frequently cultured bacteria were *S. aureus* and coagulase-negative staphylococci (*S. epidermidis and S. hominis*), so again skin commensals[68].

In conclusion, there are several hypotheses on possible pathogenic roles of bacteria in HS. First, bacteria are not the initial causative factor, but are able to aggravate the disease by creating a chronic wound infection or augmenting innate inflammatory pathways by engagement of for instance Toll like receptors (TLR), NOD-like receptors (NLR) and the inflammasome. Second, the composition of the commensal skin microbiota in HS patients may be altered to more pathogenic species, triggering the disease. Third, the immune response to commensals may be altered and easily triggered similar to the pathogenic concept of Crohn's disease as a response to the gut microbiota[69].

Immunology

The chronic inflammatory nature combined with the frequent absence of pathogenic bacteria is suspect for a key role of aberrant immunity in HS. This suspicion is further supported by the reported co-occurrence with sterile arthritis and the assumed association and similarities with Crohn´s disease[53]. 1977, Dvorak studied immune mechanisms in HS and concluded that there were no abnormalities in granulocyte function (chemotaxis, phagocytosis and intracellular killing) nor immune globulin levels, but complement levels were elevated[70]. Vasey et al. demonstrated elevated levels of immunoglobulins, C3, C4 and circulating immune complexes in patients with combined arthritis and HS[71]. Bylaite et al. studied the epidermal expression of the lysosomal proteinase Cathepsin (Cat) L and its inhibitor hurpin in several inflammatory and neoplastic skin diseases[72]. Cathepsin (Cat) L is an important lysosomal proteinase involved in a variety of cellular functions including intracellular protein turnover,

epidermal homeostasis and hair development. HS epidermis showed increased expression of Cat L and Hurpin, however, lower than in lesions of psoriasis, atopic dermatitis (AD) and squamous cell carcinoma[72]. Because of the vast numbers of neutrophilic granulocytes in HS lesions, their function has been studied to some extent. In 1982, Ginder et al. demonstrated in a single case of HS a defect in polymorphonuclear leukocyte killing of bacteria accompanied by low levels of cyclic GMP[73]. However, this patient experienced various additional infections, suggesting the defect was not HS-related[73]. Lapins et al. demonstrated a significantly higher generation of free oxygen radicals by neutrophilic granulocytes than healthy controls upon stimulation with phorbol myristate acetate[74]. Giamarellos-Bouboulis et al. demonstrated an impaired secretion of TNF-α and IL-6 upon stimulation of monocytes to bacterial compounds in patients with HS[75]. They also demonstrated a diminished percentage of natural killer cells in the blood when patients had HS for a longer period[75].

Toll-like receptors (TLR) are important pattern recognition receptors of innate immunity that play a crucial role in inducing immune responses, including the production of various cytokines. Hunger et al. demonstrated enhanced expression of TLR2 at both mRNA level and protein level on macrophages and dendritic cells (DC) within the HS infiltrate compared to healthy control skin[76].

Sartorius et al. assumed HS worsens in response to psychological stress and studied neuroendocrine involvement in HS in a limited immunohistochemical study. They observed an overall decreased density of nerve fibers in the epidermis expressing protein gene product 9.5 (PGP9.5)[77]. No difference was observed in the dermis[77]. This study did not clarify whether or not PGP9.5 positive cells play a pathological role in HS[77]. In 2010, the expression of several anti-microbial peptides (AMP) in HS lesions were studied[78]. The study demonstrated on the mRNA level in HS skin biopsies a relative deficiency of several AMP including deficiency of HBD1, HBD2, HBD3, S100A7, S100A8 and S100A978. Regulators of AMP expression include IL-22 and IL-20 mRNA levels of IL-22 and IL-20 were reduced compared to psoriasis and atopic dermatitis (AD), as well as its receptors IL-22R1, IL-20R1 and IL-20R2 whereas IL-22BP was expressed higher in HS than in psoriasis lesions[78]. IL-22 is produced by several T helper subsets but Th1, Th17 and Th22 cells were not altered in number in the peripheral blood of HS patients compared to psoriasis[78]. It was proposed that the relative IL-22 deficiency could be induced by a deficiency of cytokines essential for the generation and activation of IL-22 producing cells. However, no deficiency in mRNA of IL-12-p35, IL-12/IL-23p40, IL-23p19 IL-6 or TNF-α was found[78]. The expression of the major immunosuppressive anti-inflammatory mediator IL-10 showed a significant negative correlation with IL-22 levels[78]. No difference in lesional Foxp3 expression as a marker for regulatory T cells between psoriasis and HS was observed[78]. Since IL-10 expression and IL-1 were significantly correlated, the data suggests that IL-1β plays an important role in IL-10 expression[78]. A limitation of this study, however, was that only mRNA levels were measured and not the proteins levels[78].

Sex hormones

Whether or not sex hormones are involved in HS pathogenesis is not clear. Several disease characteristics seem to indicate that sex hormones influence the disease course. First, women are overall three times more often affected than men[1,4]. Second, the disease seems to fade out around the menopause[79,80]. Third, premenstrual flare ups have been reported in 57% of 56 female HS patients[79]. This ratio was 44% in another study including 110 females[24]. Moreover, the reported efficacy of anti-androgen therapy (cyproterone acetate) and oral contraceptives supports a sex hormonal role in HS[81-83]. However, there are just as many arguments against sex hormone involvement. First, the onset of HS is usually after puberty. Second, in HS there is no androgen-associated hyperseborrhea, and sebaceous glands are reduced in number and size[84]. Third, hyperandrogenic polycystic ovarian syndrome (PCOS) was reported in 12.5% of 64 HS cases, while 10% was expected in the healthy population. This rate suggests there is no association, although the authors concluded otherwise[83]. Fourth, pregnancy does not seem to influence disease severity. One study reported improvement during pregnancy in 2% of 110 females[24]. In a series of Jemec et al. 9/32 females reported improvement and 6/32 worsening, while 17/32 reported no severity change during pregnancy[80]. Fifth, most importantly, no aberrant hormonal balance has been demonstrated in the serum[80,85] or in apocrine glands[86] of HS patients.

Treatment

HS is notoriously difficult to treat. Most patients respond only partially or the disease rapidly re-occurs after drug cessation. The number of clinical trials in HS is limited. There are few studies with more than 10 patients (Table 4). Moreover, most clinical studies on HS treatment are of observational type rather than randomized controlled, resulting in a low level of evidence based medicine. Moreover, current HS clinical research still suffers from severe shortcomings. First is the lack of a uniform outcome measurement. Most studies use a type of a global physician assessment as primary outcome measurement. Second, since HS is a clinically highly heterogeneous disease, patient groups, especially in older studies, are ill-defined and results are therefore not comparable. Third, HS is a disease with spontaneous flare ups and remissions. Many studies lack a follow-up or have an insufficient follow-up duration. Moreover, since HS can reoccur at the same location but also at new locations, reoccurrence is not clearly defined. These complications evidently hamper generalisation of study outcomes and severely limit comparisons of studies. Only treatment modalities that have been investigated in studies including 10 or more patients will be briefly discussed below. These studies include oral and topical antibiotics, anti-TNF-α biologics, anti-androgens, retinoids and light therapy, including the neodymium-doped yttrium aluminium garnet laser (Nd-Yag), zinc substitution and the topical peeling agent resorcinol. In addition, surgery is often inevitable in more severe HS. Radical excision, whereby all fistulas and subcutaneous abscesses are removed, is considered the only method providing long term cure. Table 4 provides an

Table 4. Clinical HS trials with pharmaceuticals and light/laser therapy (minimum 10 patients)

	Trial type	n	Outcome	Ref. #
Antibiotics				
Topical clindamycin 1% QD for 12 weeks	RCT	27	Clindamycin was superior to placebo	87
Oral 500 mg tetracyclin BID vs. topical BID clindamycin 1% for 16 weeks	RCT	46	Equally effective, both drugs showed slight improvement in lesion count	88
Oral combination of clindamycin 300 mg BID and rifampicin 300 mg BID for 10 weeks	Retrospective case series	14	Improvement in 57% of patients	89
Combination of clindaycin and rifampicin in different regimens and durations	Retrospective case series	34	Improvement in 84% of patients	91
Combination of clindaycin 300 mg BID and rifampicin 600 mg QD for 10 weeks	Retrospective case series	116	Significant improvement in HS Sartorius severity score	90
Combination of rifampicin, moxifloxacin and metronidacazol in different regimens and durations	Retrospective case series	28	Complete remission in 57% of patients	92
Anti-TNF-alpha biologics				
Infliximab 5 mg/kg at week 0, 2 and 6	Prospective case series	10	54% reduction of HS severity by Sartorius score	95
Infliximab 5 mg/kg at week 0, 2, 6, 14 and 22	RCT	38	Infliximab was superior to placebo	96
Adalimumab 80 mg week 0, 40 mg EOW for 12 weeks	RCT	22	Adalimumab induced a significant reduction in HS sartorius severity score after 6 weeks	99
Adalimumab 160 mg week 0, 80 mg week 1, 40 mg EOW for 12 weeks	Prospective case series	10	Statistically clinical improvement was not observed with adalimumab	100
Etanercept 50 mg weekly for 12 weeks	Prospective case series	10	Etanercept is a safe and effective therapy for hidradenitis suppurativa	97
Etanercept 50 mg weekly for 12 weeks	Prospective case series	15	Minimal evidence of clinically significant efficacy of etanercept	98
Anti-androgens				
Ee 50 mg/ca 50 mg VS. ee 50 mg/ng 500 mg for 12 months	RCT	24	Both regimens produced similar improvement by physician global assessment	81
Antihormonal therapies, several antibiotics	Retrospective case series	64	Antihormonal therapy was superior to antibiotics (55% versus 25%)	83

overview of clinical trials with a minimum of 10 patients studying pharmaceuticals and light/laser therapy in HS.

Antibiotics

Antibiotic therapy is as a prominent form of HS treatment. This is probably because the first clinical impression of HS is that of infection although bacterial cultures are frequently negative. Interestingly the literature reveals only a handful of studies on the use of antibiotics including only two randomized controlled trials (RCT)[87,88]. A small RCT demonstrated that topical clindamycin was superior to placebo in mild forms of HS. Another RCT showed no

Table 4. (continued)

	Trial type	n	Outcome	Ref. #
Light and laser				
Nd:Yag, 3 monthly sessions	RCT	22	The Nd:Yag laser is effective for the treatment of HS, 63.3% improvement	102
PUVA twice weekly, median 25 treatments	Retrospective case series	13	In 38% of patients complete remission	104
ALA PDT weekly for 4 weeks	Prospective case series	12	Overall 29.9% reduction in lesion count	103
Retinoids				
Isotretionoine mean dose 0.56 mg/kg/day for 4-6 months	Retrospective case series	64	Disappointing results, efect 23.5% of mild cases	105
Isotretionoine mean dose 0.44 mg/kg/day for mean 7.8 months	Retrospective case series	88	Patients reported isotretinoine was not effective	106
Acitretin mean dose 0.59 mg/kg/day for mean 10.8 months	Retrospective case series	12	All patients achieved remission	108
Other				
15% resorcinol applied when necessary	Prospective case series	12	In all patients pain and lesion duration decreased	113
Zinc 90 mg/day	Prospective case series	22	All patients improved, complete remission in 8 patients	114

Antibiotics probably exert their function in HS primairily by immunomodulation and secondarily by bactericidal properties. Anti-TNF-α biologics exert their function by immunomodulation. How anti-androgens exert their effect on HS is unknown. Light and laser therapies presumably exert their effect by destroying the hair follicle, and photo dynamic therapy additionally by bactericidal properties. The retinoid acitretin might exert its effect by normalizing infundibular keratosis and immunomodulation. Resorcinol unplugs the plugged hair follicles, and zinc acts by immunomodulation. QD:once a day, BID: twice a day, EOW:every other week, PUVA:psoralen+UVA, ALA-PDT:aminolevulinic acid photodynamic therapy, RCT:randomized controlled trial.

difference between systemic tetracycline 1 g daily and topical clindamycin 1% twice daily. Both treatments resulted in improvement. Furthermore, the combination of clindamycin and rifampicin produced reasonable results[89-91] as well as rifampicin combined with moxifloxacin and metronidazole[92]. The mechanism by which antibiotics ameliorate inflammation in HS is unknown. Since bacterial cultures are often sterile, the anti-inflammatory properties of antibiotics may be more important than anti-bacterial action. The use of antibiotics has drawbacks. The chronic and recurrent nature of the disease requires the use of antibiotic cycles which can lead to the development of bacterial resistance. Furthermore a frequent side effect is diarrhoea, which often leads to therapy discontinuation.

Anti-TNF-α biologics

The best studied drugs in HS are the anti-TNF biologics which include infliximab, a chimeric monoclonal antibody, etanercept, a dimeric TNF-α decoy receptor, and adalimumab, a fully

human IgG1 monoclonal antibody. Anti-TNF biologics are generally applied only in severe cases of HS[93]. The rationale for anti-TNFα therapy in HS is based on the co-occurrence of Crohn's disease and HS. Early reports showed that in these patients both diseases responded to infliximab[94]. Infliximab therapy for treatment of moderate to severe HS is well tolerated, reduces pain intensity as well as disease severity, and improves quality of life[95,96]. Etanercept showed more conflicting results[97,98]. The efficacy of adalimumab is modest and probably lies between that of infliximab and etanercept[99-101]. This difference in efficacy might be explained by how these various biologics exert their effect. Etanercept binds only soluble TNF-α, in contrast to TNF-α antibodies (infliximab and adalimumab), which additionally target membrane bound TNF-α, resulting in cytotoxicity. The efficacy of these three biologics is comparable in psoriasis, but analogous to HS the efficacy of etanercept in Crohn's disease is less than of the other biologics.

Anti-androgens

As discussed above, hormones might play a role in the disease pathogenesis. A trial demonstrated relative success for anti-androgens such as ethinylestradiol (ee) and cyproterone acetate (ca)[81]. In addition, a retrospective study suggested that some female HS patients respond better to anti-androgen therapy than to antibiotics[83]. Since the role of hormones in HS pathogenesis is unknown, the mechanism of how these drugs would exert their effect in HS is unclear.

Light and laser therapy

Tierney et al showed in a RCT that treatment with the Nd:Yag laser is effective for HS[102]. The efficacy can be explained by the destruction of hair follicles and vasculature by Nd:Yag laser emphasising the follicular nature of the disease[102]. Sweiger et al. demonstrated some improvement in HS with ALA PDT (aminolevulinic acid photodynamic therapy), although this treatment was quite painful[103]. The mechanism of action might be by destroying the hair follicle. Shareef reported in a retrospective case series that Bath PUVA (psoralen + UVA light) also caused some improvement. Its mechanism of action in HS is unknown[104].

Retinoids

Based on the overlap of some disease features between HS and acne (e.g. open comedos and follicular occlusion), oral retinoids such as isotretinoin, etretinate and acitretin have been used in the treatment of HS. Despite its successful use in acne, isotretinoin has been found to be far less valuable in the treatment of HS[105,106]. This might be explained by the main mechanism of action of isotretionin in acne vulgaris, which is by inhibition of sebum secretion[107]. HS is not a disease in which the sebaceous glands play a significant pathogenic role. Conversely, acitretin showed to be effective in a retrospective HS case series[108]. The exact mechanism of action of acitretin is still not fully clarified. Acitretin has anti-proliferative and

immunomodulatory properties[109]. In keratinization disorders, acitretin has been reported to normalize epidermal cell proliferation, differentiation and cornification[110]. In HS acitretin may target the process of infundibular keratinisation (plugging). Furthermore, acitretin inhibits the production of vascular endothelial growth factor (VEGF) by keratinocytes and has anti-inflammatory properties such as reduction of intra-epidermal migration of neutrophils[111] and inhibition of IL-6-driven induction of Th17 cells[112].

Other treatment modalities

Resorcinol is a topical peeling agent, which in a small open study reduced pain and shortened nodule duration in HS[113]. Resorcinol might exert its efficacy by skin peeling and promoting drainage of nodules and abscesses. Furthermore, it may prevent formation of new lesions by unplugging blocked hair follicles. In a pilot study, Brocard et al. showed good results by orally supplementing zinc salts[114]. The proposed effect of zinc in HS is by immunomodulation[114]. Zinc has been shown to inhibit chemotaxis of polymorphonuclear neutrophils, to inhibit expression of ICAM-1 on keratinocytes and production of TNF-α and IL-6 by keratinocytes and to activate natural killer (NK) cells and the phagocytic function of granulocytes[114].

Surgery

In addition to drug therapies, surgery is frequently performed to control or cure HS. Surgery is generally regarded as one of the most effective treatments for HS[115]. The efficacy and recurrence rates of several types of surgical interventions have been reported in retrospective case series, but RCTs are lacking[23]. Surgical techniques are: incision and drainage or lancing, deroofing, local or wide excisions, and CO_2 laser assisted surgery[23]. Excisions can be local, with or without a healthy appearing perilesional tissue margin or removal of the complete terminal hair bearing area[23]. Reported recurrence rates between techniques vary enormously and cannot be compared since the reports employ different follow up periods and definitions of recurrence[115]. There is also no consensus on the best type of wound healing after surgery: options include healing by second intention, primary closure with suture, skin graft and skin flaps[23]. The type of surgery strongly depends on the disease stage. For abscesses, simple incision and drainage provides quick pain relief but the reported recurrence rates are almost 100%[23]. Mild HS (Hurley I and II) can be treated with limited excision or deroofing, the latter being a technique in which a nodule or abscess is probed for fistulas and the roof lesion is removed followed by secondary healing[116]. When fistulas are formed, or in case of severe disease, Hurley III surgery is accepted as the only method that can provide total disease clearance. There is no consensus of how far the excision margin should reach into healthy appearing tissue, but it should not be too limited as small margins are thought to be correlated with disease recurrence[117]. Furthermore, CO_2 assisted surgery for HS has been described as an efficacious approach[118,119]. This method has the advantage, since it is virtually

bloodless, of providing a clear view of the diseased area during surgery, so that the extent of lesions can be followed in the tissue a vue.

Surgery also has disadvantages. In the acute stage, intervention by incision and drainage can be extremely painful, since abscesses are difficult to anaesthetize. General anaesthesia brings along risk of complications. Furthermore, even wide radial excisions are not always curative since new lesions may develop next to the excised area[115]. Furthermore, in severe HS excision areas can be very extensive, increasing the risks of wound healing complications and mutilation and an extended recovery time.

AIMS AND OUTLINE OF THE THESIS

HS has been relatively neglected both clinically and scientifically; therefore it is almost 'virgin scientific territory'. This complicates reductionist approaches on immunopathogenic mechanisms and rational therapy development. The conceptual background of this thesis is therefore not limited to only one disease aspect. Instead, this thesis addresses selected major gaps in HS knowledge in terms of clinical aspects, pathomechanisms and treatment.

Clinical aspects of hidradenitis suppurativa

- Awareness about the disease severity and impact on quality of life is necessary to increase scientific interest. We hypothesized that depression is more common in HS patients than presently appreciated.
- There are striking clinical similarities between HS and Crohn's disease. Demonstration of an association may imply that parallel or similar pathogenic mechanisms contribute to both diseases. We investigated whether HS occurs more frequently in Crohn's disease patients than in the general population.
- Why HS prefers the inverse skin areas is unknown. We present a case that provides clues about the contribution of friction.

Pathomechanisms in hidradenitis suppurativa

- Characterization of the lesionalHS cytokine profile can provide a basis for development of novel therapies and improves insight into immune mechanisms. We investigated whether secretion of prototypical pro-inflammatory cytokines such as TNF-α and IL-1β is elevated in HS skin.
- Anti-TNF-α biologics show promising efficacy in HS, but the underlying mechanisms are largely unknown. We studied whether specific cytokines or inflammatory mediators are significantly affected by treatment with the anti-TNF-α biologic adalimumab.
- We showed that levels of TNF-α, IL-1β and IL-10 are increased in normal appearing perilesional HS skin. We therefore investigated the cellular source of these cytokines in non-lesional HS skin.

Treatment of hidradenitis suppurativa

- Since antibiotic therapy is the most common treatment for HS, we addressed whether combination therapy with clindamycin and rifampicin is effective in HS.
- Deroofing is a surgical method for HS, but its efficacy has not been systematically investigated. We hypothesized that deroofing would be effective in recurrent single lesions of HS.
- Colchicine is a drug with anti-inflammatory properties, and is successfully used in autoinflammatory diseases. In a pilot study we studied the efficacy of colchicine in patients with HS.

Model for HS pathomechanisms

- In chapter 5 we present a model for HS pathomechanisms, incorporating the results of this thesis and literature. In this chapter we also do suggestions for future research on HS.

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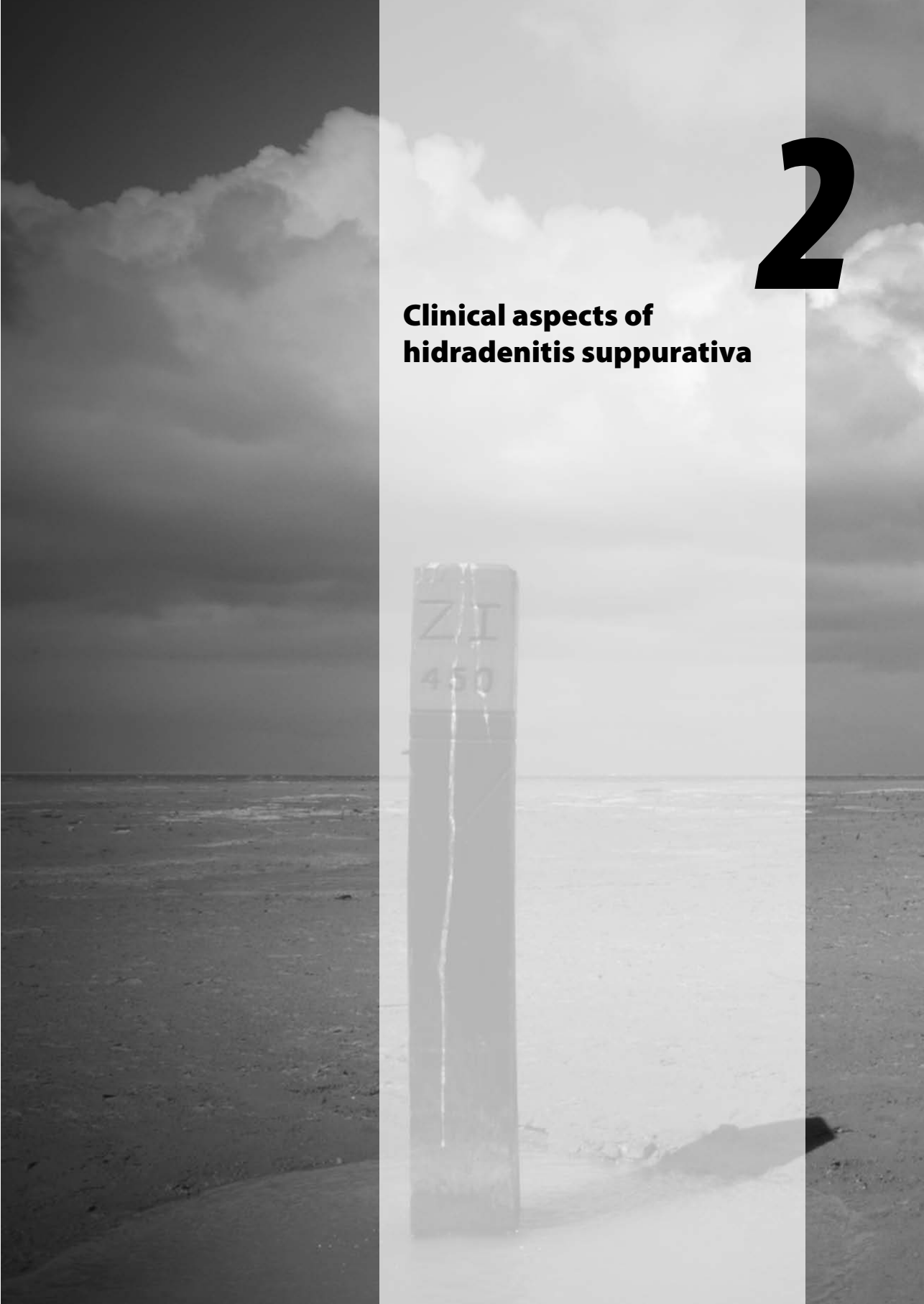

2

Clinical aspects of hidradenitis suppurativa

2.1

Depression in patients with hidradenitis suppurativa

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ABSTRACT

Background: Hidradenitis suppurativa (HS) is a chronic recurrent inflammatory skin disease with abscess formation and scarring, predominantly in the inverse areas. The disease is often difficult to treat and patients experience a decreased quality of life (QoL). It is hypothesised that depression is more common in HS than in other dermatological conditions.

Objectives: To evaluate the prevalence of depression in patients with HS.

Methods: 211 HS patients and 233 dermatological control patients were included in the study. Their QoL and depression scores were assessed using the Dermatology Life Quality Index (DLQI) and the (Major Depression Inventory) MDI questionnaires. HS severity was recorded with a questionnaire and Hurley stages were extracted from the case records.

Results: The DLQI was significantly higher for HS patients than in control patients, 8.4 $\pm$7.5 versus 4.3 $\pm$5.6 ($p<0.0001$) and correlated with Hurley stage severity. Mean MDI scores were significantly higher for HS patients, 11.0 versus 7.2 ($p<0.0001$). However, clinically defined depression rates according to ICD-10 criteria were not significantly higher in HS patients compared to controls (9% versus 6%).

Conclusions: HS has a major impact on quality of life relative to other dermatological conditions. MDI depression scores in HS patients correlated with disease severity. This correlation could indicate that the MDI depression questionnaire, although not yet widely accepted, represents a valid measure of disease-related morbidity. Its usefulness as an outcome measure in future studies needs further validation.

INTRODUCTION

Hidradenitis suppurativa (HS), also called acne inversa, is a chronic recurrent inflammatory follicular skin disease, which presents with painful suppurating lesions in the inverse skin areas[1]. It is a common disease, affecting up to 1% of the general population[2,3]. Treatment options include medical therapy, and laser as well as conventional surgery[4]. In spite of a growing number of therapeutic options the outcome is often unsatisfactory, and a considerable unmet need for therapy exists[5,6].

Current information on the psychosocial consequences of the disease is limited. This may partly be due to the clear symptomatology and partly due to the lack of specific investigations. HS affects a range of psychosocial factors and it reduces the quality of life (QoL) significantly[7-11]. The few studies addressing the QoL in HS found the disease to have a profound negative effect, often surpassing the negative QoL reported in other major dermatoses such as psoriasis and atopic dermatitis. Reduced QoL, reduced "enjoyment of life", and depressive mood has been linked to HS severity by Matusiak et al.[9]. In addition the self-reported health of HS patients is lower, and the average number of sick-days is higher than in controls[10,12]. We therefore speculated that depression is more frequent and pronounced among HS patients than among other dermatological patients, and thus a pertinent point of clinical interest and possible early intervention.

MATERIALS AND METHODS

The study was conducted in the departments of dermatology at Deventer Hospital in the Netherlands and Roskilde Hospital in Denmark, from March to November 2009, as a survey among HS patients and a random sample of other dermatological outpatients as controls. HS patients were identified from the records and they had the questionnaires mailed to them instead of handed out while attending physicians. Non-responders were accepted without further efforts and no reasons were given for non-participation. Incorrectly completed questionnaires were handled in accordance to given guidelines for the instruments used. The disease definition was in agreement with the definition suggested at the HS-Foundation research meeting in 2009: a chronic inflammatory, recurrent, debilitating skin disease that usually presents after puberty with painful, deep-seated, inflamed lesions in the apocrine gland-bearing areas of the body, most commonly the axillary, inguinal and anogenital regions. No formal power calculations were carried out as the prerequisite epidemiological data were not available for the main outcome criteria of depression. The number of patients with HS was limited to those available in the records of each department. Cases and controls were matched for age and gender at a ratio of 1:1. The controls did not have HS, (20 – 60 years old) and were selected among patients attending the out-patient department as a convenience

sample on a running schedule depending on the availability of the investigators. The controls were contacted directly by an investigator (AO or SE) who handed them the questionnaires. Bias was avoided by handing the questionnaires without interfering in their understanding of the questions or their responses. For QoL assessment the Dermatology Life Quality Index (DLQI), range 0-30, was used, and for identifying depression the Major Depression Inventory (MDI) questionnaire, was used[13,14]. The MDI has a range of 0-50 and can be used in three ways: 1) As a diagnostic instrument according to ICD-10 criteria, 2) as a diagnostic instrument according to DSM-IV criteria or 3) as a depression rating scale. In the present study the MDI scores were used as an ICD-10 diagnostic instrument, and as a depression rating scale. The MDI questionnaire has 10 questions on a Likert scale with "All of the time", "Most of the time", "More than half of the time", "Less than half of the time", "Some of the time", "At no time". Three core questions have to be scored with either "All of the time" or "Most of the time" when diagnosing depression according to the ICD-10 criteria. If they scored lower, the patient was diagnosed with no depression. In contrast, when using the MDI as a depression rating scale no limits are given.

These validated questionnaires were supplemented by simple data gathering questionnaires to characterise the patients: duration of symptoms, location of lesions, days with symptoms during the past month, number of flares during the last month and days of absence from work. In addition patients were presented with two Numeric Rating Scales (NRS) to register severity of current pain and itch on a scale from 0-10, with the anchor points no pain/itching and most severe imaginable pain/itching at the two extremes. The Hurley stage of the patients was determined from the case records[15]. The Danish Ethical committee was consulted regarding the need for approval of the study, and informed the Authors that questionnaire studies were exempt from Ethical Committee approval. Statistical descriptive analysis was performed, and the significance tested using the Wilcoxon Test. Spearman's rank correlation coefficient was used to assess the relationship between descriptive data, DLQI and MDI.

RESULTS

A total of 539 patients were contacted and 444 patients (82%) accepted participation. The distribution of the participants was 211 HS patients and 233 controls, (77% women and 23% men). The mean age of HS cases was 43 $\pm$ 11.8 years (range 16 - 70) and controls was 42 13.5 years (range 9 – 77). There were no patients with a known primary psychological disorder. The diseases in the control group included skin tumors n=62 (27%), psoriasis n=29 (12%), eczema n=45 (19%), acne n=23 (10%) and 37 other diseases n=74 (32%).

The HS patients had HS for 16.8 $\pm$11.6 (median 15, range 0-50) years, whereas controls had their skin disease for 12.7 $\pm$12.6 years (median 7.5, range 0-48) years. Using Hurley stage as

Table 1. Location of lesions

	Number	Percent
Under the arms	107	50.47
Under the breasts	27	12.74
In the crotch	165	77.83
On the buttocks	64	30.19
In the perineum/perianal	49	23.11

disease severity measure, 30.1% of HS patients were stage I, 56.4% stage II and 13.5% stage III (total n=163). The self-reported distribution of HS lesions is shown in Table 1. During the four weeks prior to answering the questionnaires HS patients had had 13.5 ±11.8 days (median 10) with flares, whereas the controls had had 18.1 ±13.7 days (median 30) with active disease (p=0.027).

At the time of answering the questionnaires the pain-score of HS patients was 3.6 ±3.2 (median 3) and controls 1.9 ±2.6 (median 0) on a 0–10 NRS (p<0.001). Using a similar scale itching was scored at 3.3 ±3.1 (median 3) by HS patients and 3.3 ±3.4 (median 3) by controls (p=1, n.s.).

The range of sick days reported by the two groups was identical 0-90 days, median=0 for both groups for the last 3 months, but the mean number of days off work was 3.1 ±13.6 for cases and 7.1 ±21.0 for controls (p=0.07, n.s.).

The DLQI was significantly higher for HS cases than for controls both in total with a mean value of 8.4 ±7.5 versus 4.3 ±5.6 (p<0.0001) as well as in sub-scores (Table 2). The DLQI correlated positively and significantly for both HS patients and controls with: MDI score, number of flares during the last month, pain, itch and sick-days during the last 3 months. For HS patients it further correlated with number of flares during the last month and Hurley severity score.

Table 2. DLQI scores

DLQI – Mean values (range 0-30)	Cases (N=211)	Controls (N=233)	
Symptoms and feelings (Q1 + Q2)	2.42 (SD 1.87) Median 2	1.67 (SD 1.77) Median 1	(P=<0.001)
Daily activities (Q3 + Q4)	1.82 (SD 1.76) Median 2	0.88 (SD 1.37) Median 0	(P=<0.001)
Leisure (Q5+Q6)	1.47 (SD 1.89) Median 1	0.71 (SD 1.35) Median 0	(P=<0.001)
Work and school (Q7)	0.67 (SD 0.98) Median 0	0.29 (SD 0,67) Median 0	(P=<0.001)
Personal relationships (Q8+Q9)	1.62 (SD 1.94) Median 1	0.57 (SD 1.30) Median 0	(P=<0.001)
Treatment (Q10)	0.35 (SD 0.74) Median 0	0.22 (SD 0,59) Median 0	(P=0.044)
Total scores	8.35 (SD 7.49) Median 6	4.34 (SD 5.58) Median 2	(P=<0.001)

Similarly, mean MDI scores were significantly higher for HS patients than controls 11.0 versus 7.2 (p<0.0001), (Table 3), with 44 HS patients (21%) having scores indicating possible depression (≥20), compared with 26 controls (11%) (p=0.006).

Table 3. MDI divided into rating scale scores and diagnostic scores

MDI (range 0-50) (p>0.0001)	Cases (N=211) Mean 11,0 (SD 10,99), Median 7		Controls (N=230) Mean 7,2 (SD 9,55), Median 3	
	Rating scale Total score	Cases ICD 10	Rating scale Total score	Controls ICD 10
No depression	167	192	204	216
Mild depression	15	5	11	3
Moderate depression	11	5	1	7
Severe depression	18	9	14	4

Using the MDI as a diagnostic tool to assess the prevalence of depression among patients according to the ICD-10 criteria, 19 HS patients (9%) were found to suffer from depression, compared with 14 controls (6%) (n.s., p=0.34).

For HS patients and controls the MDI scores as a rating scale correlated positively and significantly with: days with lesions during the past month, pain, itch, sick-days due to the skin disease during the last 3 months, and for HS patients furthermore with the number of flares during the last month. Depression scores were not significantly correlated to Hurley stage, although a near significant value was seen when using the MDI as a diagnostic tool (Table 4).

Table 4. Depression score correlated to Hurley stageing (n=163) *p=0.06

	Hurley stage I	Hurley stage II	Hurley stage III	Total	P
MDI score (mean ±SD)	7.3 ± 8.9	11.0 ±11.3	5.0 ±0.0	5.0 ±10.0	n.s.
MDI used as a rating score suggesting depression (n, %)	5 (10.2%)	21 (22.8%)	7 (31.8%)	33	n.s.
MDI used a diagnostic guide for depression (n, %)	1 (0.5%)	7 (7.6%)	4 (18.2%)	12	n.s.*
Patients (n)	49	92	22	163	n.s.

DISCUSSION

It is easy to imagine the psychosocial impact of HS, and yet in order to deal with it in an evidence-based rational manner, objective facts are helpful. This survey is the first large scale study reporting the impact of HS on quality of life. It confirms that HS has a major negative effect on the QoL of patients, also when compared to other dermatological patients. The QoL as defined by the DLQI score was significantly worse for HS than for a comparable group of dermatological patients. According to the interpretation guide proposed by Hongbo *et al.*, a difference in mean scores of this magnitude (+4) can be interpreted as a significant clinical difference[17] suggesting that HS patients form a special high-need population of dermatological patients. The differences in sub-scores reflect different aspects of QoL and all were statistically highly significant in HS patients.

A comparison with other studies shows a discrepancy in DLQI results. Matusiak *et al.*[9] They found a mean DLQI score of 12.7 ±7.7 (n = 52, median 12) with a disease duration of 10.16 ±7.64 years while von der Werth and Jemec[8] and the present study found a mean on respectively 8.9 ±8.3 and 8.4 ±7.5 with a disease duration of respectively 18.8 ±11.4 and 16.8 ±11.6 years. We speculate that the duration of HS may have some influence on self-reported QoL, but other disease specific or demographic issues could also have an impact[16].

Although surveys are only suitable to describe correlations rather than causal associations, it has been suggested that the symptomatology and poor response to therapy induces clinically significant mood changes in patients. Depression scores of HS patients were significantly higher than those of other dermatological patients, suggesting that the disease represents a significantly heavier burden for patients than other skin diseases[9]. The proportion of depressed patients according to the ICD-10 criteria in this study was 9% while 21% were suspected of having depression using the MDI as a rating scale. Matusiak et al. used the Beck Depression Inventory-Short Form (BDI) to screen for depression in a smaller cohort (n=52). In their cohort 19.5% patients had a score of at least 10 on the BDI which might suggest the co-existence of depression[9].

The MDI can also be used diagnostically to indicate the clinical level of depression, but although the mean scores were higher in HS patients and a larger proportion of HS patients scored high enough to warrant a diagnosis of depression, the prevalence of clinical depression was not significantly greater in HS patients than in other dermatological patients. This discrepancy could be explained by the limitations of the MDI relative to ICD-10. Three core questions have to be scored with either "All of the time" or "Most of the time" in case of depression. If they score lower, the patient will not be diagnosed with depression. In contrast, when calculating scores on a rating scale no limits are given. In studies that focus on the general impact of a disease it may therefore be more relevant to use the MDI as a rating scale than as a diagnostic tool. This discrepancy may also suggest that HS patients have developed better coping skills due to the often long duration of their disease, or the chronic recurrent nature of their disease. It may be speculated that acquiring of HS provides patients with a coping mechanism that allows them to prevent higher depression scores, better than other patients. Such a coping mechanism is however unproductive in the long term, and may therefore add to the general psycho-social complications of HS[18].

The MDI has not been used for other dermatological diseases so no direct comparison can be made between HS other dermatological diseases such as psoriasis. Although, depression rates established by different questionnaires cannot be compared, studying depression in 50 psoriasis patients showed that 58% of psoriasis patients scored high enough by Beck depression inventory (BDI) score to indicate depression[19]. Another study in 265 psoriasis patients the suspected depression rate was 32% measured by Center for Epidemiologic Study Depression Scale (CES-D)[20]. MDI scores of HS can however be compared to those of patients with the severe neurological disorder amyotrophic lateral sclerosis (ALS). HS demonstrated slightly

more frequent severe depression. In 51 ALS patients, 8% scored mild, 2% moderate, and 0% severe depression[21], in HS these rates were 2%, 2%, and 4% respectively.

For HS patients as well as controls the DLQI and MDI scores were, significantly correlated with frequency of flares, pain, itch, and sick-days. This also indicates that the depression scores represent a measure of disease related morbidity that may serve as out-come a measure in future studies.

There are several limitations to this study. One limitation is the selection bias by being a hospital-based sample, which restricts the generalization of the data. The use of other dermatological patients as a control group is a limitation and a strength. While limiting the generalization of the findings to the general and healthy population, it reflects the impact of this specific skin disease, underlining the burden of the disease.

In conclusion, the results of this study indicate that HS has a major impact on quality of life compared to other dermatological diseases. Dermatologists should be aware of and actively look for signs of depression and in case of severe depression refer patient for treatment. The MDI depression scores correlate with HS disease severity. The MDI questionnaire may serve as an outcome measure in future studies.

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2.2

Hidradenitis suppurativa and inflammatory bowel disease, are they associated? Results of a pilot study

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ABSTRACT

Background: The co-occurrence of hidradenitis suppurativa (HS) and Crohn's disease (CD) published in a few case reports resulted in the wide acceptance of an association between these two diseases. However the combined prevalence of these diseases is currently unknown, furthermore it is unknown whether this co-occurrence also applies for ulcerative colitis (UC). Objectives To estimate the prevalence of HS in patients with inflammatory bowel disease (IBD) living in the South-West of The Netherlands.

Methods: During an IBD patient information meeting, randomly, 158 patients with IBD were interviewed about recurrent painful boils in the axillae and/or groin and were shown illustrative clinical pictures of the appearance of HS.

Results: Of the 158 patients interviewed, 102 (64.6%) had CD and 56 (35.4%) had UC. Twenty five people 25 (15.8%) responded that they had had or still experienced painful boils in axillae and/or groin, of which 17 were CD (16.7%) and 8 UC patients (14.3%).

Conclusions: This pilot study shows for the first time that HS occurs in patients suffering from CD or UC. More prospective studies are warranted to establish the association between HS and IBD and its underlying pathogenesis.

INTRODUCTION

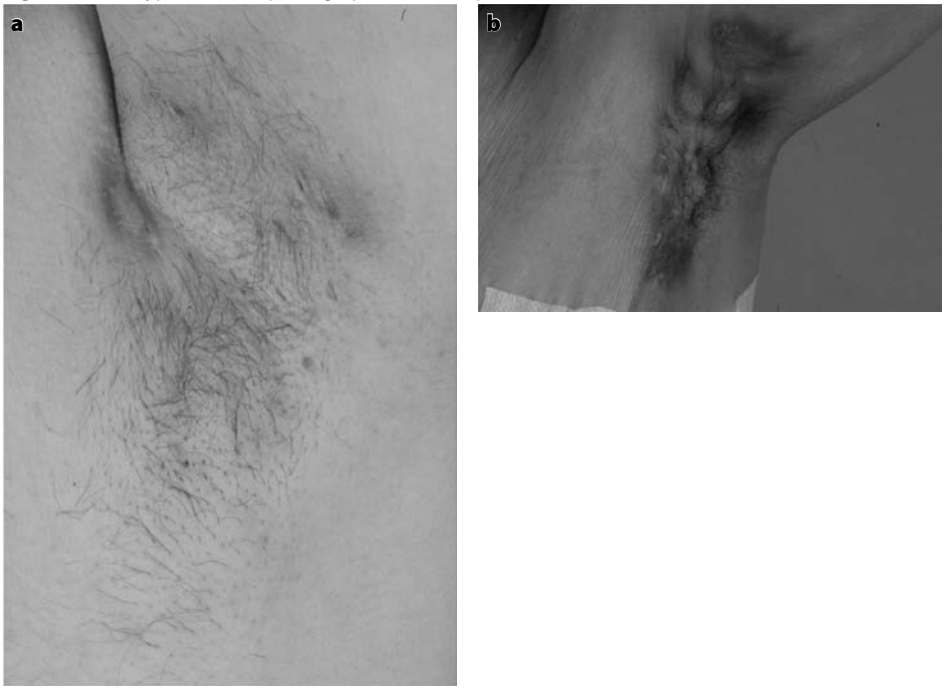
Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by recurring painful abscesses, nodules and draining sinus tracks, localized in the groin and axillae that often heal with scarring. HS usually develops during young adulthood and females are more frequently affected[1]. This painful disorder deeply affects the quality of life in these patients[2,3]. The pathogenesis of HS remains largely unknown and is probably multifactorial. The primary mechanism in the disease is thought to be hyperkeratinisation of the pilosebaceous infundibulum resulting in occlusion with subsequent inflammation[4]. Other putative pathogenic factors include hormonal influences, biomechanical forces, obesity, smoking[5,6], dysfunction of the immune system and an inappropriate response to commensal bacterial skin flora[4]. The latter is supported by a study that showed that the bacteria cultured from HS lesions are predominantly commensal skin flora[7,8]. Also genetic factors seem to be involved, since HS is a familiar disorder in 34% of cases[9]. Recently the prevalence of HS has been estimated at 1% in the general French population[10]. In Denmark a point prevalence of 4.1% and a 1 year prevalence of 1% has been reported[11]. Current treatment options include topical or systemic antibiotics and immunosuppressive agents such as anti TNF-alpha[1] in case of severe disease. Surgery forms the cornerstone of treatment, especially for advanced stages of the disease, consisting of unroofing of small lesions and total excision of large skin areas[1]. The occurrence of both Crohn's disease and HS in the same individual has been reported in over 37 cases[12]. These reports have led to generally mentioning this observed co-occurrence as an association in the literature, although the supporting evidence is weak.

Table 1. A comparison between Crohn disease, ulcerative colitis and hidradenitis suppurativa

	Inflammatory bowel disease		Hidradenitis suppurativa
	M. Crohn	Ulcerative colitis	
Localization	Entire gastrointestinal tract	Large bowel	Inverse areas of the skin
Layer of inflammation	Transmural	Surface epithelium	Deep dermal
Confluency of lesions	Skip lesions with granuloma	Continuous inflammation with crypt abcesses	Tendency towards continuous lesions
Cicatrisation	Yes, sometimes with subsequent obstruction	No	yes
Formation of fistulas	Yes	No	yes
Influence of smoking	Can aggravate	Relapse after stopping	Aggravates
Disease chronicity	Yes	Yes	Yes
Epithelia inhabited by commensal flora	Yes	Yes	Yes
Genetic predisposition	Yes	Yes	Yes
Response to anti TNF-alpha therapy	Yes	Yes	Yes

Inflammatory bowel diseases (IBD) consist of Crohn's disease (CD) and ulcerative colitis (UC). The prevalence of adult CD and UC in the USA is 0.201% and 0.238% respectively[13]. The current concept is that a dysbalance in the immune response to the commensal bowel flora in combination with genetic predisposition and other environmental circumstances can trigger IBD[14]. There are several similarities between HS and CD (Table 1). Both are chronic diseases of epithelia which are inhabited by commensal flora. Smoking has been reported in 77 to 89% of HS cases1 and has been described as a risk factor for CD[15], both diseases have a genetic predisposition and both show clincial response to anti TNF-alpha therapy. Furthermore HS and CD both are accompanied by fistula formation. These striking parallels between HS and CD led us to address two questions: Can the proposed co-occurrence of HS and CD be further substantiated from a large group of IBD patients? Does HS co-occur with UC? To date HS is still a difficult disease to treat with great unmet medical needs. Finding evidence for these associations would support the notion that parallel or similar pathogenic mechanisms contribute to HS and IBD, providing novel directions for future functional studies and therapy development.

Figure 1. Prototypical clinical photographs shown to patients



a) Mild hidradenitis suppurativa in the axilla.

b) Severe hidradenitis suppurativa in the axilla. (See also Color section, p. 196.)

SUBJECTS AND METHODS

Patients attending the Crohn's and ulcerative colitis patient information meeting in Rotterdam 2008, organised by the Dutch Society for Crohn's and ulcerative colitis patients, were randomly interviewed. All patients were asked two questions: first whether they had UC or CD and second if they ever experienced or still had recurrent painful boils in the axillae and/or groin. This question on HS closely resembles the question used in a recent HS prevalence study in France[10]. In contrast to that study focusing only on the last year of the patient's experience, we asked whether recurrent painful boils ever occurred in the patient's life. To clarify the disease patients were questioned about, they were shown 2 prototypical clinical pictures of HS in different stages in colour A4 format (Figure 1). For this type of investigation, no medical ethical committee approval is required under Dutch law.

RESULTS

We interviewed 158 patients randomly. The response rate was 100%. Of the 158 patients interviewed 102 (64.6%) had CD and 56 (35.4%) UC. Twenty five patients (15.8%) reported to have or have had recurrent painful boils in the axillae or groin, which were 17 (16.7%) patients with CD and 8 (14.3%) patients with UC (Table 2).

Table 2. Interview results

		Painful boils in groin or axillae	
	Number of patients	yes	no
Infammatory bowel disease	158	25 (15,6%)	133 (84,4%)
Crohn disease	102 (65%)	17 (16,7%)	85 (83,3%)
Ulcereative colitis	56 (35%)	8 (14,3%)	48 (85,7%)

DISCUSSION

The association between HS and CD has been reported in several case reports but has never been extensively investigated and hence cannot yet be accepted as a clinical fact. The results of this pilot study in a larger group appear to indicate that HS not only occurs more frequently than could be expected by chance in CD, but also in UC. However, this pilot study has some weaknesses. There are other causes of painful boils in the axillae and groin like furunculosis, lymphadenitis, ectopic Crohn's disease or granuloma venereum. To reduce the chance for confusion with these conditions, we specifically asked about recurring boils. Perianal cutaneous CD lesions that can mimic HS were avoided by not to asking about painful boils in the perianal area. The interviewed patients suspected of having HS were not physically examined

in order to confirm the diagnosis. Furthermore co-occurrence does not necessarily imply an association[16]. For these diseases to be associated, they must co-occur in a proportion greater than is expected by chance. To properly study an association between the diseases in both groups, one ideally should know the exact prevalence of both diseases within a population. Furthermore these two populations should both be taken from a homogeneous population because IBD has a variable prevalence among different countries. For HS such a variance in prevalence is not known. We calculated that in order to show a twofold higher prevalence of both diseases in both groups, with a power of 80%, using 0.439 prevalence rate for IBD[13] and 1% for HS[10], cohorts of 1.000 IBD and 2.000 HS patients are required. IBD patients should be biopsy-confirmed and physically examined for HS. HS patients should be interviewed about endoscopically and biopsy-confirmed IBD. If an association is present, pathogenic mechanisms in IBD could contribute to HS. However a recent limited pilot study investigating a CD-associated gene CARD15 in HS did not find a relationship between CARD15 polymorphisms and HS[17]. Our results justify further investigation into the possible association between HS and IBD in an adequate and larger patient population. These investigations will clarify whether there is a true association between the diseases, which would open new perspectives into the pathogenesis of HS and probably new treatment options.

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2.3

Hidradenitiss uppurativa-like lesions induced by wearing a leg prosthesis: evidence for mechanical stress as pathogenic factor in hidradenitis suppurativa

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Submitted for publication

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic of recurrent, inflammatory, follicular disease that usually presents after puberty with painful deep-seated, inflamed lesions in the inverse skin areas of the body. The development of fistulas is an important hallmark of severe-stage HS. Estimated prevalence rates for HS range between 1% and 4%in the Western world[1,2] and the disease is associated with a significant reduction in quality of life[3]. Clinically HS resembles infection, but often no bacteria can be cultured from typical HS lesions[4]. Putative pathogenic factors include, genetic predisposition, smoking, obesity and alterations in host defence mechanisms[5,6]. Currently, the generally accepted primary mechanism in HS is hyperkeratinisation of the follicular infundibulum resulting in follicular occlusion[5] followed by a foreign body-like inflammatory reaction when the dilated follicles rupture and keratin fibers and epithelial strands are spilled into the dermis[5]. Treatment of HS is challenging and includes topical and/or systemic antibiotics, and immunosuppressive drugs[5]. For definitive eradication of HS lesions in a specific area, surgical intervention often remains necessary. Predilection sites of HS are the inguino-femoral area which is affected in 90% of cases, axillary (69%), perineal/perianal (37%), buttocks (27%) and the breasts (18%)[7]. Because these predilection sites are rich in apocrine sweat glands, HS was initially considered an inflammatory process originating from these glands[5]. However, histological examination of HS lesions demonstrated apocrinitis only in a minority of cases, suggesting that apocrinitis is a secondary event[8]. Why HS prefers the inverse skin areas is unknown. In this report, we describe a case of persistent HS-like lesions with fistulas on a leg amputation stump, suggesting that mechanical friction and a favourable bacterial microclimate are important extrinsic pathogenic factors for HS development.

CASE

A 44-year old man presented at the outclinic dermatology department of the Erasmus MC, University Medical Center, Rotterdam, with recurrent, inflammatory deep-seated nodules, abscesses and fistulas on the amputation stump of his left leg. Five years earlier, he had been involved in a serious motorcycle accident, resulting in amputation of his left lower leg, and brain trauma, resulting in pituitary dysfunction. Since then he uses a lower leg prosthesis. Because of his pituitary dysfunction he used the following medications: metformine 500 mg daily, somatoprine 0.2 mg daily, testosteron gel 50 mg daily, clobazam 10 mg BID, budesonide/formoterol 200 µg/6 µg. The recurrent inflammatory lesions had previously been treated by several courses of oral antibiotics and several surgical incision-and-drainage interventions. Multiple adjustments were made to the prosthesis for an optimal fit. Upon physical examination actively inflamed lesions such as several draining fistulas, nodules and

abscesses were observed. In addition follicular keratotic papules and a few pustules were observed (Figure1-3). The patient was overweight with a BMI of 31.3 and was a current smoker. The patient never had HS lesions in inverse skin areas and did not have a family history of HS. A routine culture of the purulent discharge showed growth of normal commensal skin flora.

We diagnosed him with HS-like lesions induced by prosthesis-related friction. Because of follicular keratosis with occlusion he was treated with 5% resorcinol cream. The fistulas and abscesses were surgically removed using the deroofing technique under general anaesthesia (Figure 4).

Figure 1. Left lower leg stump with fistulas and follicular keratotic papules (See also Color section, p. 196.)

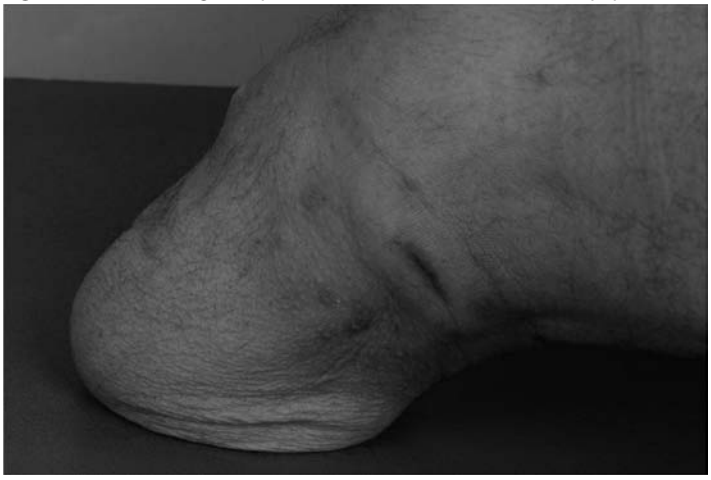


Figure 2. Detail of follicular keratotic papules on the medial side of the knee (See also Color section, p. 197.)

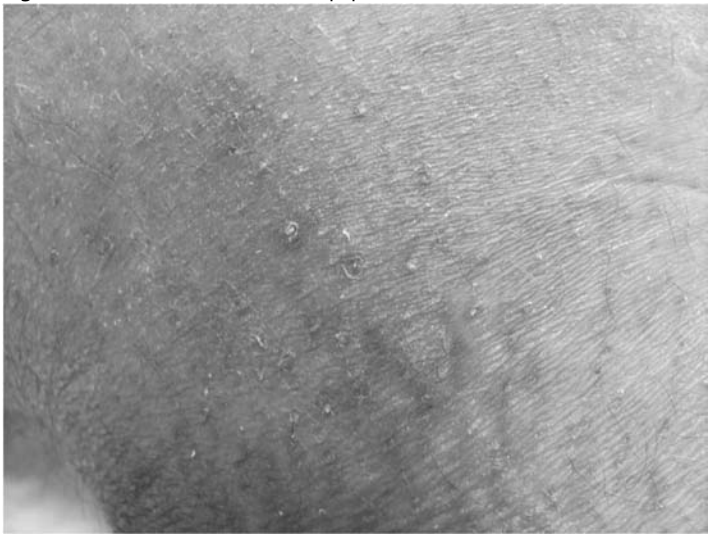


Figure 3. Probed fistula (See also Color section, p. 197.)

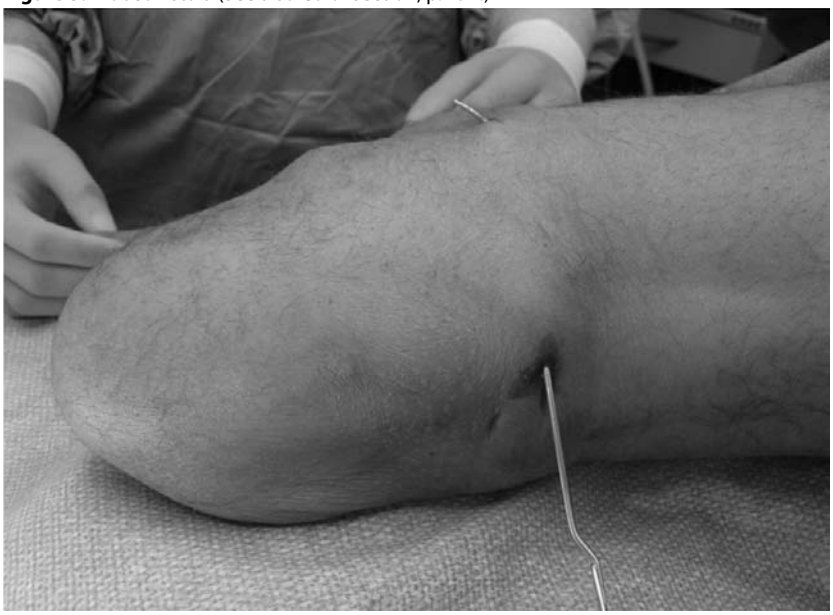
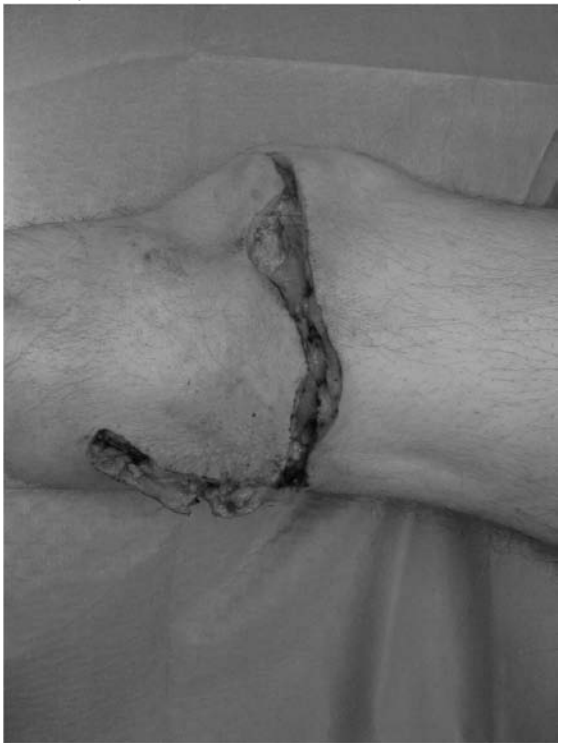


Figure 4. Extent of fistulas after surgical deroofing and slight approximation by sutures (See also Color section, p. 197.)



DISCUSSION

Lower limb amputees are frequently confronted with skin problems on their stump[9]. These stump skin problems comprise acroangiodermatitis, allergic contact dermatitis, bullous diseases, epidermal hyperplasia including epidermoid cysts and hyperkeratotic papules, hyperhidrosis, infections and ulcers[10]. Most of these stump skin problems can be traced back to the local, unnatural conditions such as shear forces and increased humidity when wearing a prosthesis[10]. The mechanical friction can lead to epidermal hyperplasia, epidermoid cysts and hyperkeratotic papules and acne mechanica in leg stumps[10,11]. However, abscessing inflammation with fistula formation has to our knowledge not been described earlier which indicates our case is distinctive. Several lesion characteristics in our patient strongly suggested a HS-like disease rather than the previously described skin problems of lower limb amputees. First, the deep-seated inflammatory abscesses, nodules and especially fistulas clinically resembled HS rather than acne mechanica. Second, culture of the lesional exudate only yielded commensal skin flora unresponsive to antibiotic treatment as is often seen in HS. Third, the patient was a heavy cigarette smoker, which is a well-known risk factor for HS as it is associated with follicular occlusion[12]. Interestingly our patient never had recurrent HS-like lesions in the axilla or groin, nor a family history of HS, which is present in 40% of HS cases[13].

The exact pathogenic mechanism by which mechanical friction contributes to HS development is unknown. Circumstantial evidence that mechanical friction is important in HS derives from a mechanical friction-related skin disorder closely resembling HS, namely 'jeep disease'. Jeep disease was characterized by abscesses, fistulas, pilonidal sinuses and cysts at the buttocks of soldiers, caused by wobbly Jeep, truck or tank rides[14.] This disease caused considerable morbidity among the US army personnel during World War II. A second example is the case of an infant with a naevus comedonicus located in the groin, in which HS-like lesions developed only after the child started to move around[15]. The authors argued that friction caused dilated hair follicles to rupture leading to inflammation[15].

A leg prosthesis creates a warm humid climate, favourable for bacterial growth. It has been postulated that skin commensal microbiota trigger an immune response that may initiate HS. We hypothesize that a similar mechanism contributed to the HS-like lesions in our case presented here. The concept of the combination of friction and a favourable bacterial climate furthermore provide a rationale why HS is more common in the obese. In the obese, HS prefers the increased and extended abdominal skinfolds as well as the inner thighs, locations characterized by enhanced mechanical friction and by a warm and humid microclimate.

We treated the patient with the surgical deroofing technique which is often used in HS and is known to yield low recurrence rates[16]. Deroofing is a technique whereby the 'roof' of an abscess, cyst or sinus tract is electro-surgically removed[16]. A probe is used to explore for subcutaneous pockets and fistulas originating from the HS nodule or abscess[16].

In summary, the patient demonstrated inflammatory lesions, strikingly similar to HS, at an ectopic location free of apocrine sweat glands and induced by wearing of a leg prosthesis. We argue that the presence of apocrine sweat glands in HS-predisposed sites is based on coincidence. Overlapping characteristics between HS predisposed sites include mechanical friction and a warm humid, favourable bacterial microclimate. Mechanical stress may promote HS in a twofold manner. First, it may promote follicular keratosis and occlusion, similar to acne mechanica. Second, it may promote rupture of fragile dilated follicles. Furthermore, the warm and humid microclimate in HS predilection sites favours bacterial growth, which may trigger or worsen HS.

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3

Pathomechanismsi n hidradenitis suppurativa

3.1

Elevated levels of TNF-α, IL-1β and IL-10 in hidradenitis suppurativa skin; a rationale for targeting TNF-α and IL-1β

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ABSTRACT

Background: The pathogenesis of hidradenitis suppurativa (HS) is largely unknown and the disease is difficult to treat. Patients are in high need of an effective treatment. Although it is not known whether the levels of TNF-α are aberrant in HS skin, anti-TNF-α biologics are used, with variable clinical efficacy.

Objectives: To determine the cytokine profile in lesional and perilesional HS skin.

Methods: We cultured 20 lesional, 10 normal appearing perilesional HS skin samples, 7 psoriasis and 6 healthy control skin samples in a transwell culture system. Two distinct cytokine bead arrays were used to measure the spectrum of inflammatory cytokines in the culture supernatant. Results from HS skin samples were compared with those of healthy and psoriasis skin.

Results: The pro inflammatory cytokines IL-1β, TNF-α as well as the anti-inflammatory cytokine IL-10 were significantly elevated in HS skin. Elevated levels of these cytokines were also found in perilesional HS skin. Fold increases relative to control skin of IL-1β, TNF-α and IL-10 in HS were 31, 5, and 34, compared to psoriasis 4, 1 and 2 respectively. Levels of all 3 cytokines showed a trend towards a positive correlation with disease severity. IL-2, IL-4, IL-5 and IFN-y were hardly detectable in HS or healthy control skin.

Conclusions: This study shows for the first time that IL-1β, TNF-α and IL-10 levels are elevated in HS skin. These data provide a rationale for therapies with biologics targeting cytokines such as TNF-α and IL-1.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, inflammatory, recurrent, debilitating follicular disease that usually presents after puberty with painful deep-seated, inflamed lesions in the inverse skin areas of the body, most commonly, the axillary, inguinal and anogenital regions. Estimated prevalence rates range between 1% and 4%[1,2]. Because HS is a painful and distressing disorder and is associated with strong feelings of shame, it significantly reduces the patients' quality of life[3-5]. Since many treatments have been unsatisfactory, and as there are many recurrences, HS remains difficult to treat. Treatment options include topical or systemic antibiotics, pain killers and immunosuppressive agents. In addition, surgical intervention is often necessary[6].

The pathogenesis of HS is largely unknown and probably multifactorial. The most likely primary mechanism is hyperkeratinisation of the follicular infundibulum which results in follicular occlusion. The dilated follicle ruptures, spilling its content including keratin and bacteria into the dermis, subsequently causing inflammation. Genetic predisposition, friction, obesity, smoking and hormonal influences are considered pathogenic factors[6,7]. Defects or alterations in host defence mechanisms may also contribute. It has been proposed that HS results from an inappropriate immune response to commensal bacterial skin flora. This concept is based on the co-occurrence and pathogenic similarities with Crohn's disease, and is furthermore supported by frequent culture of only normal skin flora and not pathogens from HS affected skin[8].

Immunosuppressive agents such as corticosteroids and anti-TNF-α biologics are used in the treatment of HS. The efficacy of anti-TNF-α biologics for HS was first reported in patients with comorbid Crohn's disease[9]. While these patients were treated with anti-TNF-α biologics for their Crohn's disease, concomitantly their HS showed clear improvement. This unexpected observation resulted in a number of case reports followed by case series and more recent by a growing number of randomized controlled trials reporting predominantly positive on efficacy of anti-TNF-α biologics[10-14].

Despite the concept of an altered immune response leading to HS and the promising addition of anti-TNF-α biologics to the HS treatment repertoire, basic immunological aspects such as cytokine profiling, particularly TNF-α have yet to be demonstrated. Hence the aim of this study was to elucidate the spectrum of cytokines in HS skin inflammation and to compare these levels to normal appearing perilesional HS skin, and the skin of healthy donors. In addition we compared the cytokine profile of HS lesions to that of psoriasis. The results provide guidance for future functional studies and contribute to the rational development of new therapeutic strategies.

MATERIALS AND METHODS

Lesional and perilesional HS skin

HS affected skin (LHS) was obtained from 20 HS patients (6 males, 14 females), undergoing radical excision surgery for their HS under general anaesthesia. Patient characteristics are shown in Table 1. HS skin was obtained from the groin 12/20, axillae 7/20 and gluteal regions 1/20. Disease severity was based on staging according to Hurley. Stage I: Abscess formation, single or multiple without sinus tracts and cicatrisation. Stage II: Recurrent abscesses with tract formation and cicatrisation, single or multiple widely separated lesions. Stage III: Diffuse or near diffuse involvement or multiple interconnected tracts or abscesses across the entire area[15]. Five of twenty patients had Hurley stage III disease, 11/20 Hurley stage II disease and 4/20 had Hurley stage I disease. Their mean age was 39.6 years, mean BMI 27.6 and 19/20 were smokers. All patients had chronic, active disease and had not been using any medication for their HS at least 3 weeks prior to surgery. The group of HS patients in this study represented the HS population at large, although in the general HS population Hurley I is the most common stage of disease. In 10 of 20 patients, the HS-affected skin was excised with a healthy appearing skin margin of 2 cm. Biopsies from this adjacent perilesional HS skin (PHS) were also obtained and cultured.

Table 1. Patient characteristics

	Lesional HS	Perilesional HS
	n=20	n=10
Gender	6 males;14 females	1 male; 9 females
Age mean (sd)	40 (15)	38 (15)
BMI mean (sd)	27.6 (4.1)	28.9 (4.5)
Smokers	n=19	n=10
Location	groin n=12	groin n=7
	axillae n=7	axillae n=3
	gluteal n=1	
Disease severity	Hurley 3 n=5	Hurley 3 n=1
	Hurley 2 n=11	Hurley 2 n=7
	Hurley 1 n=4	Hurley 1 n=2

Healthy control skin

Healthy control skin was obtained from submammary skin of 6 healthy females undergoing breastreduction surgery. We argue that skin from this region is especially suitable to function as healthy control for HS as submammary skin is an HS predilection site in female HS patients.

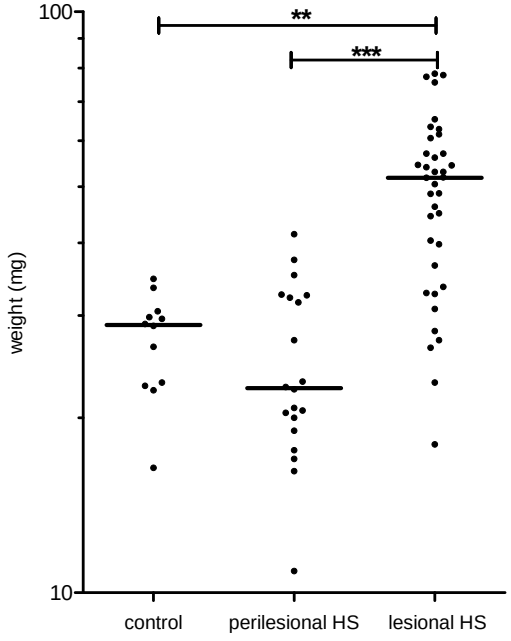
Psoriasis skin

In order to interpret the levels of cytokines produced in HS skin into pathophysiological perspective, we compared them with another inflammatory skin disease namely psoriasis. Hereto punch biopsies from lesional psoriatic plaques were obtained from 7 different patients with plaque psoriasis. These patients were not on systemic drugs for their psoriasis and they did not use any topical treatment on the biopsied psoriatic plaque for at least 2 weeks.

Biopsy procedure

All study subjects gave their informed consent as required by the ethical review board. For HS affected, perilesional and control skin we obtained six, 3-mm diameter punch biopsies. The punch biopsies of HS affected skin were taken from indurated, erythematous areas as close as possible to the same palpable, inflamed, nodule or abscess. Abscesses themselves were not sampled because of the risks of only sampling the superficial roof of the abscess, and of potential bacterial contamination in culture. The punch biopsies of perilesional skin were obtained from clinically unaffected skin 2 cm distanced from the erythematous border of an inflamed HS lesion. HS inflammatory infiltrates tend to be deep-seated, this is why all biopsies, including the ones from control skin, were taken as deep as possible (6-10 mm). For ethical reasons skin samples from the psoriatic plaques were single 4 mm diameter punch biopsies. To allow comparisons single 4 mm diameter punch biopsies were also taken

Figure 1. Weight of the biopsies in each well before culture. Lesional HS skin was significantly heavier than perilesional HS skin and healthy control skin. Means are shown.



from the healthy control skin. We expected that the punch biopsies would vary in weight, in theory this difference in weight could introduce a confounder since the more tissue is put into culture the more cytokine could be released. Therefore we assessed the combined weight of the biopsies in each well prior to culture. The combined weight of biopsies per well cultured is shown in Figure 1. LHS skin had a mean (± sd) weight of 49 mg ± 16. PHS 15 mg ± 8, and 27 mg ± 5 for healthy control. Psoriasis 25 mg ± 6 and 22 mg ± 7 healthy control skin for psoriasis. The LHS biopsies were significantly heavier than control skin ($p < 0.0001$) and heavier than PHS skin ($p < 0.0001$). This potential confounder was settled by normalizing all cytokine levels for mg tissue weight that went into culture.

Skin culture

Three, 3 mm biopsies of HS affected skin, perilesional skin or control skin were placed tightly within punctured membrane of a transwell culture system (Netwell, Costar, Cambridge, MA) in duplicate as described previously[16]. Iscove's Modified Dulbecco's Medium (IMDM, Gibco, Paisley, UK) containing 0.5% normal human serum, penicillin (100 units/ml), and streptomycin (100 ug/ml) was used as the culture medium. Since we assumed in vivo priming by DAMPS or PAMPS, no additional stimuli were added to reflect the in vivo situation as much as possible. Psoriatic skin and the control skin were cultured with a single 4 mm biopsy per well. All skin biopsies were cultured for 24 h at 37 °C, in an atmosphere of 5% CO2 and 98% humidity. After incubation the culture medium was centrifuged for 3 min at 3300 G. The supernatants were stored at -80C° until further processing.

Cytokine measurements in culture medium

Cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α and IFN-γ were measured using commercially available cytometric bead arrays (CBA Human Inflammation Kit, BD BiosciencesTM and CBA Human TH1/TH2 Cytokine Kit, BD BiosciencesTM) according to the protocol of the manufacturer. Measurements were performed on a FACS Calibur (BD Biosciences) and the analysis by FCAPTM Array Software (BD Biosciences). Assay sensitivity ranged from 5 pg/ml to 5.000 pg/ml for all cytokines. In case a cytokine level was below or higher than the detection limit, the detection limit was used for further calculations. Hereafter all cytokine levels were normalized to the tissue weight and expressed as pg/ml/mg.

Statistical analysis

For parametrically distributed values mean and standard deviation are provided, these data were analyzed by t-test. In case of non-parametrically distributed values the median with inter quartile ranges are provided, these data were analyzed by Mann Whitney U test. Tests were done two- tailed. The criterion for statistical significance was $p < 0.05$. For the statistical analysis the software GraphPad Prism 5 was used. In the figures $p < 0.05$ is presented as *, $p < 0.01$ as ** and $p < 0.001$ as ***.

RESULTS

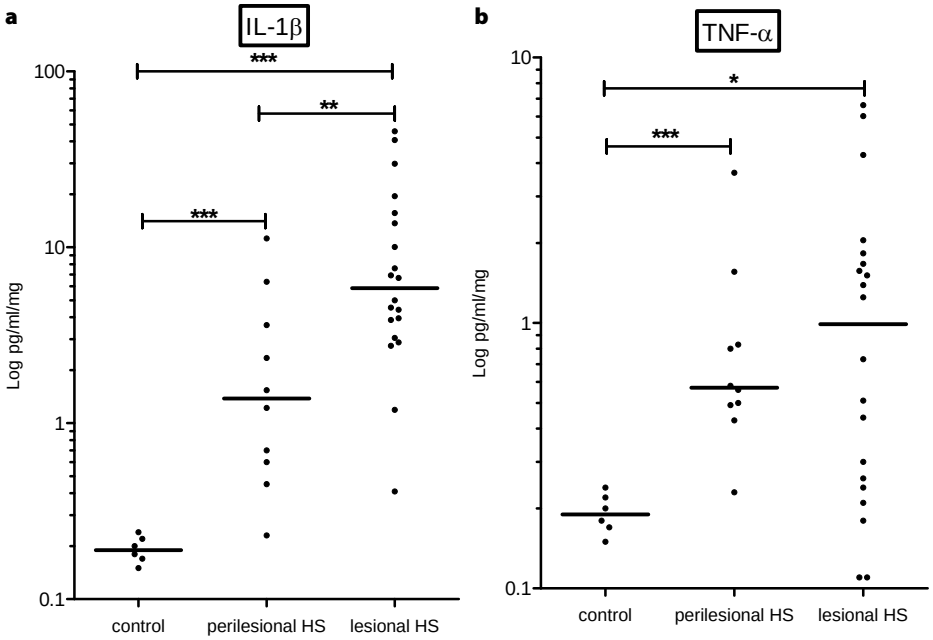
Pro inflammatory cytokines IL-1β, TNF-α and IL-10 are significantly elevated in lesional HS skin

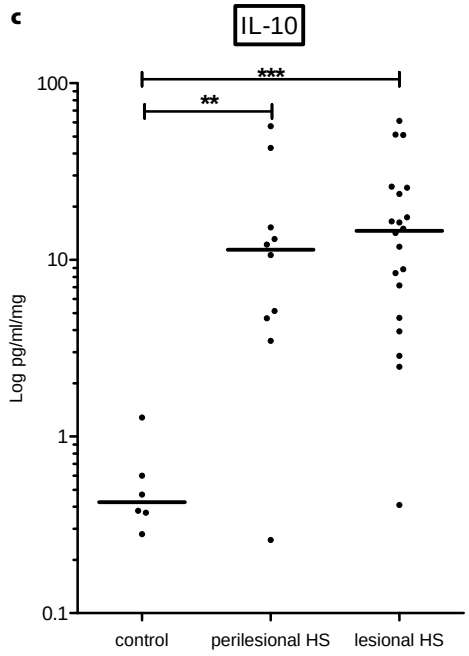
Levels of IL-1β, TNF-α and IL-10 in LHS, PHS and healthy control skin are shown in Figure 2. The median secreted IL-1β in LHS skin was 5.85 pg/ml/mg, which was significantly higher than 0.19 pg/ml/mg in the control skin (p= 0.0003). The LHS median value of secreted TNF-α of 0.99 pg/ml/mg was also significantly higher than 0.19 pg/ml/mg in the control skin (p= 0.0115). Not only the pro-inflammatory cytokines IL-1β and TNF-α were significantly elevated in LHS skin, IL-10 levels were even more strikingly elevated with a median of 14.6 pg/ml/mg compared to control skin 0.43 pg/ml/mg (p= 0.0006).

HS inflammation extends beyond the visually inflamed border

Levels of IL-1β, TNF-α and IL-10 in LHS, PHS and healthy control skin are presented in Figure. 2. Inflammatory cytokines IL-1β, TNF-α but also the anti-inflammatory IL-10 were significantly elevated in LHS skin. In PHS skin IL-1β had a median of 1.38 pg/ml/mg, TNF-α of 0.57 and of IL-10 11.43. IL-1β in LHS skin was significantly higher than in PHS skin (p= 0.0068) However, IL-1β in PHS was also significantly higher than in control healthy skin (p= 0.0005). Levels of TNF-α and IL-10 in LHS skin were not significantly higher than in PHS skin. Whereas both the

Figure 2. Levels of IL-1β (A), TNF-α (B) and IL-10 (C) in lesional HS, perilesional and in healthy control skin. Medians are shown; cytokine levels are in pg/ml/mg tissue on a log scale.





TNF-α and IL-10 levels in PHS skin were significantly higher than the healthy control skin (p=0.0005 and p=0.0075 respectively). These results show that although IL-1β, TNF-α and IL-10 levels in PHS skin are lower than LHS skin, they are still significantly elevated compared to healthy control skin.

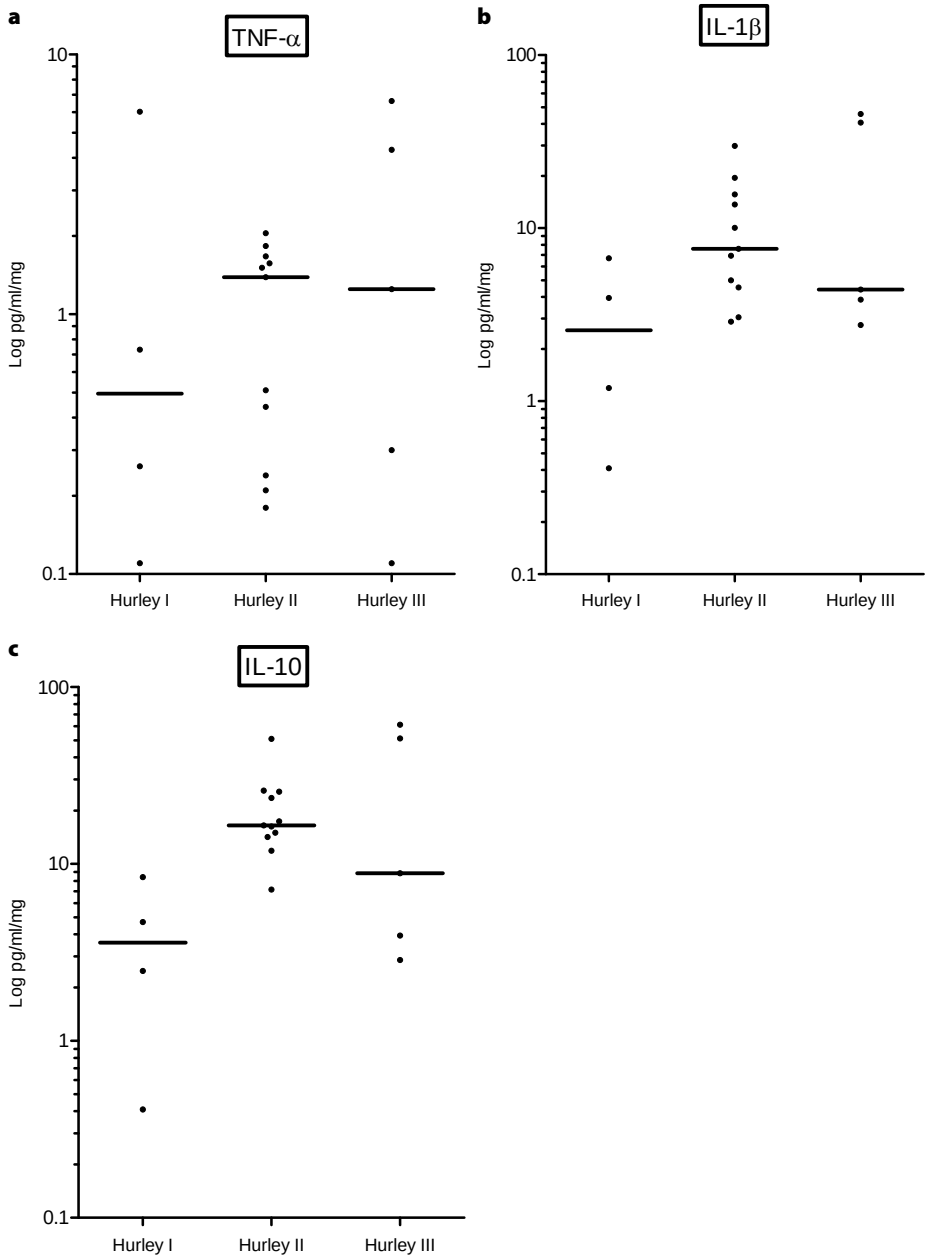
IL-1β, TNF-α and IL-10 release in HS skin tends to correlate with disease severity

Since that IL-1β, TNF-α and IL-10 were elevated in HS skin, we investigated whether the levels of these cytokines were related to disease severity. To make this comparison we ranked LHS skin according to Hurley classification. The secreted levels of IL-1β, TNF-α and IL-10 according to Hurley classification are presented in Figure. 3. We conclude that IL-1β, TNF-α and IL-10 levels tend to correlate with disease severity, but the groups became too small for statistical analysis.

IFN-γ, IL-12p70, IL-2, IL-4 and IL-5 are generally undetectable in HS skin

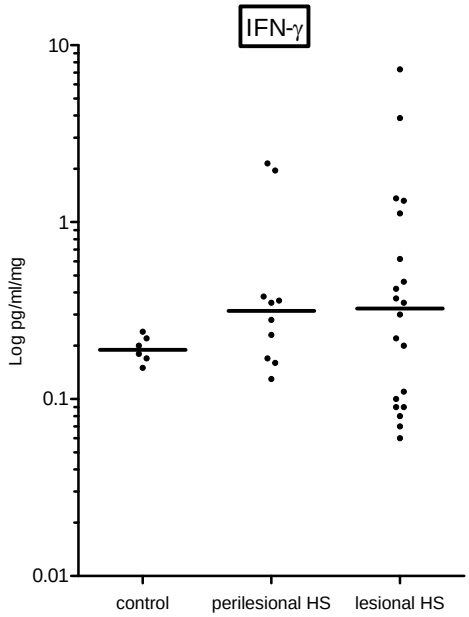
Levels of IFN-γ are shown in Figure. 4. IFN-γ was undetectable in 18 out of 40 LHS samples and in 13 out of 20 PHS samples, as well as in all healthy control samples. The median IFN-γ for LHS was 0.33 pg/ml/mg, for PHS 0.32 pg/ml/mg and for healthy controls 0.19 pg/ml/mg. The level of IFN-γ tended to be higher in LHS an PHS skin compared to healthy controls but there was no significant difference between LHS and healthy controls, PHS and healthy controls and LHS and PHS. IL-12p70 was below the detection limit in all 40 LHS samples as well as in

Figure 3. Levels of IL-1β (A), TNF-α (B) and IL-10 (C) in LHS grouped by disease severity according to Hurley staging. Hurley I n=4, Hurley II n=11 Hurley III n=5 medians are shown; cytokine levels are in pg/ml/mg tissue on a log scale.



all PHS and healthy controls. IL-2 was below detection limit in 35 out of 40 LHS samples, all PHS samples and in 11 out of 12 healthy controls. IL-4 was below detection limit in 34 out of

Figure 4. Levels of IFN-γ in lesional HS, perilesional and in healthy control skin. Medians are shown; cytokine levels are in pg/ml/mg tissue log scale.



40 LHS, in 18 out 20 PHS samples and in all 12 healthy control samples. IL-5 was beneath the detection limit in 37 out of 40 LHS samples and in all PHS and healthy control samples. These results suggest that IL-12p70, IL-2, IL-4, IL-5 and IFN-γ are not important in HS inflammation. Interleukin-6 and IL-8 levels were above the detection limit of 5000 pg/ml in all samples including healthy controls.

IL-1β, TNF-α and IL-10 production in HS skin is higher than that of psoriatic skin

To make a quantitative comparison between HS and psoriasis, cytokine levels, IL-1β, TNF-α and IL-10 were expressed as fold inductions of the medians. The IL-1β median in psoriasis was 0.47 pg/ml/mg which was not significantly different from control skin levels of 0.12 pg/ml/mg. Also TNF-α levels in psoriasis did not differ from control skin and both had a median of 0.12 pg/ml/mg. The median IL-10 in psoriasis lesions was 0.39 pg/ml/mg versus 0.18 pg/ml/mg in healthy control skin which was not significantly different. Calculations resulted in a significantly different IL-1β fold induction of 31 for HS compared to 4 for psoriasis (p=0.025), TNF-α showed a significantly different fold induction of 5 for HS compared to 1 for psoriasis (p=0.0309). The most striking difference was found for IL-10 which showed 34 fold induction of HS compared to 2 for psoriasis (p=0.0004)

DISCUSSION

Anti-TNF-α biologics are the most intensely studied drugs in HS. However, to our knowledge TNF-α levels have not been examined systematically in skin lesions of patients suffering from HS. Matusiak et al. showed that TNF-α is significantly elevated in the blood of HS patients[17]. This in contrast to another study which showed that in HS, after stimulation with LPS, peripheral blood-derived monocytes released less TNF-α and IL-6 than controls[18]. In this study we show that TNF-α is elevated in inflamed HS skin lesions, providing evidence for the use of anti-TNF-α biologics in HS. TNF-α is a pro-inflammatory cytokine produced by many different cell types, though mainly by macrophages and monocytes. It is expressed in the basal layer of the epidermis, sweat glands and hair follicles[19].

We used a cytometric bead array containing a panel of different cytokines in order to discriminate between a Th1, Th2 inflammatory profile in HS inflammation. Most cytokines in the bead arrays like IL-12p70, IL-2, IL-4, IL-5 and to a lesser extent IFN-γ were frequently below detection limit in HS skin. On the basis of these results it is unlikely that HS is prototypical Th1 or Th2 driven inflammatory disease. This predominantly innate inflammatory response is supported by the absence of enlarged lymph nodes clinically and ultrasonographically[20] although a lymphocytic infiltrate has been observed in early HS lesions[21].

We here show that IL-1β, a potent pro-inflammatory cytokine, is significantly elevated in lesional well as in perilesional HS skin. IL-1β can be produced by many different cell types but mainly monocytes and macrophages[22]. Biologically active IL-1β is generated in inflammasomes that cleave pro-IL-1 using caspase-1. Inflammasomes function as cytosolic sensors which respond to diverse types of danger signals such as damage associated molecular pattern molecules (DAMPS) and pathogen-associated molecular patterns (PAMPS). Examples of DAMPS and PAMPS which can trigger inflammasomes to convert active IL-1β, are bacterial and viral sequences, a number of host-derived molecules indicative of injury such as extracellular ATP and hyaluron, uric acid in gout and signs of metabolic stress[23]. Furthermore a number of environmental irritants such as ultraviolet B and skin irritants also activate inflammasomes[23]. We hypothesise that several of above mentioned DAMPS and PAMPS are present in HS and that they activate inflammasomes via three pathways. First by DAMPS such as host-derived molecules indicative of tissue destruction and pyroptosis, a highly inflammatory form of cell death. This concept is supported by the presence of pus and scarring in HS. Second by PAMPS, since HS lesions are frequently inhabited by commensal microbiotic flora. And third, we argue that free high molecular weight cornified keratins in the dermis, spilled by ruptured follicular cysts, can activate inflammasomes after phagocytosis, in analogy with uric acid crystals in gout. Because relatively high levels of IL-1β were found in HS skin, we argue that the inflammasome is involved in HS skin inflammation. This is a new pathogenic concept that warrants further investigations, also since the inflammasomes and the IL-1β pathway can be targeted by drugs such as colchicine, or biologics such as IL-1 receptor antagonist and IL-1β neutralizing monoclonal antibodies.

In addition to the enhanced production of IL-1β and TNF-α, HS skin also produced an increased amount of IL-10. Interleukin-10 has a central, generally anti-inflammatory role in infection and in limiting the immune response to pathogens and thereby preventing damage to the host. It furthermore has a crucial role in preventing inflammatory and autoimmune pathologies[24]. IL-10 is expressed by many cell subsets of adaptive immunity and is also expressed by innate immune cells. We speculate that macrophages in HS could be the prime producers of this vast amount of IL-10, since it was shown that bone marrow-derived macrophages stimulated by efferocytosis (clearance of apoptotic neutrophils) are characterised by high IL-10 production and in addition produce TNF-α following re-stimulation with LPS[25]. This study suggested that such regulatory macrophages play a role in resolution of inflammation[25]. There is a large overlap between this concept of IL-10 producing macrophages and what is known about HS. First macrophages and neutrophils can be found in high numbers in HS infiltrates and abscesses. Second HS lesions are frequently inhabited by commensal microbiotic flora and therefore it is credible that HS macrophages are stimulated by LPS. Third we showed that TNF-α is present in HS just as it is present in the inflammatory efferocytosis model.

Our results also demonstrate that HS inflammation extends beyond the visibly affected inflammatory borders. Histologically, an inflammation-driven process in the epidermis of HS skin is psoratiform hyperplasia[26]. It has been proposed that this psoratiform hyperplasia is a primary histological anomaly in HS[26]. Our data on the presence of inflammatory cytokines beyond the visibly affected inflammatory borders is of clinical importance since high recurrence rates of HS lesions are reported after surgical excision[27]. We argue that the occult inflammation in perilesional HS skin drives the psoratiform hyperplasia and can therefore be the cause of recurrence after surgery. This concept suggests that recurrence after excisions can be prevented by the administration of adjuvant medications which reduce inflammation and infundibular epidermal hyperplasia.

The levels of especially IL-1β, IL-10 but also TNF-α in HS were relatively higher in HS than in psoriasis. These results suggest that inflammation in HS skin is more intense than in psoriasis stressing the strong inflammatory nature of the disease.

This study shows for the first time that IL-1β, TNF-α and IL-10 production is elevated in HS skin. Our results provide a rationale for targeting these cytokines with specific biologics in HS patients.

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3.2

Adalimumab (anti-TNF-α) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study

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Submitted for publication

ABSTRACT

Background: Hidradenitis suppurativa (HS) is a difficult to manage disease. Randomized controlled trials with anti-TNF-α biologics have been conducted and in most studies disease activity was reduced. However, the mechanism of action in HS skin is so far unknown.

Objectives: To assess whether anti-TNF-α treatment affects in situ cytokine production and frequency of inflammatory cell populations in HS lesional skin.

Methods: Nine HS patients, participating in a larger placebo-controlled, double-blind phase IIb clinical trial on the efficacy and safety of adalimumab in patients with moderate to severe HS (M10-467), were randomized and treated for 16 weeks. In a mechanism of action sub-study, biopsies were obtained at fixed time points pre- and post-treatment. One part of the biopsy was cultured for 24 h for cytokine release in the culture medium, while another part was used for in situ analysis.

Results: Secretion of cytokines, including IL-1β, CXCL9 (MIG), IL-10, IL-11, BLC and IL-17A was significantly elevated in HS. Adalimumab treatment was associated with decreased cytokine production in HS skin, especially IL-1β, CXCL9 (MIG) and BLC. Treatment significantly reduced the number of $CD11c^+$, $CD14^+$ and $CD68^+$ cells in HS lesional skin. The numbers of $CD3^+$ and $CD4^+$ T cells, $CD20^+$ and $CD138^+$ B cells were also reduced by adalimumab treatment.

Conclusions: Adalimumab treatment inhibits important cytokines and inflammatory cell numbers in lesional HS skin especially inflammatory $CD11c^+$ dendritic cells and IL-1β levels.

INTRODUCTION

Hidradenitis suppurativa (HS) is an inflammatory debilitating skin disease characterized by recurrent, painful, deep-seated, inflamed boils with, in severe cases, odorous discharge from fistulas and mutilating scarring. Predisposed skin areas are the axillary, inguinal and anogenital regions. The estimated prevalence rates of HS range between 1% and 4%[1,2]. The precise pathogenesis of HS is unknown, but the most widely accepted pathogenic concept is that of hyperkeratinisation of the follicular infundibulum and follicular occlusion followed by rupture of dilated follicles causing inflammation. Besides genetic predisposition, exogenous factors such as cigarette smoking, obesity, shear forces, and hormonal influences contribute to the pathophysiology of HS[3,4]. Furthermore it has been speculated that an altered innate immune response to commensal skin microbiota contributes to or even is the primary cause of the disease[5].

Levels of TNF-α are increased in the blood of patients with HS[6], and we recently showed that both lesional HS skin and normal-appearing perilesional skin samples from HS patients released significant amounts of cytokines, including TNF-α[7]. These data provide a rationale for the therapeutic use of anti-TNF-α biologics in HS. Small randomized, double-blind trials have assessed the clinical efficacy of TNF antagonists in HS with mixed results; etanercept was frequently ineffective, but positive trends of efficacy were seen during infliximab trials and an adalimumab trial[8-13]. Despite the reported efficacy of anti-TNF-α biologics, the mechanism of action of these drugs in HS remains largely unknown. TNF-α is produced by many cell types including mast cells, macrophages, dendritic cells, T cells, granulocytes, fibroblasts and keratinocytes[14] all of which are present in HS lesions[15]. Because of its pleiotropic properties, TNF-α is able to direct inflammation in several ways. With respect to its role in supporting chronic inflammation in HS, the influence of TNF-α on monocyte activation and differentiation into inflammatory type dendritic cells, granuloma formation, accumulation of neutrophils, activation of keratinocytes and the induction of several matrix metalloproteases (MMPs) are considered important mechanisms[16].

Adalimumab is a fully human, IgG1, neutralizing monoclonal antibody specific for TNF-α. Here we report on alterations in cytokine production in lesional skin and the skin infiltrate after adalimumab treatment for HS. The patients included in this study participated in a larger placebo-controlled, double-blind phase IIb clinical trial on the efficacy and safety of adalimumab in patients with moderate to severe hidradenitis suppurativa (M10-467, ClinicalTrials.gov number: NTC00918255). The efficacy results of this RCT have been separately submitted for publication elsewhere.

MATERIALS AND METHODS

Patients and treatment

Nine HS patients participated in this study after giving their signed informed consent as required by the ethical review board. All 9 HS patients (5 males, 4 females) had chronic, active disease, stable for at least 2 months. Patient characteristics are shown in Table 1. HS lesional skin samples were obtained from the groin in 6 out of 9 patients, axillae in 1 out of 9 and gluteal region in 2 out of 9 patients. Disease severity was based on staging according to Hurley[17]. Six out of nine patients had severe HS (Hurley stage III disease), 1 had Hurley stage II disease and 2 had Hurley stage I disease. According to the protocol of the above mentioned phase IIb double blinded RCT, patients were randomly assigned to three groups. Group 1 received adalimumab 40 mg weekly (starting at week 4, after initial doses of 160 mg at week 0 and 80 mg at week 2); group 2 received adalimumab 40 mg every other week (starting at week 1, after an initial dose of 80 mg at week 0). Group 3 received matching placebo. Local alteration in disease severity was evaluated using a four stage physician global assessment (PGA) score: worsening, no difference, slight improvement and improvement. Photographs at week 0 were taken to increase sensitivity of the PGA and to exactly pinpoint the prior biopsy location.

Table 1. Patient characteristics

	Patient #	Age (years)	Gender	BMI	Smoking	Biopsy site	Hurley stage	PGA at 16 weeks
Group 1	1	47	♀	43.0	no	inguinal	3	improvement
	2	31	♀	18.3	yes	inguinal	1	improvement
	3	24	♂	27.1	yes	axilla	3	worsening
Group 2	4	32	♀	29.4	yes	pubis	3	improvement
	5	58	♀	29.7	yes	buttock	3	slight improvement
	6	58	♂	26.2	yes	pubis	2	improvement
Group 3	7	36	♂	38.5	yes	inguinal	2	slight improvement
	8	39	♂	22.8	yes	inguinal	3	no difference
	9	67	♂	22.6	yes	buttock	3	no difference

Group 1 received adalimumab 40 mg weekly (starting at week 4, after initial doses of 160 mg at week 0 and 80 mg at week 2); Group 2 received adalimumab 40 mg every other week (starting at week 1, after an initial dose of 80 mg at week 0). Group 3 received matching placebo.

Biopsy procedure

At time points 0 and 16 weeks, a 5 mm diameter punch biopsy was obtained. The punch biopsy at week 0 was taken from an indurated, erythematous, inflamed lesion as close as possible to a palpable nodule or abscess. Abscesses themselves were not sampled because of the risk of only sampling the superficial roof of the abscess. HS inflammatory infiltrates tend

Table 2. Cytokines in HS skin compared to healthy control skin

	HS		Healthy control skin			
	median	**IQR**	**median**	**IQR**	**p**	**fold induction**
IL-1β	**1.6**	**0.8 - 6.8**	**0.0**	**0.0 - 0.1**	**0.0028 ****	**54.4**
MIG (CXCL9)	**219.8**	**180.0 - 320.1**	**13.8**	**9.7 - 17.1**	**0.0028 ****	**16.0**
IL-10	**19.2**	**10.3 - 35.1**	**1.3**	**0.5 - 2.2**	**0.0028 ****	**14.8**
IL-11	**78.6**	**38.6 - 152.6**	**7.2**	**2.4 - 10.8**	**0.0056 ****	**11.0**
BLC	**8.1**	**4.6- 17.3**	**0.8**	**0.6 - 2.7**	**0.0056 ****	**10.5**
IL-17A	**8.1**	**1.9 - 36.9**	**1.1**	**0.5 - 1.4**	**0.0056 ****	**7.3**
TNF RII	**47.0**	**34.5 - 65.1**	**8.1**	**7.5 - 8.4**	**0.0028 ****	**5.8**
RANTES (CCL5)	**7.6**	**3.1 - 23.7**	**1.4**	**1.0 - 2.0**	**0.0112 ***	**5.4**
IL-16	**22.3**	**21.2 - 50.3**	**4.2**	**1.7 - 5.6**	**0.0028 ****	**5.3**
IL-6sr	**16.3**	**8.1 - 31.5**	**4.4**	**2.9 - 6.2**	**0.0028 ****	**3.7**
ICAM-1	**98.7**	**70.4 - 187.4**	**31.9**	**27.2 - 41.0**	**0.0028 ****	**3.1**
MIP-1α (CCL3)	**0.4**	**0.2 - 0.8**	**0.2**	**0.1 - 0.4**	**0.0196 ***	**2.0**
TNF RI	**78.0**	**56.3 - 90.2**	**40.2**	**30.8 - 46.3**	**0.0112 ***	**1.9**
TNF-α	**0.3**	**0.2- 0.9**	**0.2**	**0.1 - 0.2**	**0.0336 ***	**1.6**
IL-1ra	**44.0**	**37.3 - 65.6**	**29.6**	**24.1 - 32.7**	**0.0112 ***	**1.5**
Eotaxin	0.1	0.1 - 0.2	0.1	0.1 - 0.2	ns	
Eotaxin-2	3.9	0.5 - 9.2	2.5	1.7 - 9.3	ns	
G-CSF	117.8	96.4 - 196.9	96.8	75.5 - 165.9	ns	
GM-CSF	0.4	0.1 - 1.8	0.0	0.0 - 0.5	ns	
I-309	0.4	0.3 - 2.2	0.3	0.3 -1.6	ns	
IFN-γ	0.1	0.1 - 0.2	0.1	0.1 - 0.2	ns	
IL-1α	0.2	0.1 - 0.4	0.1	0.1 - 0.1	ns	
IL-2	0.8	0.4 - 1.0	0.4	0.3 - 0.6	ns	
IL-4	0.0	0.0 - 0.1	0.1	0.0 - 0.1	ns	
IL-5	0.2	0.1 - 0.2	0.2	0.2 - 0.2	ns	
IL-6	124.4	72.2 - 159.8	101.9	79.2 - 121.1	ns	
IL-7	0.4	0.3 - 0.5	0.4	0.3 - 0.5	ns	
IL-8	14,1	9.5-18.7	12.0	10.2 - 14.4	ns	
IL-12p40	0.5	0.4 - 0.6	0.4	0.3 - 0.4	ns	
IL-12p70	0.0	0.0 - 0.1	0.0	0.0 - 0.0	ns	
IL-13	0.0	0.0 - 0.1	0.1	0.0 - 0.2	ns	
IL-15	1.9	1.6 - 2.8	2.9	2.6 - 3.9	ns	
MCP-1	47.5	26.3 - 57.0	37.1	30.9 - 44.4	ns	
MCSF	0.4	0.2 - 0.8	0.2	0.1 - 0.4	ns	
MIP-1b	16.1	8.3 - 19.3	5.8	3.7 - 7.9	ns	
MIP-1d	0.1	0.1 - 0.1	0.1	0.1 - 0.4	ns	
PDGF-BB	0.5	0.3 - 1.3	0.2	0.1 - 0.3	ns	
TIMP-1	260.1	183.4 - 370.0	166.2	160.2 - 195.0	ns	
TIMP-2	989.2	680.8 - 2096.0	997.3	838.5 -1242.0	ns	
TNF-β	0.4	0.3 - 0.5	0.4	0.3 - 0.5	ns	

Significantly altered cytokines are presented in descending order by their level of induction. Non-significantly altered cytokines are ordered alphabetically. Levels are in pg/ml/mg. Medians and interquartile ranges (IQR) are shown. $p < 0.05$ is presented as *, $p < 0.01$ as ** and $p < 0.001$ as ***. ns is not significant.

to be deep-seated, therefore all biopsies, including control skin, were taken as deep as possible (6-10 mm). The punch biopsy at week 16 was taken as close as possible to the previously biopsied site. The biopsies were split whereby 1/3 was processed for immunostaining and 2/3 was cultured. Healthy mammary skin was obtained from 4 healthy females undergoing breast reduction surgery, and served as healthy control skin.

Skin culture

Two thirds of the 5 mm punch biopsy was cultured in a transwell system (Netwell, Costar, Cambridge, MA) as described previously18. In brief: biopsies were placed in holes made in the transwell filter in such a manner that the epidermis remained exposed to the air whereas the dermis was immersed in 1 ml Iscove's Modified Dulbecco's Medium (IMDM, Gibco, Paisley, UK) containing 0.5% human AB serum, penicillin (100 units/ml), and streptomycin (100 ug/ml). All skin biopsies were cultured for 24 h at 37°C, in an atmosphere of 5% CO2 and 98% humidity. After 24 h, the culture medium was collected, centrifuged for 3 min at 3300 G, and transferred to a polypropylene tube and stored at -80C° until further analysis.

Cytokine measurements in culture medium

A broad spectrum of 40 inflammation-related cytokines was simultaneously measured in the supernatant using the commercially available Quantibody Human Inflammation array 3 (RayBiotech Inc, Norcross, GA). A list of the cytokines is presented in Table 2. In addition TNF-α was measured with the more sensitive CBA Human Inflammation Kit, (BD Biosciences™, Franklin Lakes, NJ). All arrays were used according to the manufacturers' protocol.

Immunostaining procedure

One third of the 5 mm diameter punch biopsy was fixed in 4% formaldehyde and embedded in paraffin. Four micrometer thin sections were labeled with a panel of antibodies as listed in Table 3, to immunophenotype leukocyte subsets. A standard horseradish peroxidase DAB (diaminobenzidine) staining procedure was followed using a polymer detection system (K8005,

Table 3. Antibodies used in immunostaining procedure

Marker	Indicative for	Clone	Dilution	Manufacturer
CD3	T cells	polyclonal	1:150	Dako, Glostrup, Denmark
CD4	T helper cells	4B12	1:160	Monosan, Uden, The Netherlands
CD8	Cytotoxic T cells	C9/144B	1:100	Dako, Glostrup, Denmark
CD20	B cells	L26	1:400	Dako, Glostrup, Denmark
CD138	Plasma cells	B-A38	1:25	IQ Products, Groningen, The Netherlands
CD14	Monocytes	MY4	1:100	Novocastra, Newcastle, UK
CD68	Macrophages	KP1	1:160	Dako, Glostrup, Denmark
CD11c	Dendritic cells	5D11	1:60	Novocastra, Newcastle, UK

DAKO, Glostrup, Denmark). All sections were counterstained with haematoxylin (4085-9005, Klinipath, Uden, The Netherlands) to visualize cell nuclei. Antigen retrieval for all antibodies was performed by heating sections (20 min at 97 °C) in DAKO PT modules (PT 101, DAKO, Glostrup, Denmark) in Envision Flex Target Retrieval Solution high pH (DAKO, K8004). Normal antibody diluent (Scytec ABB999 Scytec laboratories, Logan, UT) was used for blocking of sections and for dilution of the primary antibodies. Human tonsil was used as positive control tissue for all antibodies and was included on each glass slide in each staining run. Negative controls comprised omission of the primary antibody and replacement with normal antibody diluent.

NanoZoomer Digital Pathology Software (Hamamatsu, Herrsching am Ammersee, Germany) was used to scan the stained sections. Leucocyte subsets within a HS infiltrate were semi quantitatively scored by two independent observers in a blinded manner, using a scale from 0 to 4, whereas a score of 0 represented no stained cells, and a score of 4 meant staining of most cells. The average score was calculated from the score of both observers.

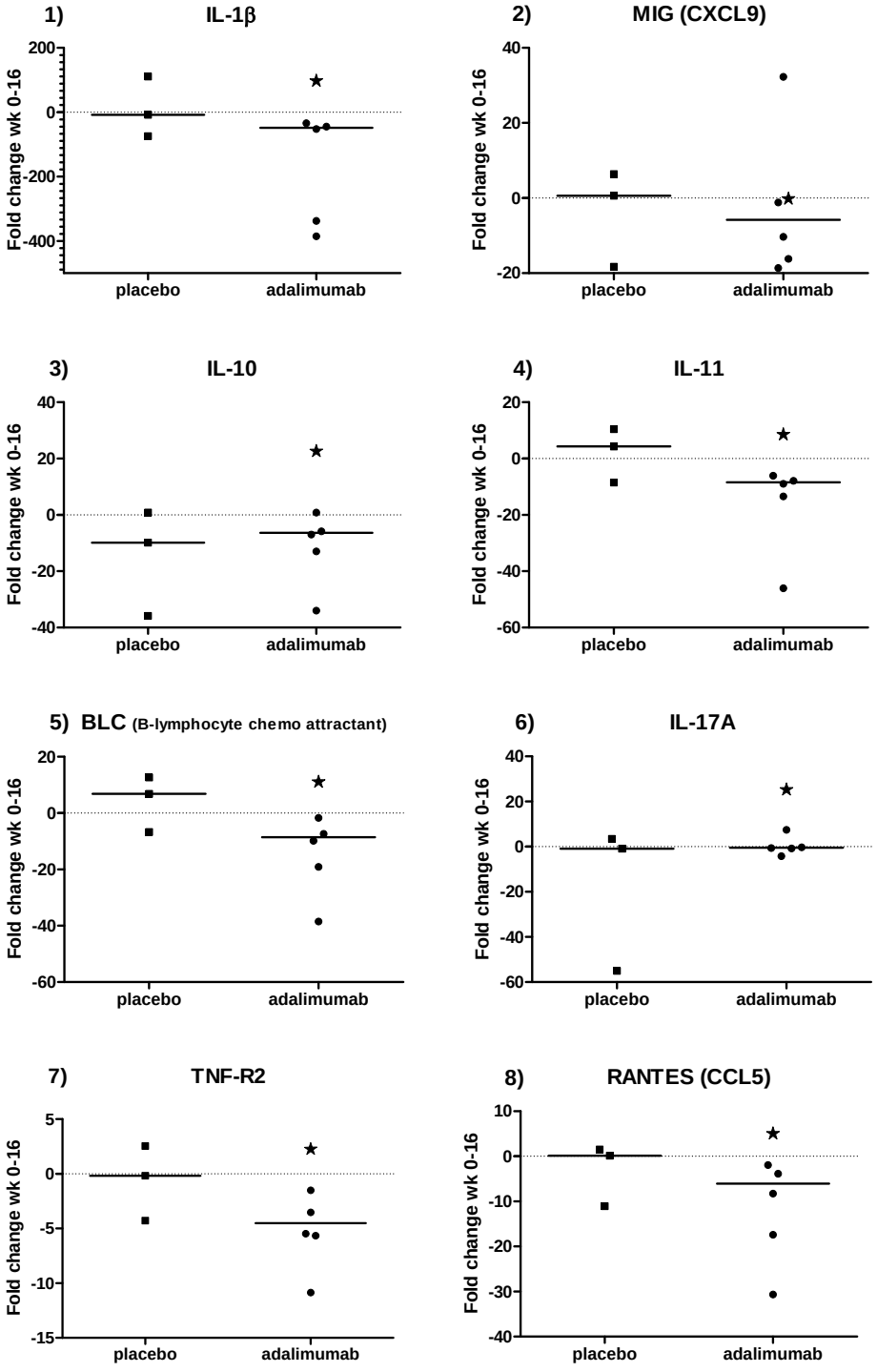
Statistical analysis

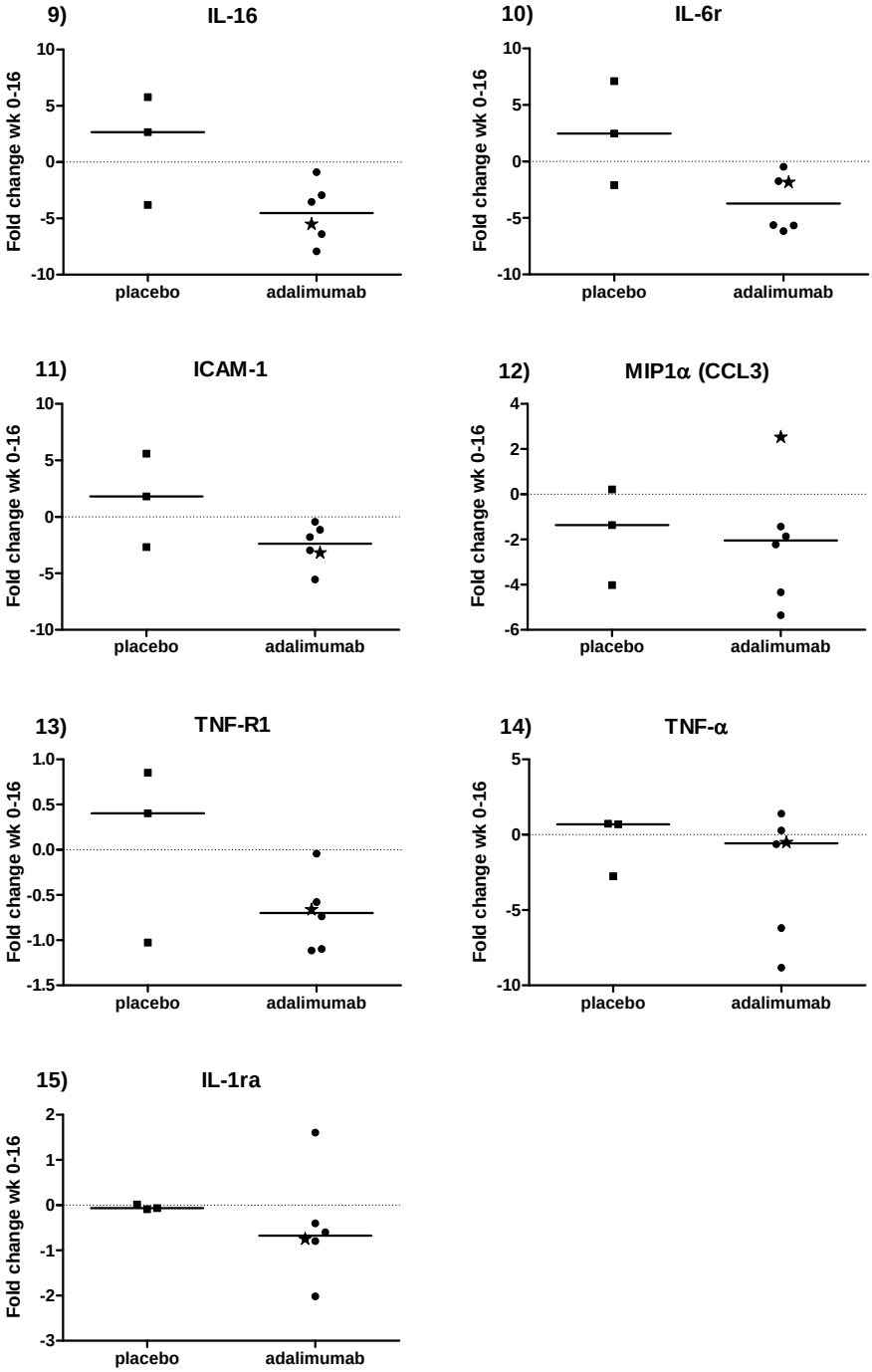
Difference in weight and volume of cultured skin samples, induced by manually cleaving the biopsies, could introduce a confounder. Therefore we assessed the weight of the tissue before culture. When detected cytokine levels were below or above the detection limit, the lower or upper detection limit was used for further calculations. All cytokine levels were normalized according to input weight and expressed in picograms per ml and per mg tissue weight (pg/ml/mg). The number of patients in the three treatment groups was too small for statistical analysis. To provide an impression of the effects of adalimumab treatment on HS lesions, patients treated with adalimumab were clustered and compared with placebo-treated patients. Medians with interquartile ranges are provided for the non-parametrically distributed values that were analysed by two-tailed Mann Whitney U test. The criterion for statistical significance was a p value less than 0.05.

RESULTS

We first compared the cytokine levels released by lesional HS skin biopsies after 24 hours of culture with that of healthy skin from controls in order to determine HS inflammation-related cytokines (Table 2). This comparison showed that the production of IL-1β was most significantly increased in HS skin, a 54-fold increase compared to healthy control skin, followed by CXCL9 (MIG; 16-fold) and IL-10 (15-fold). Other cytokines that were produced at significantly higher levels in lesional HS skin were IL-11, B lymphocyte chemo attractant (BLC), IL-17A, soluble TNF-receptor 2 (sTNF-R2), CCL5 (RANTES) IL-16, soluble IL-6 receptor (sIL-6R), soluble ICAM-1, CCL3 (MIP-1α), soluble TNF-receptor 1 (sTNF-R1), TNF-α, and IL-1 receptor antagonist (IL-1ra).

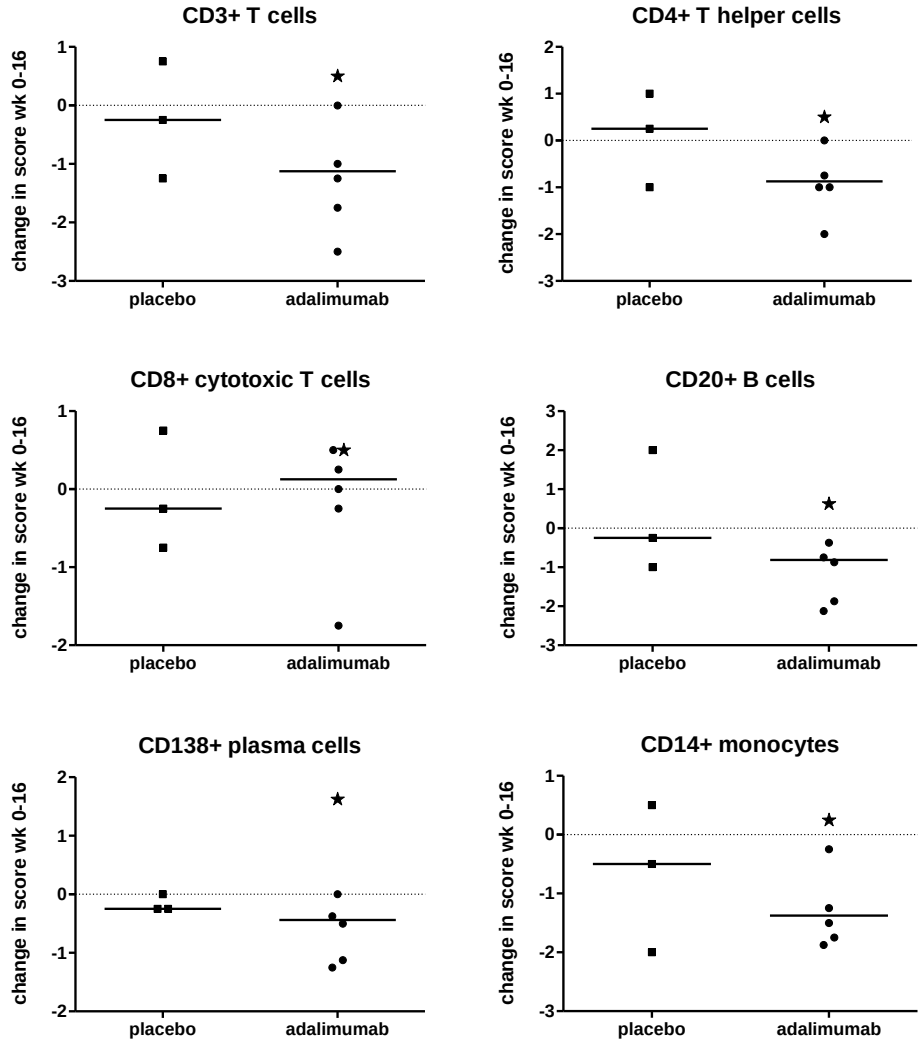
Figure 1. Alterations of cytokine levels in patients treated with adalimumab or placebo





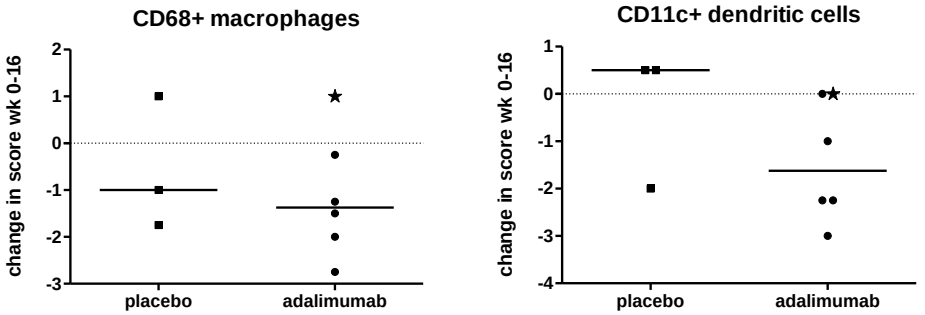
Cytokines are ordered by their level of elevation in untreated, naïve HS skin. The median is presented. The star presents patient #3 in whom HS worsened despite treatment. The dotted line represents no change between week 0 and 16.

Figure 2. Alterations in semi-quantitative scores of leucocyte subsets in patients treated with adalimumab or placebo



Subsequently we checked whether adalimumab treatment affected the levels of cytokines released from HS skin lesions. Hereto, we compared the cytokine release in culture medium between week 0 and week 16 per individual patient. The data are presented in Figure 1. IL-1β, CXCL9 (MIG), BLC, IL-11 and CCL5 (RANTES) showed the most evident decrease after adalimumab treatment compared to placebo. The production of almost all other tested cytokines also decreased upon adalimumab treatment, although to a lesser extent. The production levels of TNF-α and IL-17A were not affected by adalimumab nor placebo treatment.

Figure 2. (continued)



Scoring was performed in biopsies from week 0 and week 16 by two blinded independent investigators using a 4 point scale. Median values are shown. The star presents patient #3 in whom HS worsened despite treatment. The dotted line represents no change in the semi-quantitative score.

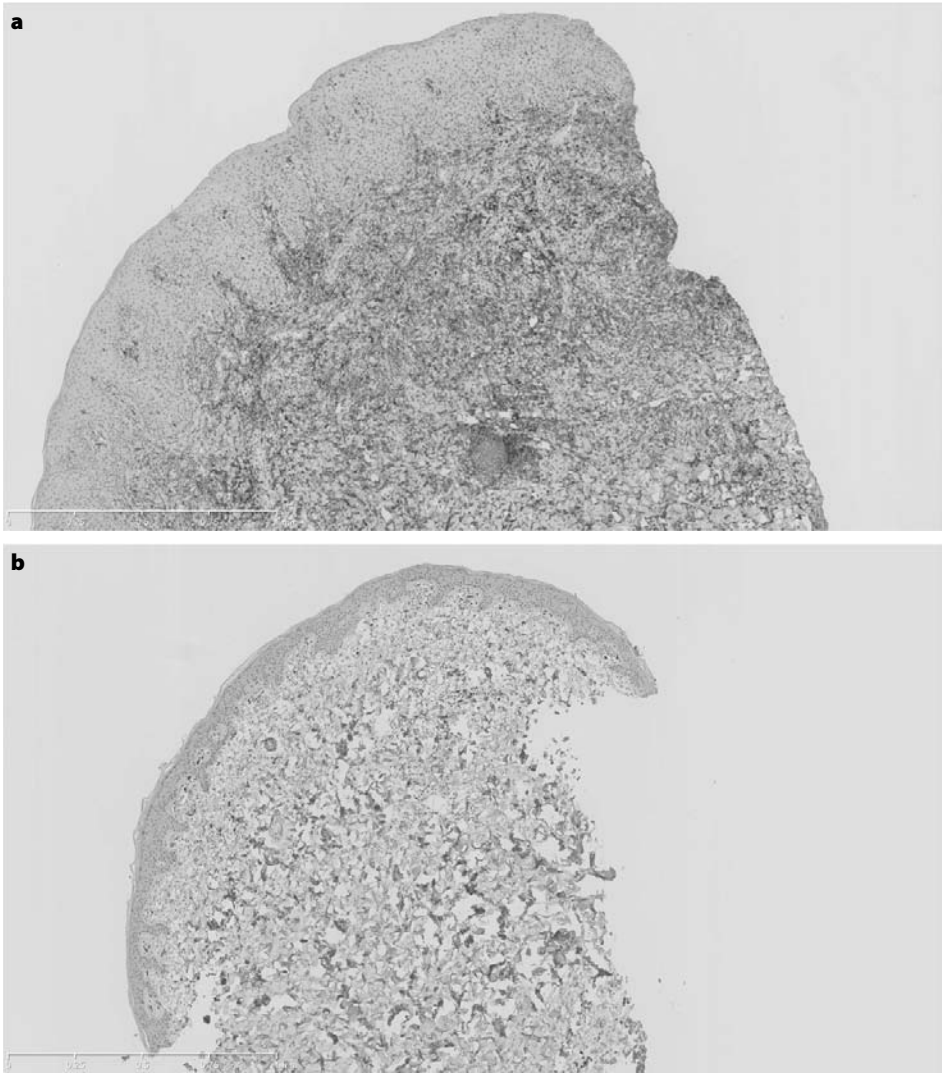
IL-1β activity is counter-regulated by its naturally occurring antagonist IL-1ra and therefore we calculated the mean IL-1ra/IL-1β ratio. In healthy control skin the median for this ratio was 1071 (IQR 336-1229) and in HS patients before adalimumab treatment the mean IL-1ra/IL-1β ratio was 23 (IQR 7-84). Sixteen weeks after initiation of adalimumab treatment this ratio increased to 142 (IQR 11-526), while the IL-1/IL-1ra ratio remained stable in placebo treated patients at 30 (IQR 7-1373).

Adalimumab treatment altered the inflammatory cell infiltrate in HS skin considerably (Figure 2). The numbers of $CD3^+$ and $CD4^+$ T cells showed a significant decrease, when compared to placebo, while no change was observed in the number of $CD8^+$ cytotoxic T cells. The number of $CD20^+$ B cells and $CD138^+$ plasma cells also decreased although to a lesser extent than that observed for T cells. Adalimumab therapy induced the most significant reduction in the number of skin infiltrating $CD11c^+$ (Figure 3), $CD14^+$, and $CD68^+$ inflammatory dendritic cells, monocytes and macrophages.

DISCUSSION

In this study, using a different multiplex-based cytokine array, we confirmed the recent finding that mRNA and protein levels of TNF-α, IL-1β and IL-10 are increased in HS[7,19]. More evidently elevated than TNF-α were its soluble receptors TNF-R1 and TNF-R2. In addition we identified other cytokines that are produced in excess in HS skin that have not been linked to its pathophysiology before. Many of these cytokines are chemotactic for a variety of immune cells. BLC is a B-lymphocyte chemo attractant and the elevated levels of BLC correspond with the observed high number of B-cells in HS skin. CCL3 (MIP1α) is chemotactic for and an activator of granulocytes which are abundant in HS lesions. CCL5 (RANTES), IL-16 and CXCL9 (MIG) are chemotactic for T cells and elevated levels of these cytokines correspond with the

Figure 3. Immunohistochemical staining of $CD11c^+$ dendritic cells in the clincally improved patient 6 in group 2: adalimumab EOW. (See also Color section, p. 198.)



a) Week 0 **b)** Week 16.

increased number of T cells in HS. ICAM-1 (intercellular adhesion molecule-1, CD54) can be induced by IL-1 and TNF-α, both of which are increased in HS. Large numbers of $CD4^+$ T helper cells were present in HS lesions. Interestingly, typical Th1 and Th2 cytokines such as IFN-γ, IL-12p70, IL-4, IL-5 and IL-13 were not elevated. This sets the inflammatory cytokine profile in HS apart from psoriasis and atopic dermatitis. But high levels of IL-17A and IL-10 were measured indicating that they theoretically are derived from a subset of Th17 or T regulatory cells which provides a rational for the use of the Th17 modulating drug Ustekinumab[20].

After 16 weeks of adalimumab treatment the most evident decrease was seen in the levels of IL-1β, CXCL9 (MIG), BLC, IL-11 and CCL5 (RANTES) and to a lesser extent IL-6R IL-16, IL-1ra, sTNF-R2, ICAM-1, IL-10, and CCL3 (MIP1α). TNF-α was only mildly increased in HS so no spectacular change after adalimumab treatment was to be expected, but the decrease in TNF-R1 and TNF-R2 was more evident. However, in this study we did not measure the levels of membrane-bound TNF-α.

The median ratio between IL-1β and IL-1 receptor antagonist showed a clearly reduced ratio of 32 for IL-1ra/IL-1β in HS, whereas 1071 was calculated for healthy skin. This aberrant ratio indicates a relative shortage of IL-1ra in HS with/or an overproduction of IL-1β. Adalimumab treatment induced a shift of the IL-1ra/IL-1β ratio to higher levels (142). The level of the anti-inflammatory cytokine IL-10 did not follow the decrease of IL-1β or other inflammatory cytokines.

The leucocyte subsets that were most clearly reduced in frequency by adalimumab treatment were dendritic cells, monocytes and macrophages followed by T helper cells and B-cells. Remarkably, the number of $CD3^+$ and $CD4^+$ T cells was clearly reduced compared to placebo, whereas no evident change was observed in the number of $CD8^+$ T cells. The decrease in B-cells paralleled the decrease in BLC production. In analogy with our findings in HS, in psoriasis adalimumab also induced a most marked decrease in the number of dendritic cells, macrophages and T cells[21].

HS remains a difficult to manage disease, which in this study is reflected by the fact that not all patients were clinical responders despite the high dose of adalimumab used. According to the intention to treat principle all patients including the non-responders were included in the figures. Lack of clinical improvement highly correlated with the lack of alterations in the leucocyte subset scores and cytokine levels. Especially the absence of clinical improvement in patient #3, group 1, closely mirrored the in situ data. This particular patient is frequently the upper outlier in the figures and hereby influences the outcomes of adalimumab treated group.

This study has several limitations. For three arms a relatively small number of patients were studied whereby statistics could not be applied. Furthermore the absence of an assessment of changes early in treatment did not allow us to determine which the primary and secondary phenomena in disease improvement were.

Clinically the patients in the placebo group showed almost no change in disease severity, and the outcomes of the leucocyte subsets and cytokine release likely reflect natural fluctuations in human biological processes. This variation is in accordance with an earlier study which showed a high variability in HS cytokine release confirming the temporal and spatial heterogeneity of the disease[7].

Although adalimumab is an anti-TNF-α biologic, TNF-α did not seem to be the cytokine most responsive to treatment. Further research is warranted to untangle the immunological processes operational in HS and how different treatment modalities influence these processes.

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3.3

Alterations in leucocyte subsets and histomorphology in normal appearing perilesional skin, early, and chronic hidradenitis suppurativa lesions

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Br J Dermatol in press

ABSTRACT

Background: The current insight into the histopathological course of events during disease progression in hidradenitis suppurativa is fragmentary.

Objectives: To identify histological alterations and leucocyte subsets in normal appearing perilesional, early, and chronic HS skins.

Methods: In this observational study we examined skin samples from 8 perilesional, 6 early and 10 chronic prototypic HS lesions, as well as 4 healthy donors by using in situ immunostaining.

Results: Perilesional skin showed mild psoriasiform hyperplasia and follicular plugging as well as a low-grade influx of tryptase$^+$ mast cells, CD3$^+$ T cells, CD138$^+$ plasma cells and FXIIIa$^+$ dendritic cells. In early HS lesions, neutrophilic abscess formation and influx of mainly macrophages, monocytes and dendritic cells predominated. In chronic disease, the infiltrate expands with noteworthy increased frequencies of CD20$^+$, CD79a$^+$ B cells and CD138$^+$ plasma cells. As in early lesions, free keratin fibers were detected in the dermis and within giant cells. Single detached keratinocytes and strands of follicular epithelium were observed in the dermis, the latter frequently expressing Ki67, indicative of active proliferation.

Conclusions: Psoriasiform hyperplasia, follicular plugging and low-grade leucocytic infiltration are already present in normal appearing perilesional skin. Keratin fibers in the dermis are associated with clinical disease. Early lesions are characterized by neutrophilic abscess formation and influx of mainly histiocytes, chronic lesions mainly by expansion of B and plasma cells in 'pseudo' follicles. Proliferating strands of follicular epithelium may initiate fistula formation. Mast cells are increased in all HS stages including perilesional skin.

INTRODUCTION

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic and recurrent inflammatory disease mainly affecting the inverse skin areas. HS is clinically characterized by recurrent, deep-seated, painful nodules and abscesses and, in severe cases fistula formation, interconnecting sinus tracts and rope-like scarring. HS has an estimated prevalence between 1% and 4%[1,2] and has a significant impact on patients' quality of life[3]. Intrinsic and environmental pathogenic factors include genetic predisposition, high body mass, smoking, shear forces and perhaps an altered immune response[4].

The pathogenic concept of HS has substantially changed over time. In 1854, the French surgeon Verneuil associated the symptoms with inflammation of the sweat glands and named the disease HS[5]. In 1990, by histological examination, this apocrinitis proved to be present in only 4/12 investigated HS specimens, and follicular occlusion was considered to be a far more constant histopathological feature[6]. Currently the generally accepted primary pathogenic mechanism is (hyper)keratinisation of the follicular infundibulum resulting in follicular occlusion and apocrinitis as a sporadic secondary event[4]. A novel histological feature of the disease, which occurs in 43% of cases, is epidermal psoriasiform hyperplasia[7]. However, despite our advancing insight, many questions remain unanswered, such as the potential existence of subclinical histomorphological alterations prior to clinical onset of the disease. Von Laffert et al. hypothesized that follicular hyperkeratosis and perifollicular inflammation represent early events in HS pathogenesis[8]. In accordance with the concept of subclinical pathogenic alterations preceding overt clinical inflammation, normal-appearing perilesional HS skin samples produce pro-inflammatory cytokines ex vivo[9]. The subclinical inflammation might lead to histomorphological changes in HS-prone skin.

Furthermore, a hallmark of moderate and severe HS is fistula formation. How or why these fistulas arise in HS and from where they primarily originate is unknown. Once fistulas have developed, inflammation often shifts from intermittent to chronic suppuration. Moreover, these fistulas and sinus tracts constitute a major therapeutic challenge, since the only method of eradication is by surgical removal[10].

The aims of this study were to assess histomorphological alterations, to phenotype infiltrating leucocyte subsets and to assess epithelial activation and/or proliferation in normal appearing perilesional HS skin, as well as in early and chronic HS lesions.

MATERIALS AND METHODS

Large, surgically resected specimens from chronic HS inflamed skin were obtained from 10 patients undergoing radical excision of their HS under general anaesthesia. 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. Six other HS patients visited the dermatology out-patient department (Deventer Hospital) with a newly emerging lesion, which the patient and an experienced dermatologist (JB) recognized as a new early HS lesion. All early HS lesions were biopsied within 4 days after onset. Healthy control skin consisted of submammary skin from 4 healthy females undergoing breast reduction surgery at the department of Plastic Surgery.

Immunostaining procedure

The skin specimens were fixed in 4% formaldehyde and embedded in paraffin. Four micrometer thin sections were stained with haematoxylin and eosin (H&E) or labeled with a panel of antibodies for immunophenotyping leukocyte subsets and for cellular proliferation. The antibodies used are listed in Table 1. A standard horseradish peroxidase DAB (diaminobenzidine) staining procedure was followed using a polymer detection system (K8005, DAKO, Glostrup, Denmark). All sections were counterstained with haematoxylin (4085-9005, Klinipath, Uden, The Netherlands). Antigen retrieval for all antibodies, except anti- FXIIIa, was performed by heating sections (20 min at 97°C) in the DAKO PT modules (PT 101, DAKO, Glostrup, Denmark) in Envision Flex Target Retrieval Solution high pH (DAKO, K8004, Glostrup, Denmark). Normal antibody diluent (ABB999, Scytec laboratories, Logan, UT) was used for blocking all sections and for dilution of the primary antibodies. Positive tissue control, placenta for FXIIIa, skin for pankeratin, and tonsil for the other antibodies was included in each staining run. Negative staining controls comprised omission of the primary antibody and replacement with normal antibody diluent.

Table 1. Antibodies used for immunostaining

Marker	Indicative for	Clone	Positive control	Dilution	Manufacturer
CD1a	Langerhans cells	O10	Skin	1:20	Immunotech, Prague, Czech Republic
CD3	T cells	polyclonal	Tonsil	1:150	Dako, Glostrup, Denmark
CD4	T helper cells	4B12	Tonsil	1:160	Monosan, Uden, The Netherlands
CD8	Cytotoxic T cells	C9/144B	Tonsil	1:100	Dako
CD11c	Dendritic cells	5D11	Tonsil	1:60	Novocastra
CD14	Monocytes	MY4	Tonsil	1:100	Novocastra
CD20	B cells	L26	Tonsil	1:400	Dako
CD56	NK cells	123C3.D5	Tonsil	1:25	Thermo Fisher Scientific, Cheshire, UK
CD68	Macrophages	KP1	Tonsil	1:160	Dako
CD79a	B cells	JCB117	Tonsil	1:100	Dako
CD138	Plasma cells	B-A38	Tonsil	1:25	IQ Products, Groningen, The Netherlands
FXIIIa	Dermal dendritic cells	AC-1A1	Placenta	1:200	Thermo Fisher Scientific
Ki67 (MIB-1)	Cell proliferation	MIB1	Tonsil	1:100	Dako
Pan keratin	Keratin	AE1/AE3	Skin	1:200	Thermo Fisher Scientific
Tryptase	Mast cells	AA-1	Tonsil	1:800	Dako

Histomorphology and quantification of stained cells

NanoZoomer Digital Pathology software (Hamamatsu, Herrsching am Ammersee, Germany) was used to scan the stained sections. All H&E-stained sections were assessed for histological features by two independent observers (HZ, EP) blinded to disease stage. Histomorphological features systematically assessed included: epidermal psoriasiform hyperplasia, parakeratosis, follicular plugging / keratosis, dilated hair follicles, presence of cysts, hyperplasia of follicular epithelia, sinus tracts/fistulas, neutrophilic abscess formation, multinucleated giant cells, keratin fibers in the dermis, apocrinitis and eccrinitis. Apocrinitis and eccrinitis were defined as an infiltration by inflammatory cells with destruction of apocrine or eccrine glandular epithelial cells. In case of some discrepancy between observers, the section was re-assessed by the observers, including an experienced dermato-pathologist (JH), to reach consensus. Leucocyte subsets within HS infiltrates tend to occur in a patchy, uneven distribution. Therefore leucocyte subsets and the proliferation marker Ki67 were scored by two independent observers (HZ, EP) in a semi-quantitative manner, using a global assessment on a continuous scale from 0 to 4, where a score of 0 represents no stained cells, and a score of 4 means staining of most cells. The average score was calculated from the score of both observers and was used for statistics. Medians with interquartile ranges are provided for the non-parametrically distributed values that were analysed by two-tailed Mann Whitney U test. The criterion for statistical significance was a p value below 0.05 ($p< 0.05$). Statistical analysis was performed using the Graphpad Prism 5 software.

RESULTS

Results of the histomorphological assessments are presented in Table 2. The semi quantitative scores for the immunostained sections are presented in Figure 1.

Normal appearing perilesional skin

Normal appearing perilesional skin already showed mild psoriasiform hyperplasia of the interfollicular epidermis and follicular plugging in 5/8 and 5/7 samples respectively. The scores for $CD3^+$ T cells, $CD138^+$ plasma cells, $FXIIIa^+$ dermal dendritic cells and $tryptase^+$ mast cells were slightly but significantly increased compared to healthy skin. These cell types were evenly distributed in the dermis.

Early HS lesions

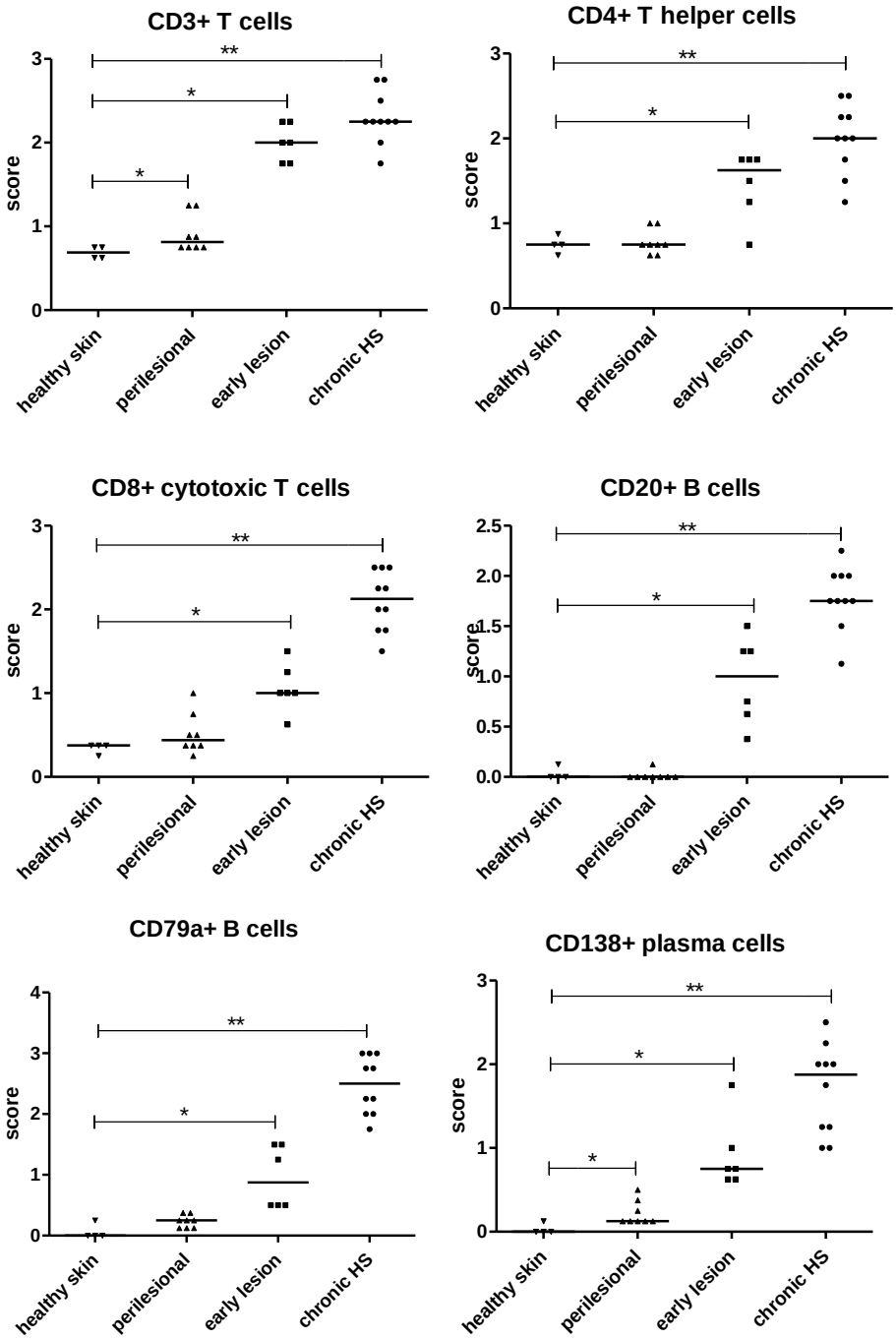
In half of the early lesions, epidermal psoriasiform hyperplasia of the interfollicular epidermis occurred. In all samples, whenever a hair follicle was assessable, follicular plugging and dilated hair follicles were observed. In all biopsies we observed a variable degree of abscess formation with massive neutrophilic infiltration. In the dermis, free 'rod-like' keratin fibers

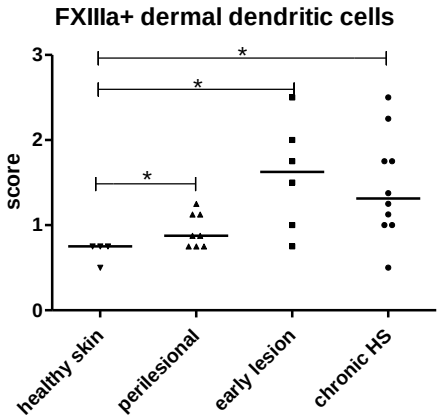
Table 2. Observed histological features n.a= not assessable

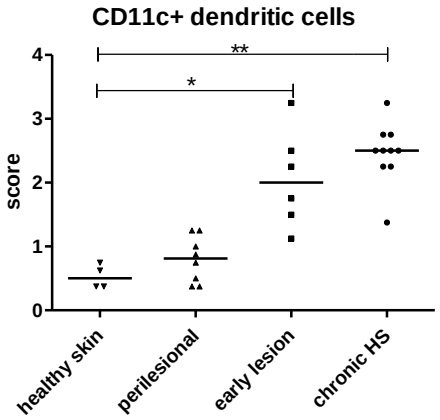
		Perilesional	Early HS	Chronic HS
Psoriasiform	no	3/8	3/6	0/10
hyperplasia	mild	4/8	1/6	1/10
	pronounced	1/8	2/6	9/10
Parakeratosis	no	8/8	1/6	6/10
	mild	0/8	5/6	4/10
Follicular	n.a.	1/8	2/6	1/10
plugging	no	2/7	0/4	0/9
	mild	3/7	1/4	1/9
	pronounced	2/7	3/4	8/9
Dilated	n.a.	1/8	2/6	1/10
hair follicle	no	2/7	0/4	0/9
	mild	4/7	1/4	3/9
	pronounced	1/7	3/4	6/9
Cyst	yes	0/8	2/6	4/10
Hyperplasia	n.a.	1/8	1/6	1/10
follicular	no	7/7	5/5	0/9
epithelia	mild	0/7	0/5	4/9
	pronounced	0/7	0/5	5/9
Sinus tract	yes	0/8	0/6	4/10
Abscessing	no	8/8	0/6	1/10
neutrophilic	mild	0/8	3/6	2/10
inflammation	pronounced	0/8	3/6	7/10
Giant cells	no	8/8	1/6	2/10
	mild	0/8	5/6	6/10
	pronounced	0/8	0/6	2/10
Keratin	no	8/8	1/6	2/10
remnants	mild	0/8	4/6	3/10
	pronounced	0/8	1/6	5/10
Apocrinitis	n.a.	3/8	0/6	4/10
	yes	0/5	2/6	3/6
Eccrinitis	n.a.	1/8	0/6	1/10
	yes	0/7	3/6	6/9

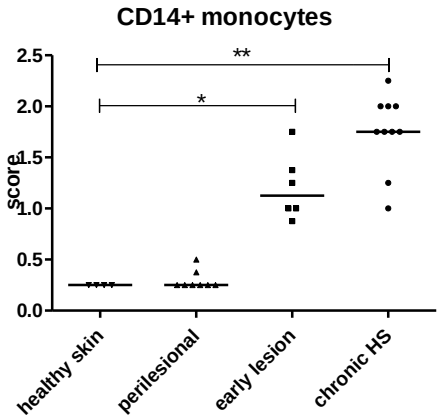
and keratinocyte remnants were detected in almost all samples. Multinucleated giant cells, regularly containing phagocytosed keratin fibers (Figure 2), were seen in almost all samples. $CD11c^+$ dendritic cells (DC) were especially abundant in abscesses. Numbers of $CD14^+$ monocytes, $CD68^+$ macrophages and $FXIIIa^+$ dermal DC were also increased, whereas $CD1a^+$ Langerhans cells showed a slight decrease. Apocrinitis was present in 2/6 and eccrinitis in 3/6 of the lesions. $CD8^+$ cytotoxic cells were regularly observed in the dermis and epidermis.

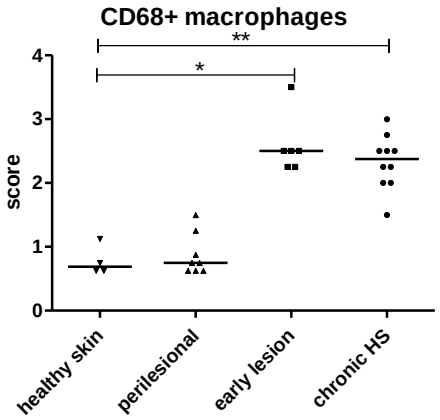
Figure 1. Semi quantitative scores for immunophenotypic leucocyte subset markers
Note the different scales in leukocyte subsets scores. $p < 0.05$ is presented as *, $p < 0.01$ as **

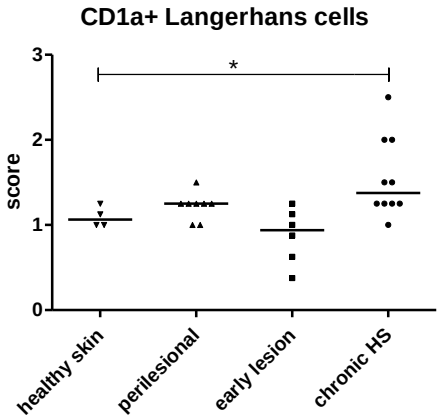


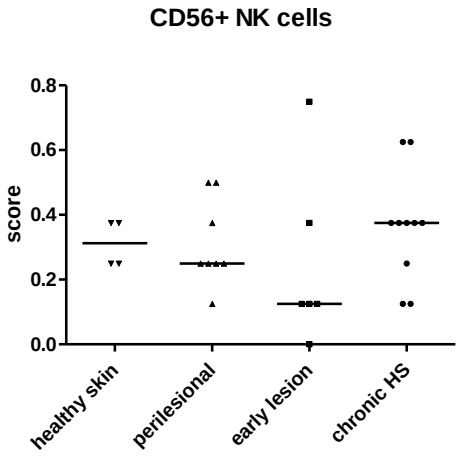

FXIIIa+ dermal dendritic cells
score
3
2
1
0
*
*
*
healthy skin
perilesional
early lesion
chronic HS

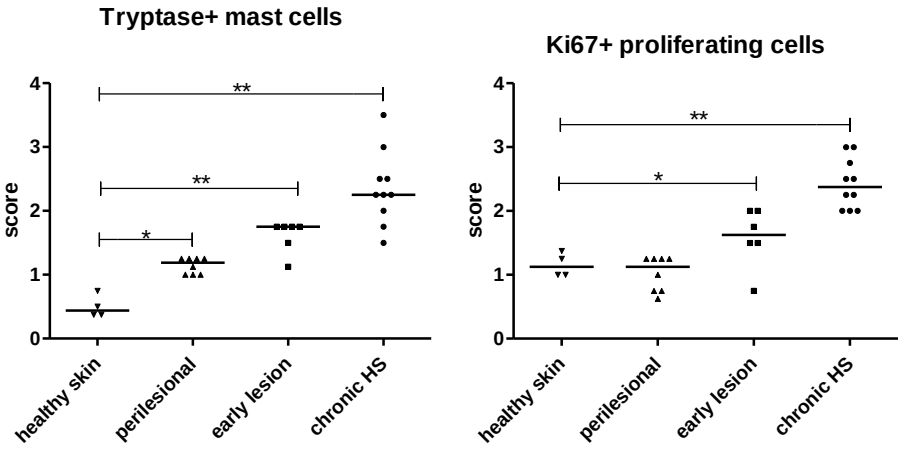

CD11c+ dendritic cells
score
4
3
2
1
0
**
*
healthy skin
perilesional
early lesion
chronic HS


CD14+ monocytes
score
2.5
2.0
1.5
1.0
0.5
0.0
**
*
healthy skin
perilesional
early lesion
chronic HS


CD68+ macrophages
score
4
3
2
1
0
**
*
healthy skin
perilesional
early lesion
chronic HS


CD1a+ Langerhans cells
score
3
2
1
0
*
healthy skin
perilesional
early lesion
chronic HS


CD56+ NK cells
score
0.8
0.6
0.4
0.2
0.0
healthy skin
perilesional
early lesion
chronic HS



Chronic HS lesions

Epidermal psoriasiform hyperplasia was present in all chronic HS lesions and was more pronounced compared to perilesional skin and early lesions. This psoriasiform hyperplasia was most pronounced in the interfollicular epidermis overlying inflammatory infiltrates (Figure 3). Mild parakeratosis was observed in 3/10 lesions. Follicular plugging, dilated hair follicles and hyperplasia of follicular epithelia were observed in all chronic HS lesions when a hair follicle was present in the sections. Four out of ten samples contained an epithelialized fistula. Fistulas were exclusively seen in chronic lesions and only in Hurley grade 2 and 3 cases. One example of Ki67+ strand of keratinocytes in the deep dermis is shown in (Figure 4). Abscess formation with neutrophilic inflammation was more extensive than in early lesions. Similar to early lesions, some multinucleated giants cells contained phagocytosed keratin fibers and keratinocyte fragments (Figure 2). Using pankeratin staining several detached and dispersed single keratinocytes were seen in the dermis, densely surrounded by neutrophils (Figure 2). Apocrine glands were not present in all samples, yet apocrinitis was observed in 2/6 samples and eccrinitis in 5/9 samples. The numbers of $CD3^+$, $CD4^+$, $CD8^+$ T cells, $CD11c^+$ DCs, $CD14^+$ monocytes, $CD1a^+$ Langerhans cells, $tryptase^+$ mast cells, and especially, $CD20^+$, CD79a B cells and $CD138^+$ plasma cells were all significantly increased compared to early lesions. In contrast, the score for $CD68^+$ macrophages and $FXIIIa^+$ dermal DC remained stable. $CD3^+$ T cells were diffusely located in the infiltrate and in case of an abscess their location was more at the periphery of the abscess. $CD4^+$ T cells were widely dispersed through the infiltrate, although clustering and epidermal infiltration was additionally observed. $CD8^+$ T cells were scattered through the infiltrate and were additionally present in the epidermis. $CD20^+$ cells were additionally diffusely located around abscesses. In addition, $CD20^+$ cells showed a very patchy distribution in the infiltrate (Figure 5). These clusters of $CD20^+$ cells also expressed CD79a. In contrast, $CD138^+$ plasma cells were quite homogeneously distributed. $FXIIIa^+$ dermal DC were

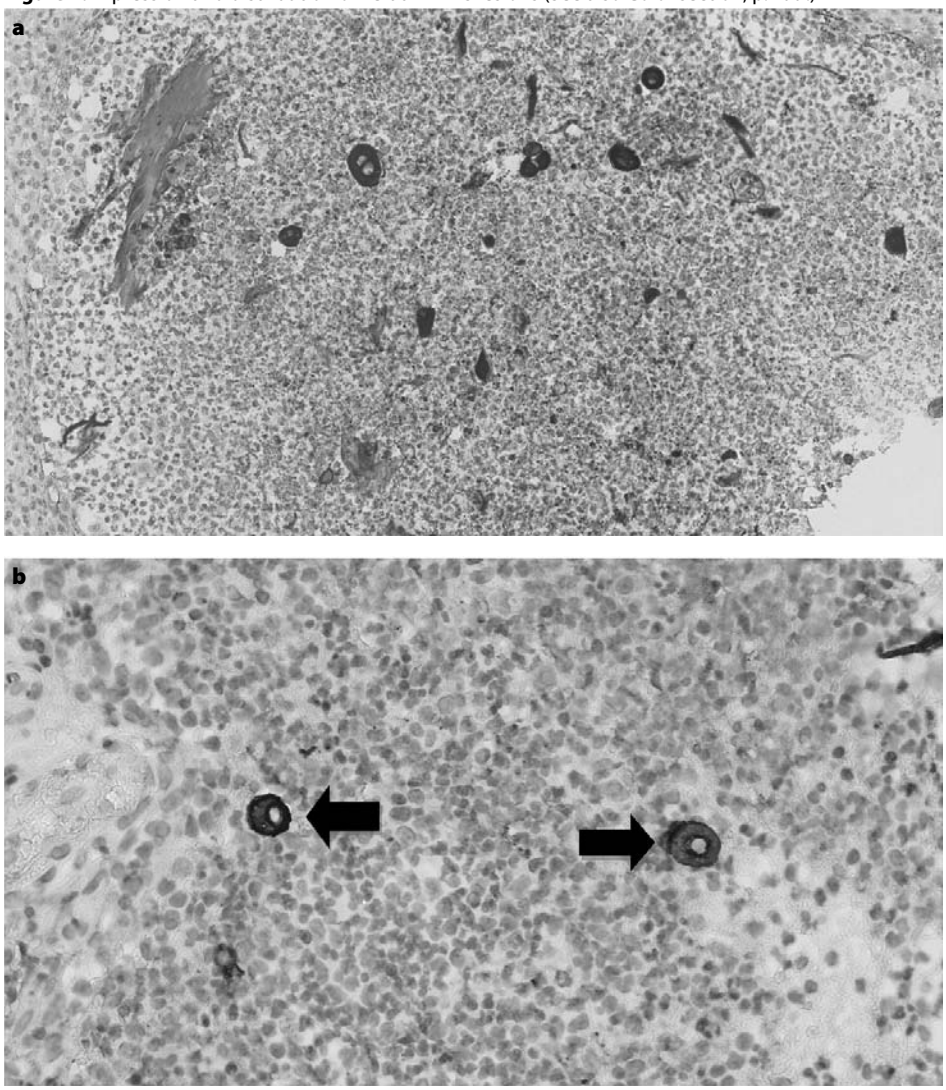
located at the periphery of the infiltrate but also along the basal membrane as well as deep in the dermis, but their numbers varied widely between samples. CD11c$^+$ DC were especially abundant within abscesses. CD14$^+$ monocytes were more diffusely spread throughout the infiltrate. Likewise in perilesional skin and early lesions tryptase$^+$ mast cells were abundantly and diffusely increased in the dermis (Figure 6). Numbers of keratinocytes, expressing Ki67 were significantly increased, especially in the epidermis overlying infiltrates (Figure. 3). Epidermal areas with increased Ki67 expression also exhibited psoriasiform hyperplasia. CD56$^+$ NK cells were observed but their frequency was neither elevated nor decreased in any HS condition compared to healthy skin.

DISCUSSION

Although the current study was conducted in a limited number of patients, these were selected as prototypic cases. Accordingly, we just as Jemec et al. observed little heterogeneity between samples, indicating the data is representative and can be extrapolated to the HS population at large[11]. Our results indicate that clinically unaffected perilesional skin already displays epidermal psoriasiform hyperplasia, follicular plugging and dilated hair follicles. These histological alterations are accompanied by a mild and significant influx of dermal CD3$^+$ T cells, CD138$^+$ plasma cells, FXIIIa$^+$ dermal DCs and tryptase$^+$ mast cells. Recently, we demonstrated increased levels of secreted IL-1β, TNF-α and IL-10 in comparable clinically unaffected perilesional skin[9]. On the basis of our data it is not possible to point out what comes first, the epithelial alterations such as psoriasiform hyperplasia and follicular/infundibular keratosis or the inflammation. We hypothesize that the mild influx of leucocytes, together with increased levels of cytokines in perilesional skin induce the epithelial alterations, possibly as a spill-over from adjacent inflammation. The subclinical inflammatory response may be initiated by an unknown trigger, possibly overgrowth of commensal bacteria. Nevertheless, we argue that these observations of subclinical inflammation and histological alterations provide a rationale for the use of keratolytics and anti-inflammatory agents in perilesional skin to prevent further disease progression. Likely candidates for such an intervention are topical resorcinol and oral acitretin, which have keratolytic as well as anti-inflammatory properties, and have both been shown to be efficacious in HS[12,13]. The preventive effect of such agents should be investigated prospectively.

In newly emerged HS lesions, not older than 4 days, we observed marked psoriasiform hyperplasia, follicular plugging and dilation, cysts, free keratin fibers in the dermis and intracellular fibers in multinucleated giant cells. This suggests that upon disease progression dilated hair follicles or cysts rupture, spilling their keratin content into the dermis. In response to this foreign material, an abscessing neutrophilic inflammation ensues at the site of rupture with an additional massive influx of CD68$^+$ macrophages, CD14$^+$ monocytes and

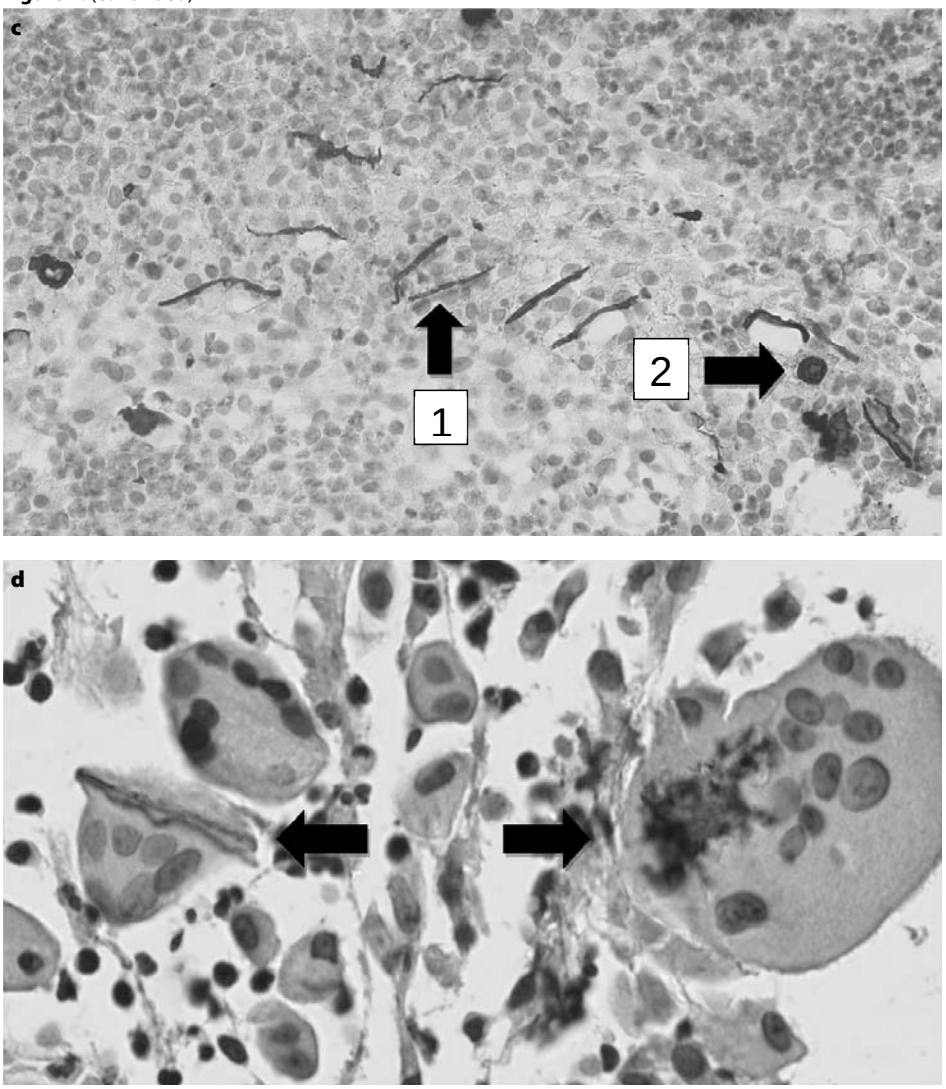
Figure 2. Expression and distribution of keratin in HS lesions (See also Color section, p. 199.)



a) Keratin fibers and isolated detached keratinocytes (brown staining) in an abscess in a chronic HS lesion.
b) Single detached keratinocytes in the dermis (arrows) in a chronic HS lesion.

CD11c$^+$ inflammatory DC. The formation of multinucleated giant cells represents an attempt of the immune system to clear dermal keratin similar to a foreign body response, although prototypical granuloma formation was not observed. In this process large numbers of CD3$^+$, T cells and B cells are attracted creating a mixed infiltrate. Previous studies showed increased levels of IL-17, IL-10 and TNF-α, but not IFN-γ, IL-2, IL-12, in active HS lesions (own data submitted for publication)[9,14]. Therefore, the signature of T helper subsets in HS is a mixed one, and does not fit into a classic Th1, Th2, Th17 or Th22 profile and as such not comparable with

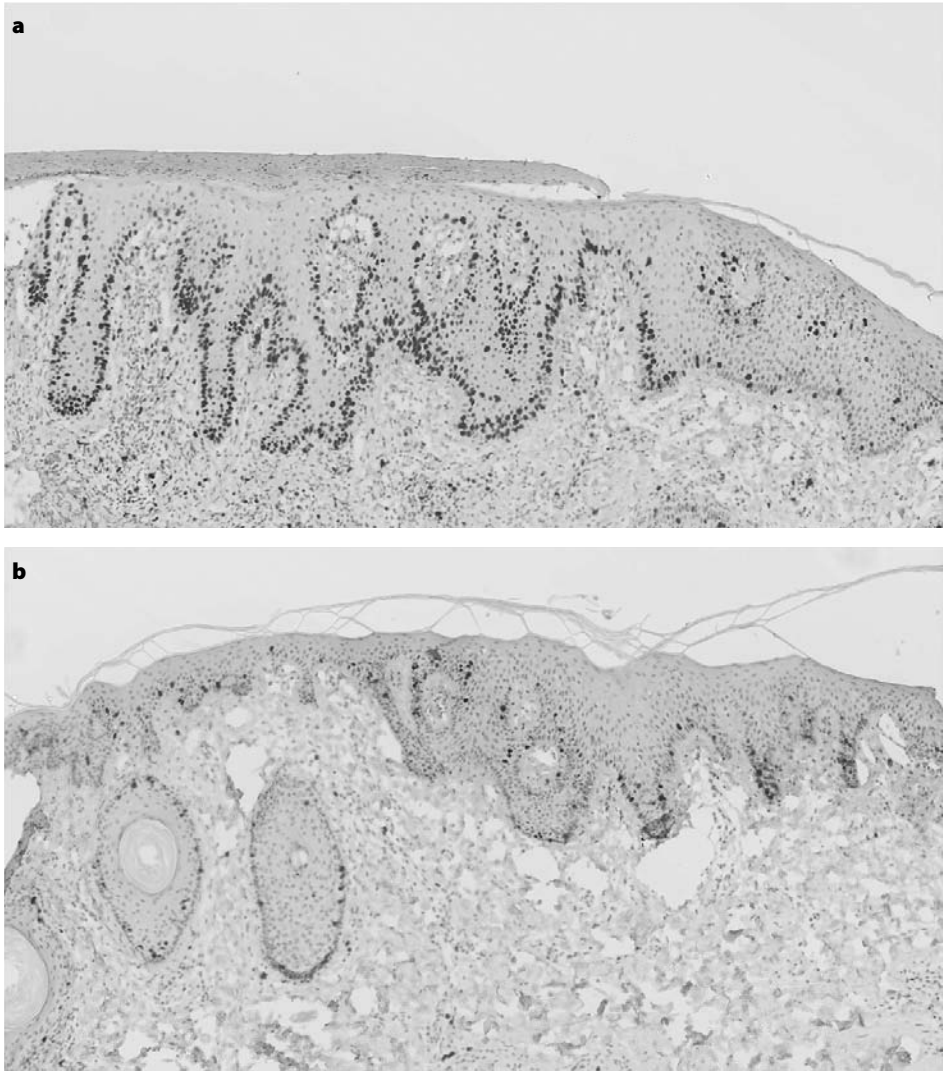
Figure 2. (continued)



c) Free keratin fibers (arrow 1) and detached keratinocytes (arrow 2) in the dermis in an early lesion.
d) Phagocytosed keratin fibers and remnants in multinucleated giant cells, in an early lesion (indicated with arrows).

psoriasis nor atopic dermatitis. As described by von Laffert[8] we also observed modest $CD8^+$ T cell epitheliotropism, for currently unknown reasons. Previously Boer and Weltevreden characterized the infiltrate in early lesions, using a limited set of immunohistological markers[15]. They demonstrated increased numbers of HLA-DR^+, $CD62L^+$ T cells and $CD3^+$ T cells in early lesions[15]. Furthermore, the tendency of $CD1a^+$ positive Langerhans cells in early lesions to decrease suggest emigration from the epidermis in response to an unknown trigger.

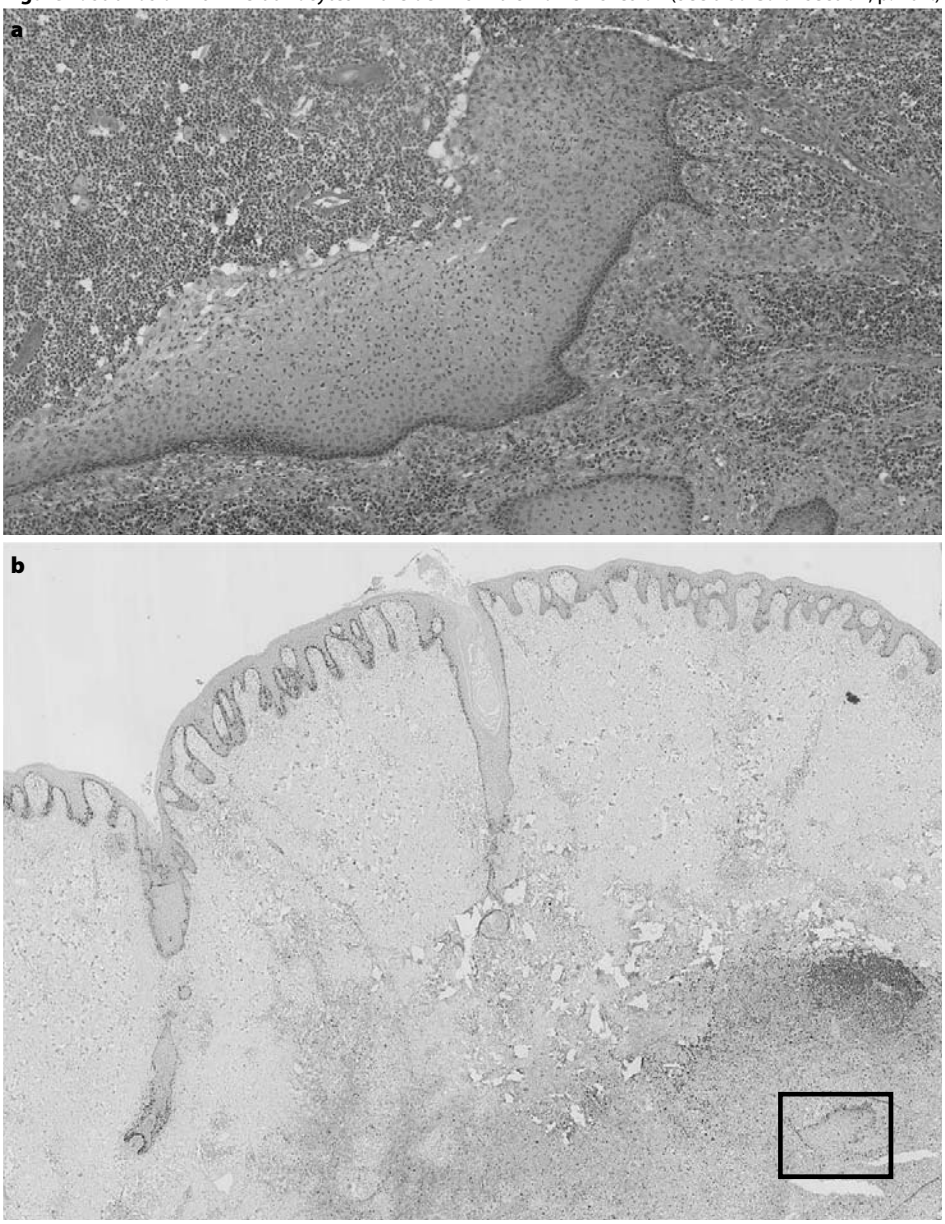
Figure 3. Psoriasiform epidermal hyperplasia (See also Color section, p. 201.)



a) Psoriasiform epidermal hyperplasia with parakeratosis overlying an infiltrate in a chronic HS lesion. Basal epidermal keratinocytes frequently express Ki67, indicating proliferation.
b) The same sample adjacent to the infiltrate shows less pronounced psoriasiform epidermal hyperplasia and fewer Ki67 positive keratinocytes.

HS lesions can become chronic. This is characterized by dramatic expansion of the infiltrate. The epidermal psoriasiform hyperplasia, follicular plugging, dilated hair follicles and cysts all become more pronounced and in contrast to Laffert et al.[7] we did observe mild parakeratosis in some samples. In addition, the follicular epithelia displayed hyperplasia. In the dermis, ectopic $CD20^+$ and $CD79a^+$ B cell 'pseudo' lymphoid follicles were observed, which might be bona fide germinal centers. These 'pseudo' lymphoid follicles were previously not described

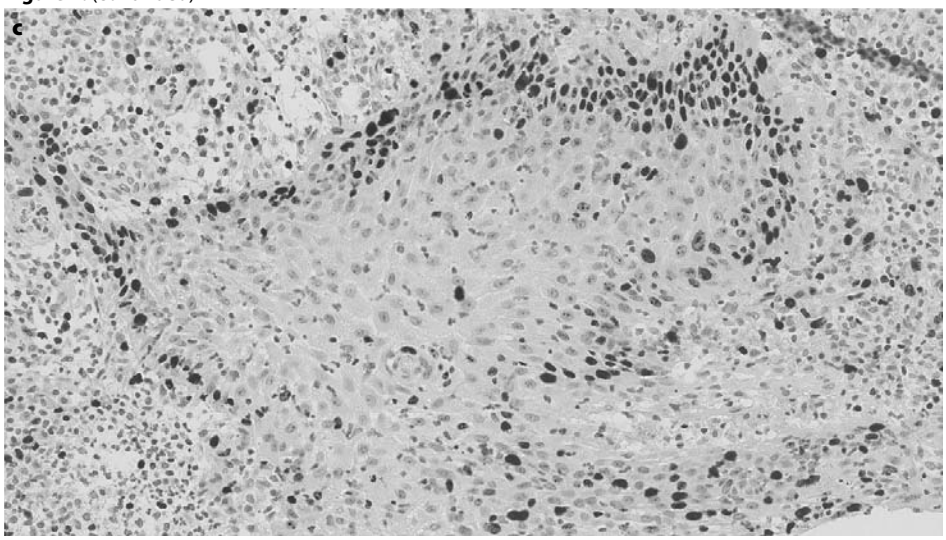
Figure 4. Strands of Ki67$^+$ keratinocytes in the dermis in a chronic HS lesion (See also Color section, p. 202.)



a) Keratinocytes, in an epidermal strand in the dermis in a chronic lesion (H&E stained).
b) Overview of the same HS lesion, Ki67 stained, with an epidermal strand in the dermis indicated by the square.

in HS and may be explained as a reaction secondary to persistent inflammation or bacterial colonization.

Figure 4. (continued)



c) Higher magnification of this epidermal strand showing multiple Ki67+ keratinocytes.

Using H&E staining keratin fibers are easily overlooked. This might explain why these fibers to our knowledge have not been described before in HS. The sustained presence of these keratin fibers in chronic HS might be explained by impaired clearance of these fibers or derived from new ruptured cysts. We argue that these rod-like keratin fibers are difficult to eradicate and the exaggerated immunologic response is comparable with that to uric

Figure 5. Scattered CD20 positive B cells, and a prototypical patch, creating 'pseudo' lymphoid follicles in a chronic HS lesion (See also Color section, p. 203.)

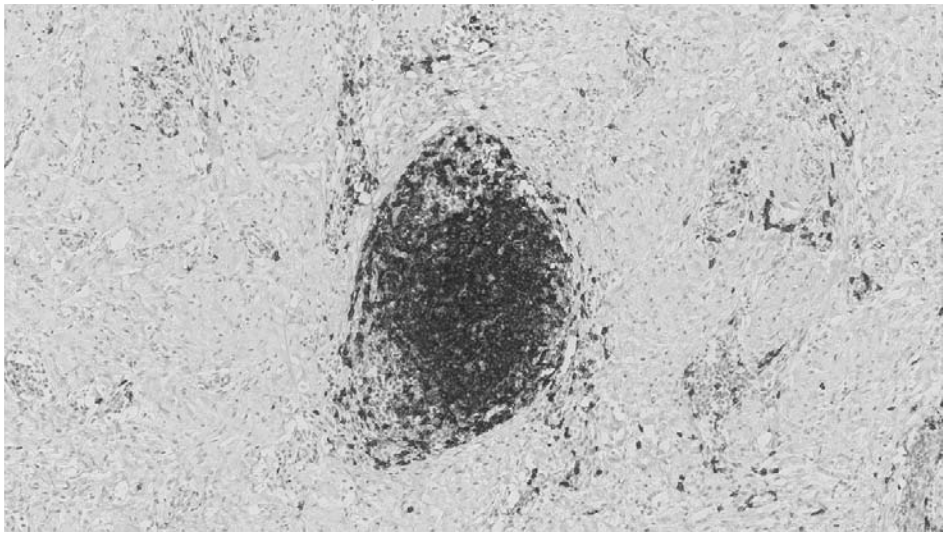
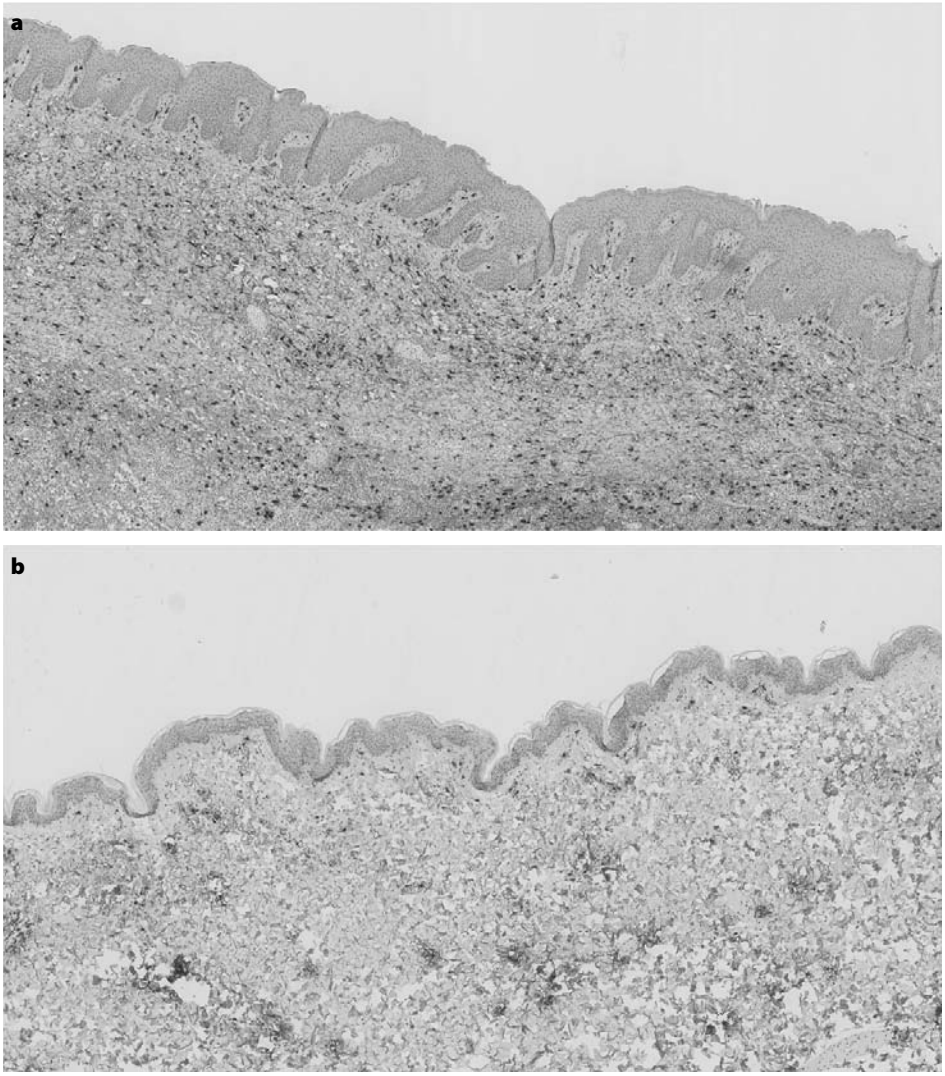


Figure 6. Increased number of tryptase positive mast cells in a chronic HS lesion (See also Color section, p. 204.)



a) Tryptase positive mast cells in a chronic HS lesion.
b) Tryptase positive mast cells in healthy control skin.

acid crystals in gout activating the inflammasome. Thus, these phagocytosed keratin fibers activate the inflammasome, resulting in activation of caspase 1, cleavage of pro-IL-1 into IL-1β, generating the high levels of bioactive IL-1β in HS lesions[9]. The inflammasome may be further activated by other damage-associated molecular patterns (DAMP)[16] from necrotic cellular debris that is abundantly present in abscessing HS lesions.

In chronic HS the formation of epithelialized fistulas indicates a new phase in HS disease severity. It is currently unknown how and from where these epithelialized fistulas originate. It is

has been postulated that fistula formation originates from the leaf like dermal protrusions from the hyperplastic epidermis[17] or from keratinocyte stem cells[18]. We here show a marked increase of $Ki67^+$ proliferating keratinocytes in strands of epithelia, and single detached keratinocytes which are probably launched into the dermis from ruptured cysts. We propose that these epithelial strands are the forerunners of fistulas, as they may form dermal epithelial tubes.

Initially HS was thought to be a disease of the apocrine sweat glands. However, in accordance with the recent literature, we observed apocrinitis and eccrinitis in 42% and 60% of cases, when assessable, and always as part of massive dermal inflammation, never as an isolated or specific event. These percentages are slightly more than reported by Jemec and Hansen, who observed apocrinitis in 12% and eccrinitis in 25% of cases[19]. Because eccrinitis was more frequently observed than apocrinitis, it seems unlikely that the apocrine glands play an important role in the disease pathogenesis. We suspect that sweat glands are secondarily inflamed by chance, as bystanders. The still current term 'apocrine-bearing skin' to describe the predilection site for HS should therefore be considered a misnomer. Other characteristics of HS predilection sites such as mechanical friction forces, a 'loose' dermal composition and a warm, humid, bacteria-friendly microclimate seem more relevant.

HS patients frequently complain at a variable degree of concomitant itch[20]. To our knowledge, we demonstrate for the first time the presence of high numbers of tryptase positive mast cells in HS lesional skin. These excessive numbers of mast cells might be responsible for the patient reported itch. Furthermore, increased numbers of mast cells were not only present in chronic and early HS but were also abundant in perilesional skin. This suggests that mast cells are also important in the initial, subclinical phase of HS pathogenesis.

Our data indicate new potential therapeutic targets in HS and validate the use of current treatments for HS. The demonstration of ectopic 'pseudo' B cell follicles in chronic HS lesions opens the novel option of therapeutic interventions aimed at depletion or blocking of B cells. Neutrophils could be targeted by drugs that inhibit neutrophil chemotaxis such as anti-inflammatory antibiotics and Dapsone. However, a recent retrospective case series demonstrated improvement only in 9 out of 24 patients treated with Dapsone[21]. Anti-TNF-α biologics also inhibit neutrophil chemotaxis and are already in use for the treatment of moderate to severe HS. Furthermore mast cells might be a target for future intervention.

In conclusion, there are probably pathogenic mechanisms at work prior to clinical disease manifestation, indicated by follicular plugging and psoriasiform hyperplasia in the presence of low grade influx of a mixed infiltrate in normal appearing perilesional skin. The appearance of painful nodules could be the result of an immunological response to breached dilated hair follicles or cysts. We stress that in almost all clinical lesions, early and chronic, multinucleated giant cells and keratin fibrils were observed indicating that, along with follicular plugging and psoriasiform hyperplasia, these are the most constant histological hallmarks of HS. Additional research is warranted to identify the cellular source of cytokines that promote follicular plugging, and whether this process can be ameliorated by therapeutic intervention.

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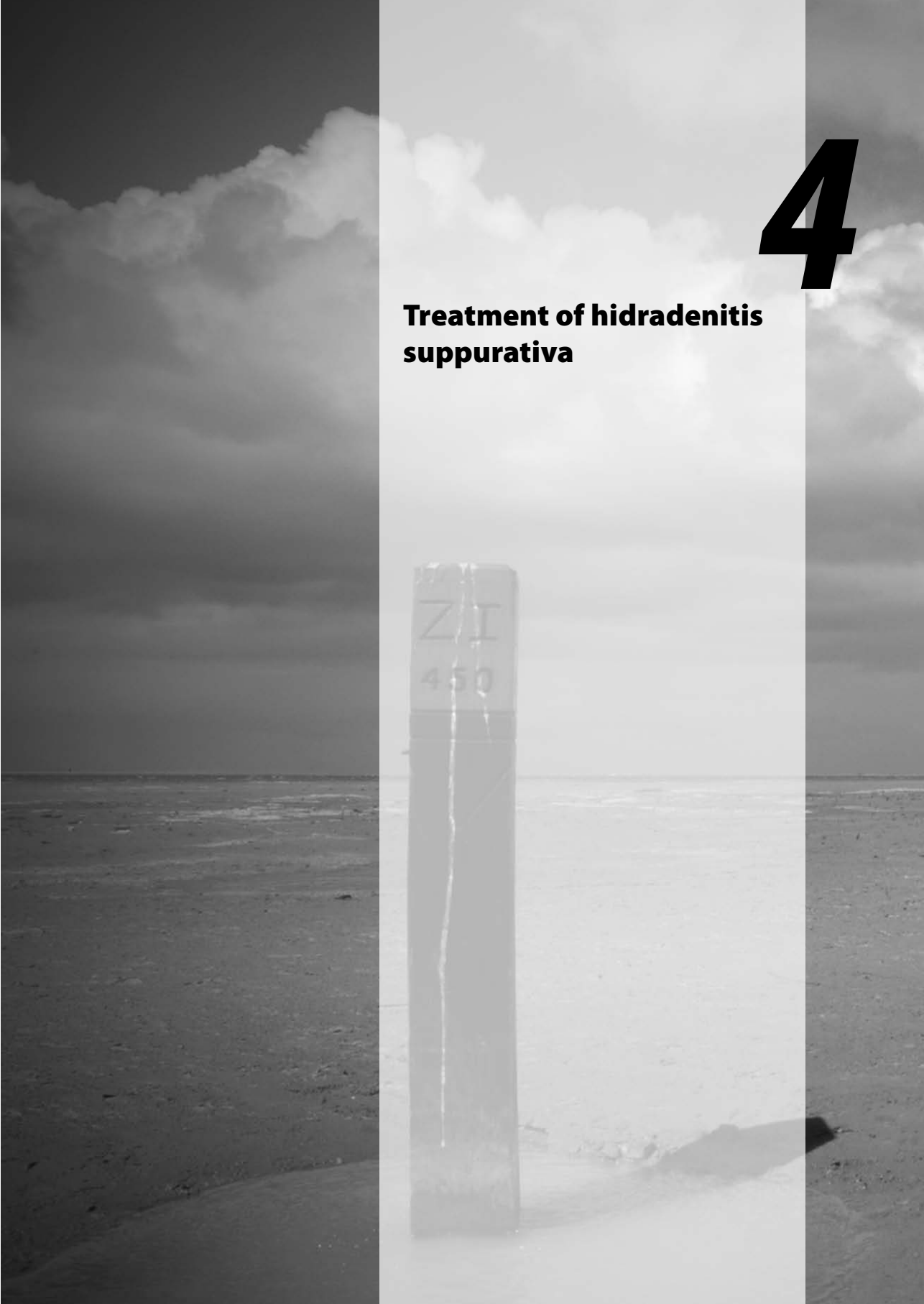

4

Treatment of hidradenitis suppurativa

4.1

The effect of combined treatment with oral clindamycin and oral rifampicin in patients with hidradenitis suppurativa

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Dermatology 2009; **219**:143-7

ABSTRACT

Background: A previous limited study showed promising results of combined oral treatment with rifampicin 600 mg and clindamycin 600 mg for 10 weeks.

Objective: To expand and to validate the basis for this therapy we reviewed the response to different treatment durations.

Method: A retrospective study in 34 patients.

Results: Twenty eight of 34 patients (82%) experienced at least partial improvement, and 16 (47%) showed a total remission. The maximum effect of treatment appeared within 10 weeks. Following total remission, 8 of 13 (61.5%) of patients treated with above mentioned treatment schedule experienced a relapse after a mean period of 5.0 months. Non-responders were predominantly patients with severe disease.

Conclusion: Combination treatment with oral rifampicin and clindamycin is a promising treatment option for HS, despite the frequently occurrence of diarrhea as a side effect. The length and the dosage of treatment are not yet firmly established.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by recurring painful abscesses and draining sinuses in the ano-genital-inguinal area and axillae, with a prevalence of 1%[1]. It has a significant impact on the patients' quality of life[2,3]. The exact cause of HS remains unclear[4]. Histopathologic studies have suggested occlusion of the follicular infundibulum as an important factor[5,6]. A large variety of microorganisms can be isolated from the lesions, but often lesions appear to be sterile[7,8]. *Staphylococcus epidermidis* species are the most frequently cultured bacteria and *Staphylococcus aureus* is found in a minority of lesions[7,8]. Other data however suggest that HS patients may suffer from low-grade bacteraemia, indicating that bacteria may play a role[9]. HS is currently thought of as being a sterile inflammation but bacteria are suspected of playing a role in the disease process although not that of a simple infection. It is speculated that bacteria may play part through immune mediated mechanisms of inflammation in association with a dysregulated immune response in the hair follicles[4]. Antibiotic therapy is however widely used and mentioned in all textbooks of dermatology as a prominent form of treatment for HS. Curiously the literature reveals only a handful of studies on the use of antibiotics. Topical clindamycin was found to be superior to placebo in a randomized double blind clinical trial and in another randomized clinical trial there was no difference between systemic tetracycline 1 gr daily and topical clindamycin 1% twice daily[10,11]. A case report of two patients using systemic clindamycin in a high dosage of 1200 mg and 2400 mg respectively showed good improvement albeit with relapse on cessation of treatment[12]. The combination therapy with oral clindamycin and oral rifampicin has been suggested to be an effective treatment for other follicular occlusion disorders such as folliculitis decalvans[13,14]. Combined treatment with oral rifampicin 300 mg B.I.D. and oral clindamicin 300 mg B.I.D. for 10 weeks has been advocated for HS[15,16]. In order to assess the validity of these claims a review was made of the outcomes of combined oral clindamycin and oral rifampicin treatment of consecutive patients in two dermatology centers.

SUBJECTS AND METHODS

In total 47 patients diagnosed with HS were treated with the therapy of combined oral clindamycin and oral rifampicin at the dermatology departments of the Deventer Hospital, the Netherlands and Roskilde Hospital Denmark between 2006 and 2007. Information about age, sex, duration of HS, sites of inflammation, previous treatment, the outcome of clindamycin and rifampicin treatment, side effects and follow-up were extracted from the records. All included patients had active disease for many months or years and had failed several other HS treatments including topical clindamycin, other oral antibiotics and surgery prior to the clindamycin and rifampicin combination treatment (Table 1). The combination treatment

used in this study was not first choice of disease management. The decision to use this treatment was taken by JB (Deventer) or GJ (Roskilde). Thirteen patients were excluded because of concomitant oral or topical medication. Descriptive staging of disease severity based on the assessment of scarring and inflammation according to Hurley was made[17]. Stage 1: Abscess formation, single or multiple without sinus tracts and cicatrisation. Stage 2: Recurrent abscesses with tract formation and cicatrisation, single or multiple widely separated lesions. Stage 3: Diffuse or near diffuse involvement or multiple interconnected tracts or abscesses across the entire area. Patient characteristics are given in Table 1. Outcomes were presented as a physician global assessment and were classified according to the effect of the treatment on inflammation including suppuration, whereas the presence of non-inflamed lesions such

Table 1. Patient characteristics. Figures in parentheses are percentages.

Number	34
Gender (F/M)	29/5
Age, years	
Mean	39.9
Range	19-59
Duration of HS, years	
Mean	17.8
Range	1-52
Disease severity	
Hurley 1	4 (11.8)
Hurley 2	20 (58.8)
Hurley 3	10 (29.4)
Affected areas	
Groin	26 (77.5)
Axillae	12 (35.3)
Gentitalia	8 (23.5)
Buttocks	8 (23.5)
Perianal	4 (11.8)
Other	2 (5.9)
Previous medication	
Oral antbiotics	23 (67.6)
Surgery	22 (64.7)
Isotretinoin	12 (35.3)
Resorcinol	10 (29.5)
Topical antibiotics	9 (26.5)
Acitretin	3 (8.8)
Infliximab	1 (2.9)
Prednisone	1 (2.9)

as sinus tracts or scars was not considered. Partial improvement was defined as less than 75% clinical improvement from baseline, whereas total remission was defined as total clearance or at least improvement by more than 75%.

RESULTS

Five different dosage regimens were used. Four dosage regimens contained only 7 or fewer patients. Outcomes are presented according to intention to treat principle. In the group as a total, 28 of 34 patients (82.4%) responded to treatment, 12 (35.3%) showed partial improvement and 16 (47.1%) total remission. Six (17.6%) patients showed no improvement (Table 2). No cases of worsening of the disease were observed during the treatment period. There was not much difference in outcomes between patients treated for 10 weeks or longer compared to patients that were treated shorter than 10 weeks. Outcomes according to the Hurley classification at onset are shown in Table 3. Patients with no response were predominantly patients with severe disease. Adverse side-effects occurred in 13 of 34 patients (38.2%), of which diarrhea was the most common, 9 patients (26%). In addition 2 patients experienced a Candida vaginitis, 2 nausea, 2 dizziness, and 1 glossodynia. Nine patients (26%) stopped treatment due to the side-effects. Six of these 9 patients discontinued due to diarrhea. The largest subgroup of patients studied was treated according to the scheme of Mendonça and Griffiths[15] (n=23). This group was therefore further analyzed. Thirteen (56.5%) patients experienced total remission, 7 (30.4%) partial improvement and 3 (13%) no improvement. All non-responders in this group were Hurley stage 3. Following total remission in this group, 8 of 13 patients (61.5%) experienced a relapse of the disease after a mean of 5.0 months (range

Table 2. Outcomes according to different treatment durations. Figures in parentheses are percentages.

	Any dosage of clindamycin + rifampicin combination			Clindamycin 2x300 mg rifampicin 2x300 mg		
	weeks <10 (n=13)	weeks 10≥ (n=21)	any weeks (n=34)	weeks <10 (n=7)	weeks 10 (n=16)	any weeks (n=23)
No improvement	1 (7.7)	5 (23.8)	6 (17.6)	0	3 (16.8)	3 (13)
Partial improvement	6 (46.2)	6 (28.6)	12 (35.3)	3 (42.9)	4 (25)	7 (30.4)
Total remission	6 (46.2)	10 (47.6)	16 (47.1)	4 (57.1)	9 (56)	13 (56.5)

Table 3. Outcomes according to Hurley score at onset. Figures in parentheses are percentages.

	Any dosage of clindamycin rifampicin combination			Clindamycin 2x300 mg rifampicin 2x300 mg		
	Hurley 1 (n= 4)	Hurley 2 (n=20)	Hurley 3 (n=10)	Hurley 1 (n=1)	Hurley 2 (n=15)	Hurley 3 (n=7)
No improvement	1 (25)	1 (5)	4 (40)	0	0	3 (43)
Partial improvement	1 (25)	9 (45)	2 (20)	0	6 (40)	1 (14)
Total remission	2 (50)	10 (50)	4 (40%)	1 (100)	9 (60)	2 (29)

0.3-18 months). Three patients (23.1%) were still in remission at the end of study after a mean follow-up of 7.3 months (range 1-12 months). Two (15.4%) patients with total remission were lost to follow up.

DISCUSSION

This study shows that treatment of HS with combined oral clindamycin and oral rifampicin results in clinical improvement in 28 of 34 patients (82.4%). Our results are in agreement with those of Mendonça and Griffiths[15] as well as Gener et al.[16]. Mendonça and Griffiths[15] showed that the combined treatment with oral clindamycin 300 mg BID and oral rifampicin 300 mg BID for 10 weeks was effective in 10 of 14 (71.4%) treated patients. Gener et al.[16], showed in a in large series of 116 consecutive patients complete remission in 8 of the finally analysed 70 patients (11%), improvement in 60 of 70 patients and worsening in 2 of 70 patients after 10 weeks of the combined treatment. Our study has some limitations because it was a retrospective study and further there was heterogeneity in the group of HS patients included, it does however reflect clinical practice and variation. Although a 10 week treatment period seems rather short for a chronic fluctuating disease like HS, we did not observe big differences in outcomes between patients treated 10 weeks and longer and patients treated for a shorter period. Actually, a higher percentage of non-responders were observed in the group treated 10 weeks or longer, indicating that shorter treatment duration may be effective. Furthermore the response to treatment tends to correlate with the disease severity as all non-responders in the 10 week course[15] were Stage 3, i.e. those with significant scarring of a larger affected area. However, not many Hurley 1 patients were treated making a more precise estimate of the influence of disease severity on outcome more difficult. Most patients who achieved total remission at the end of treatment in the 10 week course[15] also experienced a relapse after a mean of 5.0 months (range 0.3-18 months). This may indicate that the used antibiotics do not cure the disease but relieve the symptoms. A 5 month disease free period does not look long but for these chronic HS patients it was quite a relief. The number of patients experiencing side effects in this study was quite high, 13 of 34 patients (38.2%) making 9 of 34 patients (26%) to discontinue this regimen. This side effect rate was higher than Gener et al.[16] who showed that 10 of 70 patients (14%) experienced side effects, making 8 of 70 (11%) to discontinue this regimen. This high incidence of side effects forms the major drawback of this combination treatment schedule and hampers the usefulness in daily practice. Clindamycin usage is associated with the development of *Clostridium difficile* colitis. None of the patients with diarrhoea in this study experienced a *Clostridium difficile* colitis. The mechanism of action of these two drugs in HS needs further elucidation. A significant proportion of HS lesions appear to be sterile[7,8] and anti-inflammatory therapy with e.g. cyclosporine[18], dapson[19], methotrexate[20], prednisone[21] or biologicals[22] has been described as an alternative therapy to antibiotics in HS.

Moreover, besides their bactericidal effects the antibiotics used in the treatment of HS also have immunomodulatory properties[23], suggesting that the latter property is also responsible for their beneficial effect in HS. Rifampicin is a derivative of *Streptomyces mediterranei*. It is a lipid-soluble, broad spectrum antibacterial agent which acts by binding to and inactivating bacterial deoxyribonucleic acid-dependant ribonucleic acid polymerase[24]. It can sterilize staphylococcal abscesses[25] and maintains its bacteriolytic effect even when the targets are engulfed in phagocytic cells[26]. It inhibits the growth of the majority of gram positive bacteria as well as many gram negative microorganisms[27]. Rifampicin is highly active against both *S. aureus* and coagulase negative staphylococci. Furthermore, bacterial resistance can occur rapidly when rifampicin is used as monotherapy. In addition to its antimicrobial effects it also modifies cell mediated hypersensitivity by suppressing antigen-induced transformation of sensitized lymphocytes, and T-cell function[23]. Clindamycin is the chlorine substituted successor to lincomycin. It binds to the 50s subunit of the bacterial ribosome and inhibits the early stages of protein synthesis. The antimicrobial effect is primarily bacteriostatic. It is active against gram-positive cocci except enterococci and most anaerobic bacteria[28]. Like rifampicin, clindamycin has the potential to modify or suppress inflammation. It suppresses the complement-derived chemotaxis of polymorphonuclear leukocytes in vitro, reducing inflammation[29]. In addition rifampicin and clindamycin have effective bactericidal action when given together[30]. HS is a notoriously difficult to treat disease, and the encouraging results of this retrospective case series and that of Gener et al.[16] emphasize the need for a large prospective, dose finding, randomized controlled clinical trial.

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4.2

Deroofing: A tissue-saving surgical technique for the treatment of mild to moderate hidradenitis suppurativa lesions

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ABSTRACT

Background: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease, often refractory to treatment. HS patients as well as dermatologists are in need of an effective, fast surgical intervention technique. Deroofing is a tissue saving technique, whereby the "roof" of an abscess, cyst or sinus tract is electro-surgically removed. The use of a probe is mandatory in order to explore the full extent of a lesion.

Objective: To evaluate the efficacy and patient satisfaction of the deroofing technique for recurrent Hurley 1 (mild) or Hurley 2 (moderate) graded HS lesions at fixed locations.

Methods: An open study consisting of 88 deroofed lesions in 44 consecutive HS patients, treated by a single clinician with a follow-up time of up to 5 years.

Results: Fifteen out of 88 (17%) treated lesions showed a recurrence after a median of 4.6 months. Seventy three treated lesions (83%) did not show a recurrence after a median follow-up of 34 months. The median patient satisfaction with the procedure rated 8 on a scale from 0 to 10. Ninety percent of the treated patients would recommend the deroofing technique to other HS patients. One side effect occurred in the form of post-operative bleeding.

Limitations: Some patients were lost to follow up.

Conclusions: The deroofing technique is an effective, simple, minimally invasive, tissue-saving surgical intervention for the treatment of mild to moderate HS lesions at fixed locations and it is suitable as an office procedure.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic, inflammatory, recurrent, debilitating follicular skin disease that usually presents after puberty with painful deep-seated, inflamed lesions in the apocrine gland-bearing areas of the body, most commonly, the axillary, inguinal and anogenital regions[1]. Prevalence rates of up to 4% have been estimated[2]. This painful disorder deeply affects the quality of life of affected patients[3]. The exact pathogenesis of HS remains largely unknown and is probably multifactorial[4]. The primary mechanism in the disease is thought to be hyperkeratinisation of the pilosebaceous infundibulum resulting in occlusion of the follicle followed by its rupture with subsequent inflammation[5]. To date, HS remains difficult to treat, with often unsatisfactory clinical results. Current pharmaceutical options include topical or oral antibiotics, hormonal therapies, anti TNF-alpha biologics[1] and topical resorcinol[5]. Retinoids in the form of oral isotretinoin have limited clinical efficacy[1]. In addition to pharmaceutical drugs, surgical interventions are often used. Surgical interventions include incision with drainage, limited excision using cold steel[6] or electro surgery and CO_2 laser evaporation[7,8]. Sometimes large skin areas are excised *en bloc.* Defects may be sutured, closed with grafts, local or distant flaps or left open for healing by secondary intention. Rigorous surgery is considered to be more effective than treatments with drugs and conservative surgery. The treatment of choice depends on severity, disease course and the wishes or preferences of the patient. In chronic, severe cases, surgery is considered mandatory[1]. In mild and moderate HS, patients may benefit from topical or systemic treatment, surgery, or not uncommon, a combination of both. Treating the disease at an early stage is considered essential as delay in treatment could lead to a situation whereby disease activity has got out of control making wide surgical excision necessary. There is need for an effective and fast surgical technique, other than simple incision and drainage, which is suitable as an office procedure. We therefore propose the deroofing technique. This technique converts, with limited surgery and maximal preservation of the surrounding healthy tissue, painful recurrent lesions into cosmetically acceptable scars. The deroofing technique was first described by Mullins[9] as early as 1959. Ever since, deroofing has been mentioned in many reviews and dermatology textbooks but its efficacy has never been properly investigated. Nevertheless dermatologists in The Netherlands adopted this technique and it is now widely used and yielding positive results, which were mostly published as case reports[10-12]. Therefore, the aim of this study was to evaluate the efficacy and patient satisfaction of the deroofing technique in a larger population of patients with HS. Here, we report the results of an open study on the deroofing intervention in 44 patients with Hurley grade I and II, with a follow-up of up to 5 years.

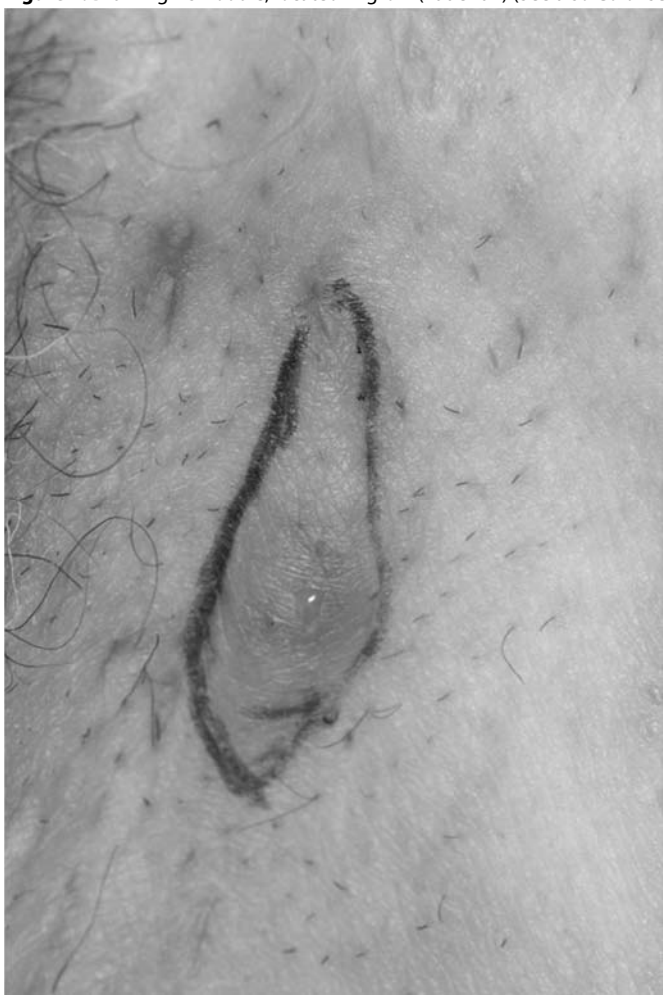
PATIENTS AND METHODS

Forty-four consecutive HS patients were treated in an open trial with the deroofing technique in the outpatient department of Dermatology, Deventer Hospital in the period 2003-2007. The criteria used to establish the diagnosis of HS were: Presence of typical lesions, i.e. deep-seated painful nodules, abscesses, draining sinuses, bridged scars and 'tombstone' open comedones in secondary lesions. Typical topography, i.e. axillae, groin, perineal and perianal region, buttocks, infra and inter mammary folds and a history of chronicity and recurrences[1]. The physical examination, the decision to perform deroofing intervention and follow-up were all done by the same investigator (JB). Descriptive staging of the disease severity according to Hurley was made in all patients[13]. Stage 1: Abscess formation, single or multiple, without sinus tracts and cicatrization. Stage 2: Recurrent abscesses with tracts and scar formation single or multiple, widely separated lesions. Stage 3: Diffuse or near diffuse involvement or multiple interconnected tracts or abscesses across the entire area. The following questions were always asked: Are recurrences of the HS lesions located at exactly the same "fixed" locations? at various locations? or a combination of both. Lesions at fixed locations and that were assessed as Hurley 1 or 2 were considered suitable for deroofing. All patients gave their informed consent. Age, age at onset of the disease, sex, BMI, treated area, length of the created defect, patient-reported healing time, complications and recurrences at follow-up were recorded and monitored. All patients were initially seen 6 weeks after deroofing, patients were asked the number of days required for complete closure of the surgical defect. Recurrence of a deroofed lesion was clinically assessed and asked at each follow-up visit. A recurrence was defined as an inflammatory boil in, or less than 0.5 cm adjacent to the scar. The patients were interviewed by telephone in 2008 or 2009 for their views on the long term outcomes of the deroofing treatment. The questionnaire consisted of the following questions: "On a scale of 1-10 how satisfied are you with the treatment (0, very dissatisfied; 10, very satisfied)"? Would you recommend this treatment to other patients with HS? The telephone interview was conducted by one of the authors (HZ).

The deroofing technique

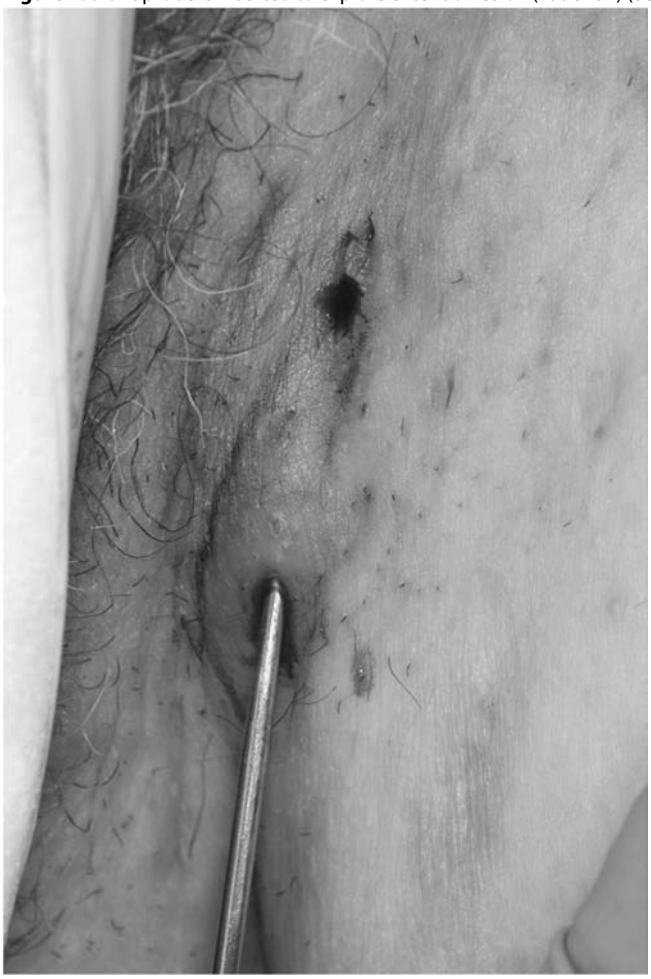
Pre-operatively HS lesions to be deroofed were identified by visual inspection and palpation, and were marked with ink (Figure 1). The skin was disinfected with 0.05 mg/ml chlorohexidine solution. Local anaesthesia was performed in two steps: First, anaesthesia solution, lidocaine 1% (10 mg/ml) plus adrenaline 1:2000.000 (5 µg/ml) (AstraZeneca, Södertälje, Sweden) was injected to infiltrate the surrounding area. Secondly, the nodule and/or sinus tract was also injected with the same local anaesthetic. The latter procedure provides good demarcation of the extent of the lesion. Because the anaesthesia injections were experienced as painful, in some cases lidocaine prilocaine cream (EMLA, AstraZeneca, Södertälje, Sweden) was applied one hour before the injections. For electrosurgical cutting, the Erbotom ICC50 (Erbe

Figure 1. Draining HS nodule, located in groin (Patient 1) (See also Color section, p. 205.)



Surgical systems, Marietta, GA,) operating at 35 W, with a manually controlled hand-piece fitted with a loop was used. A hyfrecator with a sharp tip and used in the fulguration mode would probably give a comparable effect. A blunt probe was inserted in sinus openings that were discharging purulent exudate. In case openings were not detectable a small incision was made to introduce the probe. The lesion was then explored with the probe in all directions in order to find and explore all communicating tracts (Figure 2). It was common to find considerable areas of undermined skin with tracks sometimes running at different depths (Figure 3). Care was taken not to create false passages with the probe. In case a blunt probe is not available the blunt tip of a closed, fine forceps or 'mosquito' could be used as a probe. Then the roof of the lesion was surgically removed using the probe as a guide, leaving the floor of the lesion exposed (Figure 4). The walls were carefully probed again for other

Figure 2. Blunt probe is inserted to explore extent of lesion (Patient 1) (See also Color section, p. 205.)



remaining communicating sinus tracts, in order not to miss those tracts. The gelatinous and sanguinolent material on the floor of the exposed and inflamed lesions was carefully scraped away with a disposable curette. The created defects were left open for healing by secondary intention. Postoperative wound care consisted of once daily application of mupirocine ointment in the defect, together with a non-adhering (Mepitel®) dressing that served as a wedge to keep the wound open, for five days. Thereafter the patient switched to application of daily dressings consisting of iodine ointment containing gauzes (Betadine®) until the defect was closed. Patients were instructed to rinse the defect twice daily using the shower. Generally the defects healed into cosmetically acceptable scars. (Figure 5)

Figure 3. Sinus tracts can be unexpectedly long (Patient 2) (See also Color section, p. 205.)

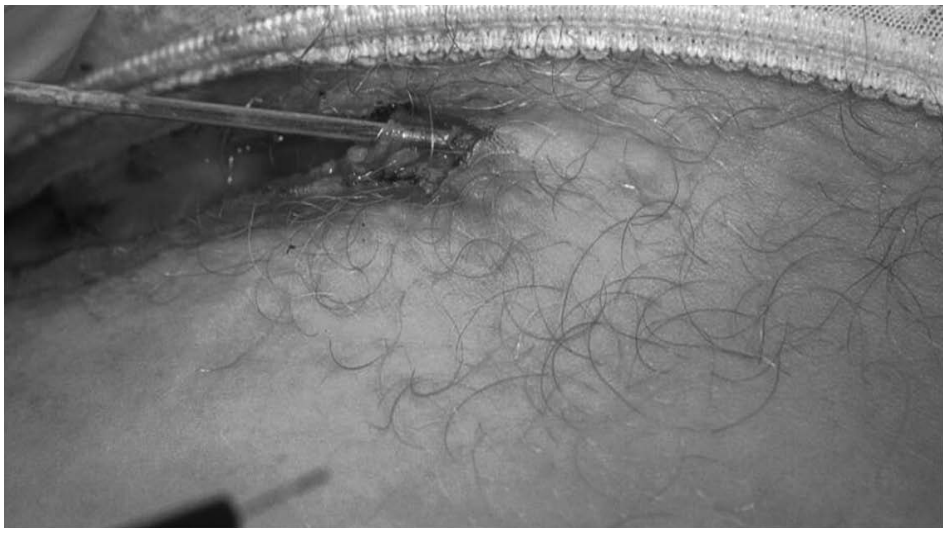


Figure 4. The roof of the lesion is removed and left for healing by second intention, after removal of debris by curette (Patient 2) (See also Color section, p. 206.)

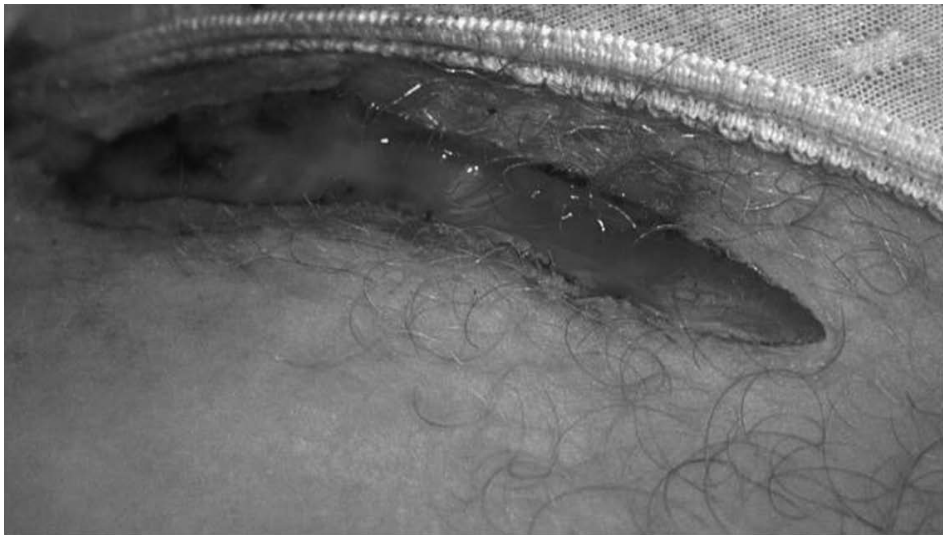
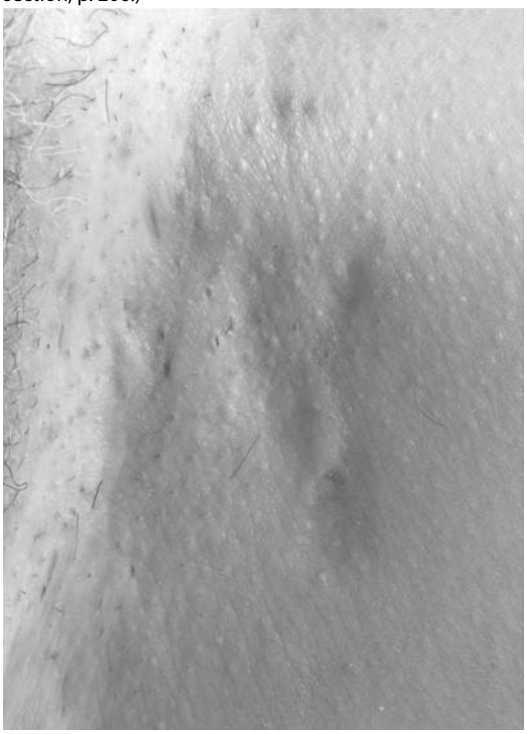


Figure 5. Lesion healed into a cosmetically acceptable scar. Result after 1 year (patient 1) (See also Color section, p. 206.)



RESULTS

Forty-four HS patients, 3 males and 41 females, with in total 88 lesions were deroofed during the investigated period. No deroofed patients were excluded from the study. Patient characteristics are given in Table 1. All patients had a history of active long-standing HS. The median age of disease onset was 28 years. The median age at time of deroofing was 35 years with a median BMI of 26.8.

Lesion characteristics are given in Table 2. Most of the deroofed lesions, 41 (47%), were located in the groin followed by the axillae, 39 lesions (44%). Eight treated lesions were located on the buttocks. The mean length of the created defect was 3.0 cm. The mean healing time was 14 days. Fifteen out of 88 (17%) deroofed lesions showed a recurrence, after a median

Table 1. Patient characteristics

Patients, n = 44 (41 female, 3 male)	Median (interquartile range)
Age of disease onset, y	28 (20-37)
Age, y	35 (28-43)
Body mass index	26.8 (22.3-30.9)

Table 2. Characteristics of treated lesions. *Mean with SD

Treated lesions	n = 88
Location of treated lesion	Axillae 44.3%
	Groin 46.6%
	Buttocks 9.1%
Size of defect directly post-operatively, cm	3.0 ± 1.7* (range 1-10)
Healing time, d	14.1± 7.8* (range 2-35)

of 4.6 months (interquartile range 1.2- 6.2). Seventy three deroofed lesions (83%) did not show a recurrence after a median follow-up of 34 months (interquartile range 24-44). One complication occurred in the form of a post-operative bleeding. No infections were observed, also no impairment of movement occurred due to post-operative scarring. Thirty-seven out of 44 patients (84%) were contacted and interviewed by telephone, 7 patients could not be traced by any means and were considered lost to follow up. The median satisfaction rate for deroofing was 8.0. Patients without a recurrence evaluated the technique higher than patients with a recurrence, 8.0 versus 7.0 respectively. Ninety percent of treated patients would recommend the deroofing technique to other HS patients (Table 3). Interestingly, patients with a recurrence recommended the procedure almost as frequent as patients without a recurrence: 92% vs. 82%.

Table 3. Patient satisfaction with deroofing procedure. *Median and interquartile range

	All patients n=44	No recurrence n=29	Recurrence n=15
Satisfaction score (0 to10)	8 (7-9)*	8 (7-9)*	7 (4-8)*
Score 6	16%	8%	36%
Recommending deroofing to other patients	90%	92%	82%

DISCUSSION

The treatment repertoire for HS consists of medical (topical, systemic) and surgical interventions. Early intervention is considered mandatory in order to prevent disease activity getting out of control with consequences such as fibrosis, scarring and sinus tract formation causing therapy resistance and major quality of life impairment. Several surgical interventions have been described for HS often with considerable recurrence rates. The reported recurrence rates after incision with drainage are up to almost 100%, for limited excision using cold steel 43% and for wide excisions 27%[14]. Here we show that after a median follow up of 34 months from 88 deroofed lesions 83% did not recur. Systematic comparison of studies is hampered by variable definitions of recurrence. The deroofing technique, as used in this study, was first described in 1959 by Mullins et al.[9] In 1983 Culp et al.[15] reported on deroofing in a group of

30 patients with HS of the anal region. Unfortunately recurrences and follow-up were not studied and the diagnosis of HS was questionable. However, a novelty was that in contrast to Mullins[9], Culp[15] left the floor of the exposed lesions untouched. The authors stated that preservation of the exposed lesion floor was essential for its epithelial regenerative elements. In 1986 Brown et al.[16] presented three HS cases treated with deroofing. In their opinion, like Culp[15], epithelial cells from sweat glands and hair follicles remnants were present in the debris and at the floor of the exposed lesion which could rapidly re-epithelialize the defect. We agree with Mullins et al.[9] that the debris on the floor should be removed, because keratinous debris or viable epithelial remnants could get entrapped deep within the dermis when not removed and may cause recurrence[8]. It is known that other sites for recurrences are irradically excised lesions, which appear to be the major cause for recurrence after conventional surgery[17]. Excision of HS lesions should be done "*en bloc*" with all its communicating sinus tracts. Therefore we argue that the use of a probe is mandatory. Ninety percent of our deroofed patients would recommend deroofing to other HS patients. This was the same percentage as Madan et al.[7] who treated 9 HS patients with the CO_2 laser. They achieved a total clearance in 7 out of 9 patients (78%) after a 12 months of follow-up, and a patient satisfaction rate of 8.5 in contrast to 8.0 with deroofing. In comparison with our method, Madan et al.[7] treated all HS lesions in one session under general anaesthesia. In contrast to deroofing, CO_2 laser treatment is more time consuming, rather expensive and must be performed by experienced hands. Recently Aksakal and Adişen[18] reported on the use of electro surgery for HS areas assessed as Hurley 1 and 2. Thirty lesions in 12 patients were treated. The efficacy of the technique was measured by post-operative wound infection, recurrence rate was however not reported. In comparison to the deroofing technique, Aksakal and Adişen[18] did not use a probe. This approach resembles normal excision with cold steel and is therefore less tissue-saving. Due to the pathophysiology of HS, new lesions can always occur in the predisposed HS areas. Some HS lesions spontaneously resolve, never to come back at the exact location. Another type of HS lesion stays at exactly the same location, is a non-tender nodule when in remission, but can flame up periodically. This last type of lesion is especially suited for deroofing. Deroofing of such lesions can be applied both when it is inflamed or in remission. Lesions in Hurley 3 areas are not deroofed in this way in our clinic, since we argue that deroofing does not add any advantages in these cases. In our opinion these areas can only be treated with radical wide excision, mostly under general anaesthesia. Because the deroofing technique does not take much time, our HS patients are generally deroofed during the same consultation in which they present with their lesions. We argue that early intervention with deroofing can prevent disease aggravation, and so prevent radical surgery. The deroofing technique generally makes small defects so no general anaesthesia is needed. In addition, single-lesion surgical treatment offers low morbidity and moreover the chance of creating unacceptable cosmetically results is clearly diminished and scar contractures are prevented. This is supported by the high patient satisfaction rate and recommending rate. Due to the

use of the electro surgical loop good haemostasis is achieved, allowing good visualisation of the operative area. In conclusion the deroofing method is easy to conduct and cheap. We provide evidence that it is safe to conduct with prolonged effectiveness for recurrent HS lesions at fixed locations in Hurley 1 or 2 areas. We argue that the deroofing technique is superior over incision with drainage and simple excisions with cold steel.

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4.3

Thea nti-inflammatory drug colchicine lacks efficacy in hidradenitis suppurativa

HH van der Zee and EP Prens

Dermatology in press

ABSTRACT

Background: Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease. Because current treatments are unsatisfactory for many patients, there is a high need for effective drugs for this debilitating disease. Recent pathogenic insights suggest inflammasome activation and IL-1β production are important in HS. Colchicine is efficacious in the IL-1β- and inflammasome-mediated diseases gout, familial Mediterranean fever and Behçet's disease, and therefore a potentially effective drug in HS.

Objective: To investigate the efficacy of colchicine in HS.

Methods: In an open prospective pilot study, 8 HS patients were treated with the accepted gout maintenance regimen of 0.5 mg colchicine b.i.d. orally up to 4 months. Efficacy was assessed by a physician global assessment.

Results: Colchicine treatment did not result in a clinical relevant improvement of disease severity. Three patients experienced nausea and diarrhea as known side effects.

Conclusion: Colchicine in the used dose regimen does not ameliorate HS severity.

INTRODUCTION

Hidradenitis suppurativa (HS) is an inflammatory, debilitating follicular skin disease that usually presents after puberty with painful deep-seated, inflamed lesions in the axillary, inguinal and anogenital areas of the body. HS is not uncommon with prevalence rates ranging between 1% and 4% in European countries[1,2]. It significantly compromises quality of life in affected patients, even more than alopecia, chronic urticaria, psoriasis or atopic dermatitis[3,4]. Evidence-based therapies for HS are scarce, making HS difficult to treat. Moreover, the chronic and often refractory nature makes HS treatment even more challenging.

Randomized controlled trials in HS are scarce. Topical clindamycin was superior to placebo[5]. Oral tetracycline was equally effective as topical clindamycin[6]. Oral contraconceptives were shown to improve HS to some degree in women[7], Infliximab and adalimumab both demonstrated significant improvement[8,9] and also 3 monthly sessions with the Nd:Yag laser improved HS[10].

Other relative successful treatment options include combination therapy of clindamycin and rifampicin[11], in milder cases topical resorcinol[12] and the surgical deroofing method[13]. Oral isotretinoin showed very limited efficacy in HS[14] but acitretin demonstrated improvement by physician global assessment in 12/12 patients[15].

Our knowledge of the pathomechanisms underlying HS is still in its infancy. It has been suggested that HS is an auto-inflammatory disease[16] with comparable pathologic mechanisms as diseases such as gout, familial Mediterranean fever (FMF) and Behçet's disease. HS is probably the result of a mixture of intrinsic and environmental factors in a genetically predisposed individual. It is thought that the disease starts with occult skin inflammation causing follicular infundibular keratosis[17]. The hair follicles become blocked, and cyst formation ensues. Abscesses and inflammatory nodules acutely manifest when these occluded and dilated hair follicles or cysts rupture. This event subsequently causes an inflammatory abscessing response in an attempt to clear the foreign material such as keratin fibers and commensal bacteria. Recently we demonstrated that IL-1β secretion is significantly increased in HS lesions which strongly suggests the involvement of the IL-1β/inflammasome pathway[17]. IL-1β and the inflammasome play a crucial role in gout, FMF, Behçet's disease and other autoinflammatory diseases[18,19].

We argue that the spilled keratin fibers in HS trigger an inflammasome-mediated inflammatory response similar to the activation of the inflammasome by uric acid crystals in gout[18]. Colchicine is a well-recognized valid therapy for gouty arthritis, FMF and Behçet's disease[20,21]. Thus we hypothesized that colchicine could improve HS inflammation by reduction of inflammasome activity.

MATERIALS AND METHODS

In an open prospective pilot study the efficacy of 0.5 mg colchicine b.i.d. (twice a day) was investigated in 8 patients with moderate to severe HS. Informed consent was obtained. All included patients had previously been refractory to several other HS treatments including oral antibiotics, oral anti-conceptives, isotretinoin, resorcinol and even surgical deroofing or wide excisions. Thus colchicine was used as a last resort. All included patients had active disease for at least a year. After 1 month, in case of insufficient improvement, patients were offered a dose escalation to 0.5 mg t.i.d. (three times a day). Baseline characteristics including, age, previous treatments, BMI, smoking habit and disease staging according to Hurley are presented in Table 1. Blood samples for monitoring of routine lab parameters such as liver and kidney function as well as hematological markers, were collected prior to initiation of treatment and at each visit. At each visit, patients were asked about side effects and clinical photographs were taken. The outcome was assessed by the same investigator (HZ) using a physician global assessment (PGA) compared to baseline -2 corresponded with clear worsening, -1 slight worsening, 0 no change, 1 slight improvement, 2 clear improvement en 3 total clearance of inflammatory lesions.

Table 1. Patient characteristics and outcomes by PGA score

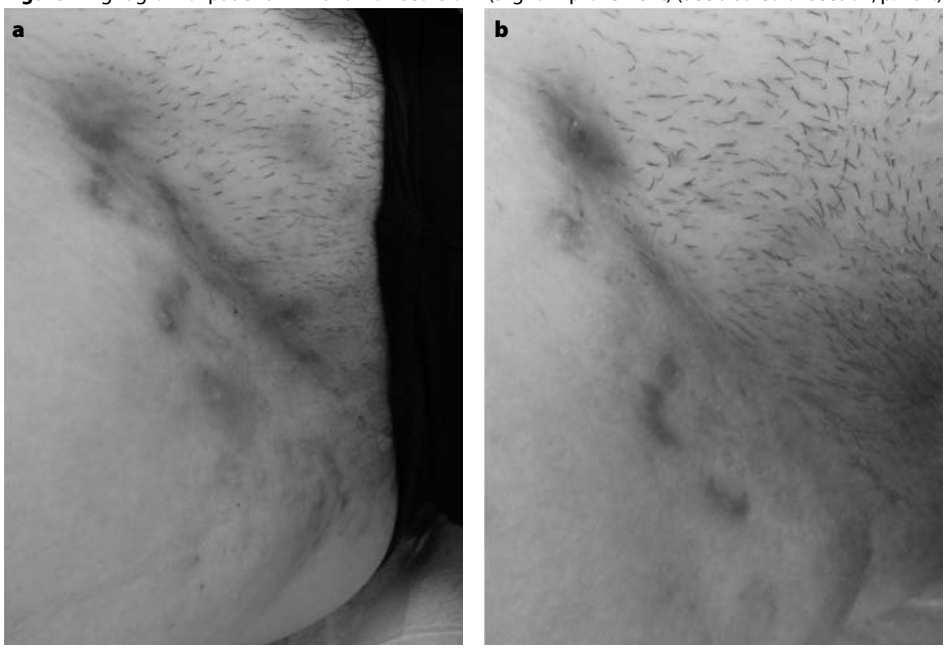
Patient #	Gender	Age	BMI	Hurley stage	Current smoker	Previous treatments	1 month PGA	2 month PGA	4 month PGA
1	♀	24	31.2	2	no	AB, resor, OC	0	1*	1*
2	♀	27	29.1	1	yes	AB, deroof, resor, OC	0	0	-
3	♀	37	26.2	1	yes	AB, deroof, resor, roac, OC	0	-	-
4	♂	54	27.4	2	no	AB, deroof, resor, roac	0	0	-
5	♂	34	25.7	3	yes	AB, surg, resor	0	-1*	-
6	♀	19	24.2	3	yes	AB, surg, resor, OC	1	-	-
7	♀	58	20.0	3	yes	AB, surg, resor	1	1	-
8	♀	46	29.8	3	yes	AB, surg, resor	0	1	-1

AB: oral antibiotics, deroof: deroofing, OC: oral contraceptive, resor: topical resorcinol, surg: wide excisions, roac: isotretinine, * 0.5 mg colchicine t.i.d.

RESULTS

Outcomes by PGA assessment are presented in Table 1. After 1 month, 2/8 patients experienced slight improvement of their symptoms whereas 6/8 experienced no change. Six patients completed 2 months of treatment. Three of six patients experienced slight improvement; one patient deteriorated and two patients showed no change. Two patients were able to tolerate the increased dosage of 0.5 mg t.i.d. Only two patients were treated for 4 months; one patient slightly improved the other one deteriorated compared to baseline (Figure 1). Three patients experienced some level of nausea and diarrhea as a side effect, for patient #3

Figure 1 Right groin of patient #1 with a PGA score of 1 (slight improvement) (See also Color section, p. 207.)



a) at baseline; **b**) at 4 months

this was the argument to stop colchicine treatment after one month. Other patients dropping out of the study reported lack of efficacy as the argument for cessation. No patient experienced clinically significant changes in blood parameters during treatment.

DISCUSSION

Colchicine has been used in medicine for a long time and is a natural product that can be extracted from plants of the lily family[20]. Colchicine is lipophilic and is absorbed in the jejunum and ileum, and it is excreted via the bile tract and kidneys[20,22]. The bioavailability ranges between 24% and 88% and steady state plasma levels are reached within 8 days[20]. Colchicine has a narrow therapeutic to toxicity window. The therapeutic plasma levels are achieved by intake of 0.015 to 0.03 mg/kg which generally corresponds with 1-2 mg colchicine per day[20]. The narrow therapeutic-toxicity window and the interindividual bioavailability variation make colchicine clinically difficult to titrate. The therapeutic mechanism of action of colchicine is not fully understood. Colchicine inhibits microtubule polymerization inhibiting several cytokine signalling pathways[22]. In gout, colchicine suppresses inflammasome-driven caspase-1 activation, IL-1β processing and release[18]. Colchicine also accumulates in neutrophils[23], inhibiting neutrophil expression of cell adhesion molecules and decreasing neutrophil

degranulation, chemotaxis and phagocytosis[20]. Its efficacy in neutrophilic auto-inflammatory diseases is attributed to its effect on neutrophils in addition to the inhibition of the inflammasome[20]. Since colchicine is metabolised through the CYP3A4 and P glycoprotein system, avoidance of drug interactions is essential. For example macrolides used for treating HS such as clarithromycin inhibit P-glycoprotein and CYP3A4 and therefore likely impair colchicine elimination[20]. Although antibiotics are frequently used to manage HS, the patients in this study did not use any concomitant antibiotics.

In gout, colchicine is used as an intervention therapy and as a maintenance therapy. The treatment of gout flares recommended by the FDA is 1.2 mg at onset followed by 0.6 mg in 1 h[24]. The maintenance therapy of gout is between 0.5 and 1.0 mg daily[24]. For treatment of FMF and Behçet's disease only a maintenance therapy is used of an oral dosage of 1-2 mg per day[20]. The therapeutic efficacy of colchicine is normally seen within one week of treatment.

We administered 1 mg of colchicine daily and patients #1 and #5 received 1.5 mg daily after the first month. The lack of efficacy in HS might be explained by initial underdosing. Perhaps a higher intervention/start dose as in gout for the first day followed by the maintenance dosage of 1 mg daily would improve efficacy. However, data from auto-inflammatory diseases have shown that five percent of patients with either FMF or Behçet's disease do not respond to a daily dose of 2 mg of colchicine[20]. We argue that the observed variation of HS disease activity in time is due to the natural course of the disease rather than induced by colchicine.

Common side effects of colchicine are of gastro-intestinal nature; such as nausea and diarrhea. In this study 3 patients reported some level of nausea and diarrhea. Even higher side effect rates are to be expected when a higher dose will be used.

The plasma level of colchicine was not measured, so it is unknown if the patients reached therapeutic colchicine levels. However the occurrence of side effects indicates that sufficient levels had been reached.

A limitation of this pilot study is the small sample size of only 8 patients and the fact that selection of patients was restricted to patients refractory to other regular therapies.

The efficacy of an intervention dose of colchicine should be studied in a larger patient cohort. We suggest that colchicine treatment should start with 1 mg followed by 0.5 mg every 2 hours with a maximum of 5 mg cumulative, followed by 1 mg daily. The plasma levels of colchicine should be monitored and the dose should be adjusted accordingly. In Behçet's disease the addition of pimecrolius cream increases the efficacy of colchicine, although not significantly[21]. It would be worthwhile to also investigate the addition of pimecrolius cream to colchicine in HS in a randomized controlled trial. Furthermore since IL-1β secretion is significantly increased in HS lesions[17] other drugs modulating the IL-1 pathway such as Anakinra (an IL-1 receptor antagonist) and Canakinumab (a human monoclonal antibody targeting IL-β) are potential treatment modalities for HS as well.

In conclusion, this prospective pilot study suggests that treatment with colchicine 0.5 mg b.i.d. does not result in a significant clinical improvement in HS disease activity.

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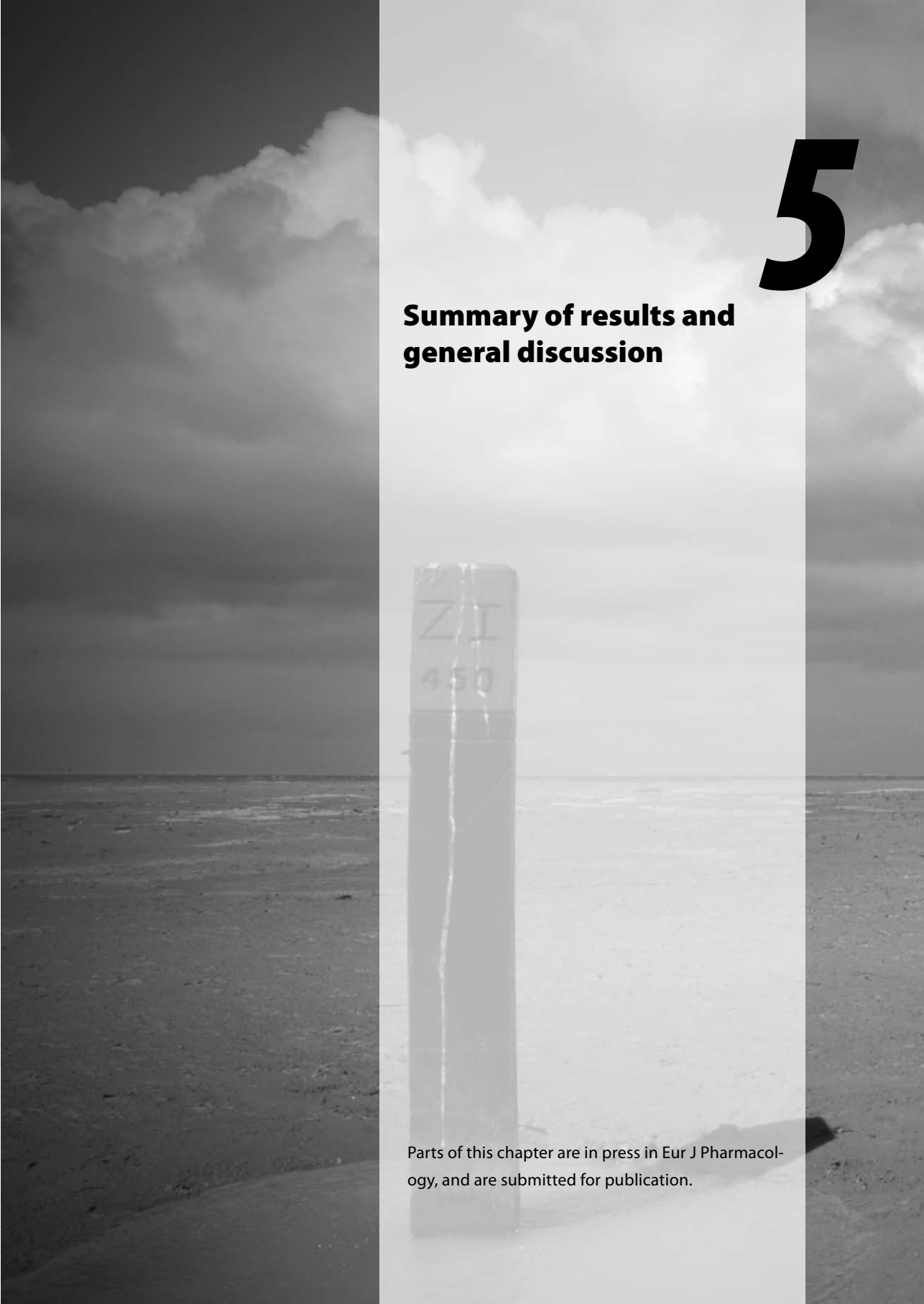
5

Summary of results and general discussion



Parts of this chapter are in press in Eur J Pharmacology, and are submitted for publication.

Hidradenitis suppurativa (HS) was, until recently, a scientifically underestimated and clinically poorly recognized disease, despite its prevalence, severity and its high unmet medical need. The aim of this thesis was not restricted to a single aspect of the disease, but instead took a broad approach on the aetiology, pathomechanisms and treatment.

In this chapter we first recapitulate the specific aims of this thesis. Second, we summarize the key results and present the main conclusions in Box 1. Third, the lack of an animal model for HS is discussed. Fourth we systematically discuss the topics addressed in this thesis and present a comprehensive view on HS pathogenesis. This chapter finishes with future perspectives on HS research.

The specific aims and research questions of this thesis were the following:

Clinical aspects of hidradenitis suppurativa

Awareness about the disease severity and impact on quality of life is necessary to increase scientific interest. We hypothesized that depression is more common in HS patients than presently appreciated.

There are striking clinical similarities between HS and Crohn's disease. Demonstration of an association may imply that parallel or similar pathogenic mechanisms contribute to both

Box 1. Main conclusions of this thesis

Clinical aspects of hidradenitis suppurativa

- HS has a major impact on quality of life compared to other dermatological diseases. Depression scores in patients with HS correlate with disease severity scores.
- HS is probably associated with IBD and vice versa. HS occurs in about 15% of patients suffering from Crohn's disease or ulcerative colitis.
- We argue that the presence of apocrine sweat glands in HS-predisposed sites is based on coincidence. Overlapping characteristics between HS predisposed sites include mechanical friction and a warm humid microclimate, permissive to bacterial growth.

Pathomechanisms in hidradenitis suppurativa

- Secreted levels of IL-1β, TNF-α and IL-10 protein are highly elevated in lesional HS skin as well as in normal appearing perilesional skin.
- Psoriasiform hyperplasia, follicular plugging and low-grade leucocyte infiltration are present in normal appearing perilesional skin and may precede clinical disease. Detached keratinocytes and strands of follicular epithelium may represent the first steps in fistula formation.
- Adalimumab treatment inhibits important cytokines and inflammatory cell numbers in lesional HS skin, especially CD11c+ dendritic cells and IL-1β levels.

Treatment of hidradenitis suppurativa

- Combination treatment with oral rifampicin and clindamycin is an effective treatment option for HS, despite the frequent occurrence of diarrhoea as a side effect.
- The surgical deroofing technique is an effective, simple, minimally invasive, tissue-saving intervention for the treatment of single or isolated mild to moderate HS lesions at fixed locations.
- Colchicine in the 0.5 mg b.i.d. dose regimen does not ameliorate HS severity.

diseases. We investigated whether HS occurs more frequently in Crohn's disease patients than in the general population.

Why HS preferable affects the inverse skin areas is unknown. We present a case that provides clues about the contribution of friction.

Pathomechanisms in hidradenitis suppurativa

Characterization of the HS cytokine profile can provide a basis for development of novel therapies. We investigated whether secretion of prototypical pro-inflammatory cytokines such as TNF-α and IL-1β is elevated in HS skin.

Anti-TNF-α biologics show promising efficacy in HS, but the underlying mechanisms are largely unknown. We investigated whether specific cytokines or inflammatory mediators were significantly affected by treatment with the anti-TNF-α biologic adalimumab.

We showed that levels of TNF-α, IL-1β and IL-10 are increased in HS lesions and normal appearing perilesional HS skin. We therefore investigated the possible cellular source of these cytokines.

Treatment of hidradenitis suppurativa

Since antibiotic therapy is the most common treatment for HS, we addressed whether combination therapy with clindamycin and rifampicin is effective in HS.

Deroofing is a surgical method for HS, but its efficacy has not been systematically investigated. We hypothesized that deroofing would be effective in recurrent single lesions of HS.

Colchicine is a drug with anti-inflammatory properties, and is successfully used in autoinflammatory diseases. In a pilot study we investigated the efficacy of colchicine in patients with HS.

MAIN RESULTS

The quality of life of HS patients, as assessed by the Dermatology Life Quality Index (DLQI), was significantly reduced compared to control patients $p<0.0001$ (8.4 ± 7.5 versus 4.3 ± 5.6). Depression scores, as assessed by the Major Depression Inventory (MDI), were significantly higher for HS patients, 11.0 versus 7.2 ($p<0.0001$). However, actual depression rates as defined by the International Classification of Diseases-10 (ICD-10) criteria were not significantly higher in HS patients compared to controls (9% versus 6%).

In a group of 158 patients with inflammatory bowel disease (64.6% Crohn's and 35.4% ulcerative colitis patients), 25 patients (15.8%) (7 with Crohn's and 8 patients with ulcerative colitis) reported the occurrence of painful boils in the axillae and/or groin suggestive for HS.

We described a patient with inflammatory lesions, strikingly similar to HS, at an ectopic location free of apocrine sweat glands and induced by wearing of a leg prosthesis.

Secretion of the cytokines IL-1β, TNF-α, CXCL9 (MIG), IL-10, IL-11, BLC (B-lymphocyte chemoattractant) and IL-17A as well as the anti-inflammatory cytokine IL-10 were significantly elevated in HS skin. Elevated levels of IL-1β (31-fold), TNF-α (5-fold) and IL-10 (34-fold) were also found in perilesional HS skin. Levels of the latter three cytokines showed a weak positive correlation with disease severity according to the Hurley stage. Remarkably, IL-2, IL-4, IL-5 and IFN-γ were almost undetectable in HS skin.

Treatment with adalimumab resulted in decreased cytokine production in HS skin, especially IL-1β, CXCL9 (MIG), BLC, and a significantly reduced number of inflammatory $CD11c^+$ dendritic cells (DC), $CD14^+$ monocytes and $CD68^+$ macrophages in HS lesional skin.

Histologically, in early HS lesions, neutrophilic abscess formation and influx of mainly macrophages, monocytes and DC predominate. In chronic disease, additionally increased numbers of $CD20^+$, $CD79a^+$ B cells and $CD138^+$ plasma cells are seen. Free keratin fibers were detected in the dermis and within giant cells in all phases of HS inflammation. Single detached keratinocytes and strands of actively proliferating follicular epithelium were observed in the dermis. Perilesional skin showed mild psoriasiform hyperplasia and follicular plugging as well as a low-grade influx of $tryptase^+$ mast cells, $CD3^+$ T cells, $CD138^+$ plasma cells and $FXIIIa^+$ dendritic cells.

Combination therapy with clindamycin and rifampicin resulted in improvement in 82% of patients including a temporary complete remission in 47%. The maximum effect was seen within 10 weeks. Following complete remission, 62% of patients treated with a 10-week schedule experienced a relapse after a mean of 5.0 months. Non-responders were predominantly patients with severe disease.

The surgical deroofing technique yielded a recurrence rate of 17% that appeared after a mean of 5 months after surgery. Eighty three percent of deroofed lesions did not show a recurrence after a median follow-up period of 34 months.

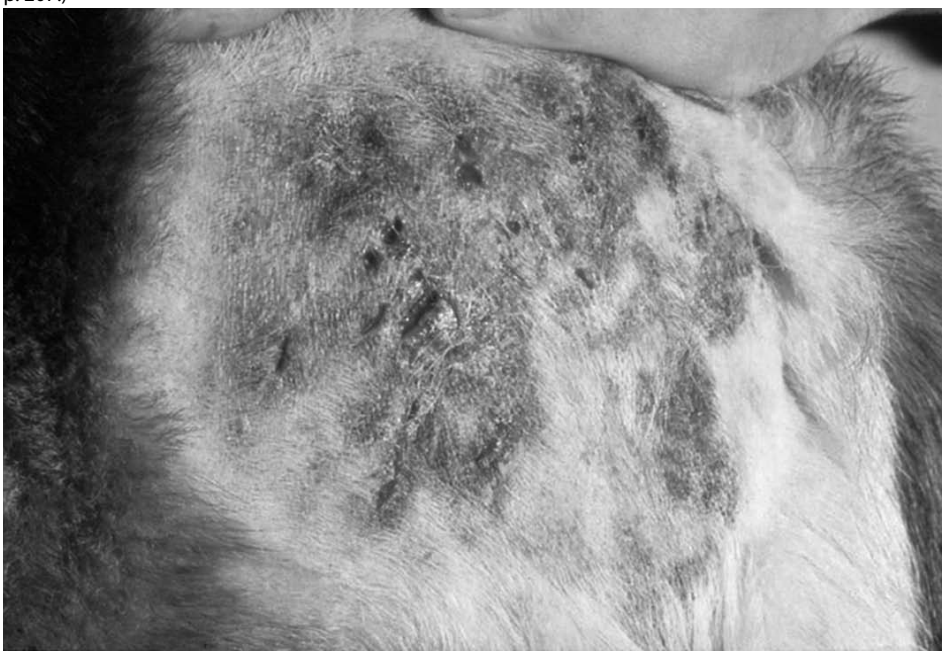
Treatment with the TNF-α antagonist adalimumab showed a better clinical response at week 16 in patients receiving weekly injections versus injections every other week (17.6% *vs*. 9.6%). A decline in the response rate was seen following the switch from weekly dosing to every other week.

Colchicine treatment, in a dose of 0.5 mg b.i.d. orally for up to 4 months, did not result in a clinical relevant improvement of disease severity in 8 patients with HS.

Can HS-like disease in animals serve as a model?

An animal model mimicking HS would greatly facilitate the study of pathogenic mechanisms and rational design of therapeutics. Ideally, such an animal model would exhibit the cardinal clinical as well as immunopathological characteristics of HS. However, no such animal model is currently available. To our knowledge 5 cases of spontaneous canine HS have been reported[1,2]. However, these reports were published in 1969 and 1973 when human HS was still considered apocrinitis rather than follicular occlusion. The main criterion for the diagnosis of

Figure 1. The back of a German shepherd dog with GSP (source Dr. M.A. Wisselink) (See also Color section, p. 207.)



canine HS was therefore apocrinitis. Canine HS looked clinically distinct from human HS and was characterized as recurrent suppurative, sharply marginated, erythematous, non-tender warm plaques at the inner thighs, groin scrotum, vulva or axilla. There were no deep-seated nodules or fistulas as in human HS. Histological examination showed polymorphic neutrophil invasion of the apocrine glands. *Staphylococcus aureus* was cultured in all cases. Hence, we argue that this canine disease is probably not comparable to human HS.

Another naturally occurring canine skin disease, closely resembling human HS, is deep pyoderma in German shepherd dogs also known as German shepherd dog pyoderma (GSP) (Figure 1). GSP is rare and closely mimics the clinical aspects of human HS. GSP is characterized by deep seated chronic and inflammatory lesions in the dermis and subcutis resulting in ulceration, fistulisation, necrosis with hemopurulent exudate and oedema[3]. Main histopathological findings include acanthosis, ulcerations and an inflammatory infiltrate consisting of mainly neutrophils and mononuclear cells[3]. Frequently this infiltrate is present around the adnexa with frequent destruction of the hair follicles and sweat glands[3]. GSP commonly starts in the lumbar region. Opportunistic pathogenic coagulase-positive Staphylococci are frequently cultured from the lesions, although these bacteria can also be found on normal canine skin. Like in human HS, particular genetic traits and immunological dysfunctions are the suspected pathogenic factors[4,5]. Like HS, the efficacy of antibiotics and/or glucocorticoids is poor or temporary[3]. Although these similarities are striking, there are also some discrepancies.

The most notable dissonance is the histological lack of follicular plugging with cyst formation and rupture. Furthermore, the skin areas most liable to mechanical stress do not seem to be predisposed areas. Recently, mutations in γ-secretase genes were discovered in familial HS[6,7]. In that study and the corresponding editorial, mouse models of gamma-secretase signalling were proposed as animal models for HS[6,7]. These mouse models would mimic HS and show follicular and epidermal abnormalities. However, these transgenic mice formed follicular cysts but none of these models showed other distinct clinical HS characteristics such as inflammation, abscesses, fistulas and scarring. Moreover, the mice in the model of Pan et al. shed hair, something which is not seen in HS, and they lacked the psoriasiform hyperplasia of the epidermis[8]. The mouse models of Xia et al. and Li et al. spontaneously developed squamous cell carcinomas[9,10]. Squamous cell carcinoma is rare in HS and is considered as a sequela of longstanding inflammation and scarring. The mouse model of Nicolas et al. developed basal cell carcinomas[11], something which is not seen in HS. Hence we argue that there are too many discrepancies between these mouse models and human HS. In conclusion, a valid animal model for HS is currently not available and the many discrepancies between human HS and the possible models are daunting. Therefore the prospects of developing a valid animal model at the short term seem poor.

Does HS lead to depression?

Information on the psychosocial consequences of HS is limited. Compared to other dermatoses such as severe psoriasis and hand eczema, HS causes a greater impairment of quality of life (QoL)[12-16]. Relative depression rates for HS are unknown. In case depression would be a frequent event in HS patients, dermatologists should be attentive and, when needed, consult or refer to a psychiatrist for appropriate help.

In chapter 2.1 we studied depression in HS. We show that the QoL as defined by the DLQI score is significantly worse in patients with HS than in a comparable group of general dermatological patients.

We also show that the depression scores of HS patients are significantly higher than those of other dermatological patients. This suggests that HS represents a heavier burden than other skin diseases. The high depression scores in HS were in agreement with those described by Matusiak et al. who used the Beck Depression Inventory-SF[16].

A limitation of our study was a selection bias. We investigated a hospital-based patient population which restricts generalization of the data.

We used the major depression inventory score (MDI) to study depression. The MDI can be used to diagnose depression using the ICD-10 criteria, but also as a rating scale to measure depression severity[17,18]. The mean MDI scores used as a rating scale were higher in HS patients than the group of general dermatological patients. However, when the MDI was used as a diagnostic tool, the prevalence of clinical depression was not significantly higher in HS patients than in other dermatological patients. This discrepancy might indicate that

the MDI score is a better severity rating tool than a diagnostic tool for depression. It may also indicate that HS patients have developed effective coping skills. The MDI score has not been used before for other dermatological diseases, but comparing HS to the severe neurological disorder amyotrophic lateral sclerosis (ALS), HS demonstrated comparable severe depression[19]. In conclusion, the results of our study indicate that HS has a major impact on quality of life compared to general dermatological patients. Depression scores in patients with HS correlate with disease severity scores. Depression scores may serve as outcome measures in future studies and dermatologists should be aware of and actively search for signs of depression in patients with HS.

Is HS associated with inflammatory bowel disease?

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC)[20].

The occurrence of both Crohn's disease and HS in the same individual has previously been reported in over 37 individual cases[21]. Based on these sporadic case reports an association was assumed in the literature. Yet, in attempts to find a common genetic basis, two limited studies investigating the CD-associated gene CARD15, also known as NOD2, did not demonstrate CARD15 polymorphisms in HS[22,23]. The current pathogenic concept of IBD is that a disbalance in the immune response to commensal bowel flora together with a specific genetic predisposition and other environmental factors trigger IBD[24]. An association between HS and CD may imply that parallel or similar pathogenic mechanisms are operative in both diseases.

In chapter 2.2 we investigated the co-occurrence of HS in IBD patients. The results of this pilot study indicate that HS not only occurs frequently in CD (16.7%), but also in UC (14.3%).

However, the study had some limitations. First, other diseases may also cause painful boils such as furunculosis, lymphadenitis, ectopic Crohn's disease, or granuloma venereum. We reduced the chance of confusion with these conditions by specifically asking for recurrent lesions and showing clinical photographs of prototypical HS lesions. Because perianal cutaneous Crohn's lesions can mimic HS, we did not ask for painful boils in the perianal area in order to avoid confusion. Second, the interviewed patients suspected of having HS were not physically examined to confirm the diagnosis. Third, co-occurrence in a small sample does not necessarily imply an association.

In conclusion, this study suggests that HS occurs not only in Crohn's disease but also in patients suffering from ulcerative colitis.

Is the presence of apocrine sweat glands necessary to develop HS lesions?

In chapter 2.3 we presented a patient who developed HS-like lesions on a leg stump wearing a leg prosthesis. We argue that the lesions on the leg stump in this patient were not compatible with acne mechanica or other known skin problems in leg amputees[25]. Especially the presence of fistulas strongly suggested a HS-like disease. Furthermore, other characteristics conform HS included a lesional culture demonstrating a commensal skin flora rather than

pathogens. In addition the patient was a heavy cigarette smoker, which is a well-known risk factor for HS as it stimulates follicular occlusion[26]. On the other hand, the patient never had recurrent HS lesions in the axilla or groin, nor a family history of HS, which is present in 40% of HS cases[27].

The skin of a leg stump is exposed to mechanical friction and a warm humid climate of the prosthesis. We argue that the presence of apocrine sweat glands in HS-predisposed sites is based on coincidence. Overlapping characteristics between HS predisposed sites include mechanical friction and a warm humid, microclimate promoting bacterial growth. Mechanical stress may promote follicular keratosis/occlusion and rupture of fragile dilated follicles. Furthermore, the microclimate in HS predilection sites favours bacterial growth, which may trigger or worsen HS.

Which cytokines are important in HS skin inflammation?

Little is known about the spectrum and the quantity of cytokines that are produced in HS lesions. Until recently the use of anti-TNF-α biologics in HS was therefore solely based on empirical grounds.

In chapter 3.1 and chapter 3.2 we assessed the ex vivo cytokine production by lesional HS biopsies in a transwell culture system. The cytokine signature of HS might provide clues about activated inflammatory pathways and thereby support development of potential therapeutic targets.

In chapter 3.1 we show that the pro-inflammatory cytokines IL-1β, TNF-α as well as the anti-inflammatory cytokine IL-10 were significantly elevated in HS lesions. Compared to psoriasis, the levels of especially IL-1β, IL-10 but also TNF-α were higher in HS lesions. Fold increases relative to control skin for IL-1β, TNF-α and IL-10 were for HS 31, 5, and 34, and for psoriasis 4, 1 and 2, respectively. IL-1β is a potent pro-inflammatory cytokine, that is produced by many different cell types, but monocytes and macrophages in particular[28]. Biologically active IL-1β is generated in the inflammasome by cleavage of pro-IL-1 by caspase-1 which was formerly known as ICE, an acronym for interleukin-1 converting enzyme[29]. The relatively high level of IL-1β in HS skin is suggestive for involvement of the inflammasome in HS skin inflammation. The inflammasome functions as a cytosolic sensors that respond to diverse types of danger signals such as damage associated molecular pattern molecules (DAMP) and pathogen-associated molecular patterns (PAMP)[30]. Some examples of PAMP and DAMP are bacterial and viral DNA and RNA sequences, host-derived molecules indicative of injury such as extracellular ATP, hyaluronic acid, uric acid crystals and metabolic stress[31]. We argue that several such DAMP and PAMP are abundantly present in HS and that they may activate the inflammasome in three ways. First, by DAMP such as host-derived molecules indicative of tissue destruction and pyroptosis (a highly inflammatory form of cell death), which is supported by the occurrence of pus and scarring in HS. Second, by PAMP, since HS lesions are frequently inhabited by commensal microbiota[32]. And third, by free high molecular weight cornified keratins in

the dermis, spilled by ruptured cysts. We propose these can activate the inflammasome after phagocytosis, in analogy with uric acid crystals in gout[33,34]. Remarkably, HS skin also produces an increased level of IL-10[35]. IL-10 generally has an anti-inflammatory role, limiting the immune response to pathogens and thereby preventing excess damage to the tissue[36]. It furthermore has a crucial role in preventing inflammatory and auto-immune pathologies[35]. IL-10 is expressed by many cell subsets of adaptive immunity and is also expressed by innate immune cells. We speculate that macrophages in HS could be the prime producers of this vast amount of IL-10. It has been shown that bone marrow-derived macrophages stimulated by efferocytosis (clearance of apoptotic neutrophils) are characterised by high IL-10 production and in addition produce TNF-α following re-stimulation with LPS[37]. This corresponds with the inflammatory reaction observed in HS. First, macrophages and neutrophils are present in high numbers in HS infiltrates and abscesses. Second, HS lesions are frequently inhabited by commensal microbiota, therefore it is conceivable that HS macrophages are exposed to LPS and other microbial PAMP. Third, we showed that TNF-α is elevated in HS lesions.

In chapter 2.2 we complement the list of cytokines increased in HS with CXCL9 (MIG), IL-11, BLC, IL-17A, TNF-R2, CCL5 (RANTES), IL-16, IL-6sR, ICAM-1, CCL3 (MIP-1α), TNF-R1 and IL-1ra. Many of these cytokines are chemotactic for a broad spectrum of immune cells. The arrays used contained a panel of cytokines in order to discriminate between a Th1 or Th2 inflammatory profile. Typical Th1 and Th2 cytokines such as IL-2, IL-4, IL-5 and IFN-γ were hardly detectable in HS. Another array that was described in chapter 3.1 again did not detect significant levels of Th1 nor Th2 cytokines. On the basis of these results we conclude that HS is not a prototypically Th1 or Th2 driven inflammatory disease. The high levels of IL-17A and IL-10 indicate that theoretically Th17 and Treg cells play a role in HS pathogenesis. In a highly recent paper increased levels of IL-17 in HS lesions was confirmed by semiquantitative real-time polymerase chain reaction, furthermore a cellular source of this cytokine were $CD4^+$ T helper cells demonstrated by double immunofluorescence[38].

Elevated levels of IL-1β, TNF-α and IL-10 were also detected in perilesional HS skin. This indicates that HS inflammation extends beyond the visible borders of affected lesions. The presence of inflammatory cytokines in perilesional areas can be of clinical importance since high recurrence rates of HS lesions have been reported after surgical excision[39]. We argue that the occult perilesional inflammation drives follicular keratinisation and plugging and may therefore contribute to perilesional recurrences after surgery. This concept further suggests that recurrence after surgery may be prevented by the administration of adjuvant medications which reduce inflammation and infundibular epidermal hyperplasia and keratosis.

In conclusion, we show that among other cytokines especially TNF-α, IL-1β, IL-10 are elevated in inflamed HS skin lesions. The elevated levels of TNF-α provide evidence for the rational use of anti-TNF-α biologics in HS. In addition we show that HS inflammation extends beyond the visibly affected inflammatory borders.

Are histological and leucocyte subset alterations present in clinically normal appearing skin?

In chapter 3.2 we demonstrated that levels of IL-1β, TNF-α and IL-10 are elevated perilesionally. In chapter 3.3 we investigated histological characteristics in the clinically normal skin in the periphery of active HS lesions. We show that clinically unaffected perilesional skin already displays epidermal psoriasiform hyperplasia, follicular plugging and dilatation of hair follicles. These histological alterations are accompanied by a mild influx of $CD3^+$ T cells, $CD138^+$ plasma cells, $FXIIIa^+$ dermal DC and $tryptase^+$ mast cells. The mild influx of leucocytes, together with increased levels of cytokines in perilesional skin, represents a subclinical inflammatory response, possibly due to diffusion of chemokines from the actively inflamed lesion that paves the wayfor expansion of a pre-existing HS lesion. This subclinical inflammation may induce psoriasiform hyperplasia and infundibular keratosis. In newly emerged HS lesions, we observed cysts, free keratin fibers in the dermis, and intracellular fibers in multinucleated giant cells. This indicates that during progression of the disease, dilated hair follicles or cysts rupture and spill their keratin contents into the dermis. This foreign material induces an abscessing neutrophilic inflammation at the site of rupture with an additional massive influx of $CD14^+$ monocytes and $CD11c^+$ DC. Also multinucleated giant cells develop, which may indicate that these cells attempt to clear and sequester foreign keratin material. In this process large numbers of $CD3^+$ T cells and B cells are attracted.

When HS becomes chronic, a dramatic expansion of the infiltrate is observed. In addition, the follicular epithelium displays focal hyperplasia. We also demonstrated ectopic $CD20^+$ and $CD79a^+$ B cell 'pseudo' lymphoid follicles in the dermis of chronic lesions. These 'pseudo' lymphoid follicles were previously not described in HS. We argue that these lymphoid follicles appear as a reaction secondary to persistent bacterial colonization or to chronic inflammation. It is known that in chronic inflammation e.g. in rheumatoid arthritis and multiple sclerosis, lymphoid follicles can be formed in the inflamed tissue[40,41].

In chapter 3.1 we hypothesize that the high levels of IL-1β are the result from inflammasome activation by free keratin fibers comparable with that of uric acid crystals in gout[33,34]. In this study we demonstrate these keratin fibers deep in the dermis of clinical HS, supporting our hypothesis.

A characteristic of moderate or severe HS is fistula formation. How or why fistulas arise in HS and from where they primarily originate is unknown. It is has been postulated that fistula formation originates from downward expansion of the leaflike dermal protrusions from the hyperplastic epidermis[42] or from keratinocyte stem cells[43]. We have demonstrated ectopic strands of $Ki67^+$ proliferating epithelia, and single detached keratinocytes deep in the dermis, that were probably derived from ruptured cysts. We propose that these strands of epithelial lining and single keratinocytes are the initiators of epithelialized fistulas, similar to the artificially induced fistulas in acne, induced by puncturing a comedo with a needle[44].

In the past, HS was thought to be primarily an apocrinitis. In our study we observed apocrinitis as often eccrinitis (50% and 67%) and always as part of massive dermal inflammation, never as an isolated event. Therefore the term 'apocrine-bearing skin' to describe the predilection site for HS should be considered a misnomer and should be abandoned. Other characteristics of HS predilection sites such as mechanical friction, the 'loose' dermal structure and a favorable bacterial microclimate are likely more relevant.

Clinically we noted that many HS patients complain about itch. We demonstrated for the first time the presence of high numbers of tryptase positive mast cells in HS early and established lesions, and also in perilesional skin. Hence it is tempting to speculate these mast cells are the cause of the itch.

These observations of subclinical inflammation and histological alterations in perilesional skin provide a rationale for the use of keratolytics and anti-inflammatory agents to prevent further disease progression even though the disease is not visibly active at that time. Likely candidates for such an intervention would be topical resorcinol and oral acitretin, which have keratolytic as well as anti-inflammatory properties, both already shown to be of benefit in HS[45,46]. The increased numbers of mast cells might also be a future target for therapeutic intervention via modulation of IL-9. Recently a humanized anti-IL-9 biologic demonstrated promising results in asthma patients[47].

A possible limitation of this study is the limited number of patient samples analysed. But since big excised lesions were serially sectioned and little heterogeneity was observed between samples, we argue that the results are likely representative for the HS population at large.

In conclusion we demonstrate that different pathogenic mechanisms are operational in perilesional skin. In chronic clinical disease we demonstrate 'pseudo' lymphoid follicles as well as increased numbers of mast cells. The appearance of painful nodules is likely the result of an immunological response to breached dilated hair follicles or cysts. The free keratin fibers in active lesions might activate the inflammasome to produce bioactive IL-1β. We argue that fistulas might originate from observed detached keratinocytes and strands of follicle epithelium.

How effective is combination therapy with clindamycin and rifampicin?

Currently, antibiotic therapy is still the most common form of treatment for HS. The combined treatment with oral rifampicin 300 mg and oral clindamycin 300 mg, both twice daily, has previously been advocated for HS based on a small case series[48].

In chapter 4.1 we studied the efficacy of this antibiotic regime in a larger HS population and showed that this combination therapy resulted in clinical improvement in 82% of patients. The results are in agreement with previously published case series and also with those of Gener et al., whose study was published back to back with our study[48,49]. However, 61.5% of patients treated for 10 weeks experienced a relapse after a mean period of 5.0 months. This

indicates that this antibiotic combination does not cure the disease but relieves symptoms and reduces inflammation.

A limitation of our study is the retrospective approach. We did not observe major differences in outcomes between patients treated 10 weeks and longer and patients treated for a shorter period, indicating that treatment shorter than 10 weeks may also be effective. We argue that the mechanism of action of these antibiotics in HS is probably more immunomodulatory than bactericidal or bacteriostatic, since bacterial cultures from HS lesions are frequently negative[32]. A drawback of this combination treatment was the occurrence of side effects in 38% of patients, most frequently diarrhoea.

In conclusion, the combination treatment with oral rifampicin and clindamycin induces an improvement of symptoms in 84% of cases and is therefore an effective treatment for HS, despite the frequent occurrence of diarrhoea as a side effect.

Is surgical deroofing effective?

The treatment repertoire for HS consists not only of pharmaceutical but also of surgical interventions. Several surgical interventions have been described for HS, often with considerable recurrence rates. The reported recurrence rates after incision with drainage are up to almost 100%, for limited excision 43% and for wide excisions 27%[39]. Two methods of deroofing have been described in the literature. One method leaves the floor of the exposed lesions untouched assuming that preservation of the exposed lesion floor is essential for its epithelial regenerative elements[50,51]. The other method argues that the debris on the floor of lesions should be removed, because viable epithelial strands could get entrapped and cause recurrence[52].

In chapter 4.2 we studied the efficacy of the latter deroofing method in HS. We showed that deroofing results in an 83% cure rate after a median follow up of 34 months. The median patient satisfaction with the procedure rated a 8 on a scale from 0 to 10 and 90% of treated patients would recommend the deroofing technique to other HS patients. The deroofing procedure may be performed by using electrosurgery, cold steel or with the CO_2 laser[53,54]. Compared to electrosurgical deroofing, CO_2 laser-assisted surgery is more time consuming and rather expensive. The use of a probe during the procedure is necessary in order to detect subcutaneous fistulas.

Among the different types of HS lesions, a type is the chronic lesion that recurs at exactly the same location. We argue that this type of lesion is especially suited for deroofing. In our opinion Hurley 3 lesion areas are not suitable for deroofing, since it would require deroofing of the entire affected area and would be almost similar to radical excision. We argue that early intervention by deroofing can prevent disease aggravation. Advantages of the deroofing technique include: small defects avoiding the need for general anaesthesia, limited chance of creating extensive scarring, single-lesion surgical treatment gives low morbidity and the

use of electrosurgery provides good haemostasis which is highly needed because HS lesions are well vascularized.

In conclusion the deroofing method is cheap, simple, safe, and has a prolonged efficacy for recurrent HS lesions at fixed locations in Hurley 1 or 2 areas. The deroofing technique yields superior outcomes, and hence is preferable over incision with drainage and simple excisions with cold steel.

Does the anti-TNF-α biologic adalimumab reduce HS inflammation?

HS is often refractory to treatment. The efficacy of anti-tumor necrosis factor alpha (TNF-α) infliximab was first shown in single cases, later in case series and in small RCTs[55].

Mechanisms of action of TNF-α antagonists were unknown. In chapter 3.3 we investigated whether adalimumab affects the in situ cytokine production and the frequency of different inflammatory cell populations in HS lesions. We showed that after 16 weeks of adalimumab treatment the most evident decrease was seen in the levels of IL-1β, CXCL9 (MIG), BLC, IL-11 and CCL5 (RANTES) and to a lesser extent for IL-6R IL-16, IL-1ra, sTNF-R2, ICAM-1, IL-10, and CCL3 (MIP1α). Adalimumab treatment induced a shift most pronounced in the numbers of dendritic cells, monocytes and macrophages, followed by T helper and B-cells. These in situ results generally correlated with the clinical response of individual patients. By which mechanisms adalimumab ameliorates inflammation in HS is unknown. In psoriasis adalimumab is thought to exert its function by inhibiting the p38 mitogen-activated protein kinase (MAPK) pathway[56,57]. P38 MAPK is a kinase that regulates the expression of inflammatory cytokines such as IL-1β, IL-8, and TNF-α, IL-12/IL-23(p40), IL-23(p19) and IL-20[57]. Adalimumab might ameliorate inflammation in HS in a similar fashion.

This in situ study had several limitations. From each of three dosing arms, a relatively small number of patients were included so that statistics could not be applied. Furthermore, sequential biopsies were not taken during treatment. So it was not possible to link in situ inflammatory changes to the clinical response.

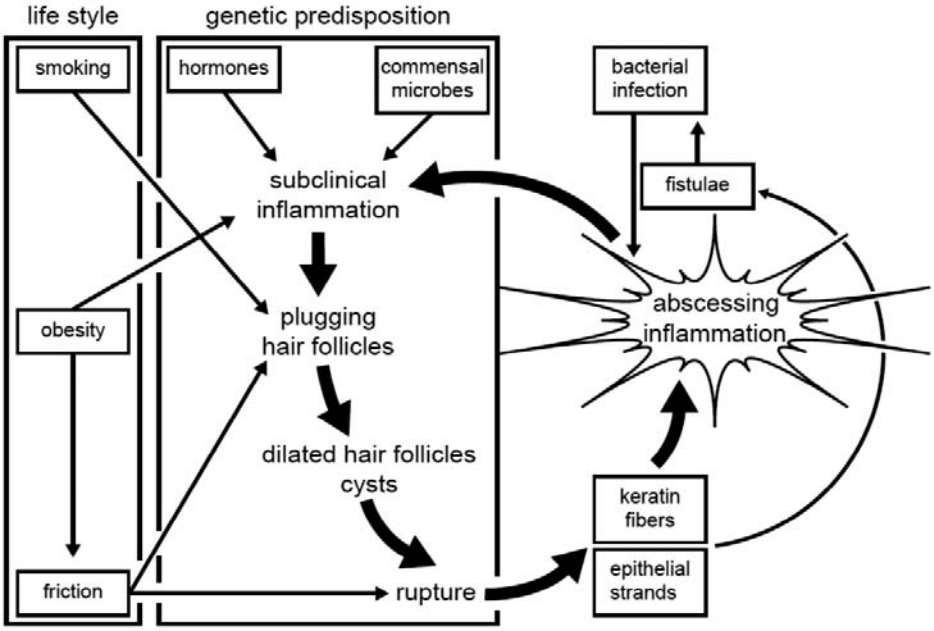
In conclusion, Adalimumab treatment inhibits cytokine production and reduces inflammatory cell numbers in lesional HS skin. The most pronounced reduction was observed in IL-1β levels and in the number of inflammatory DC.

Is the anti-inflammatory drug colchicine effective?

In chapter 4.3 we demonstrated that IL-1β secretion is significantly increased in HS lesions suggesting activation of the IL-1β/inflammasome pathway[58]. IL-1β and the inflammasome play a crucial role in gout, familial Mediterranean fever (FMF) and Behçet's disease[34,59]. Colchicine is a well-recognized therapy for gouty arthritis, FMF and Behçet's disease[60]. Therefor we hypothesized that colchicine could improve HS inflammation by reducing inflammasome activity. In a prospective pilot study we investigated the efficacy of 0.5 mg colchicine b.i.d. in 8 patients for up to 4 months. This daily dose is frequently used as a maintenance therapy in

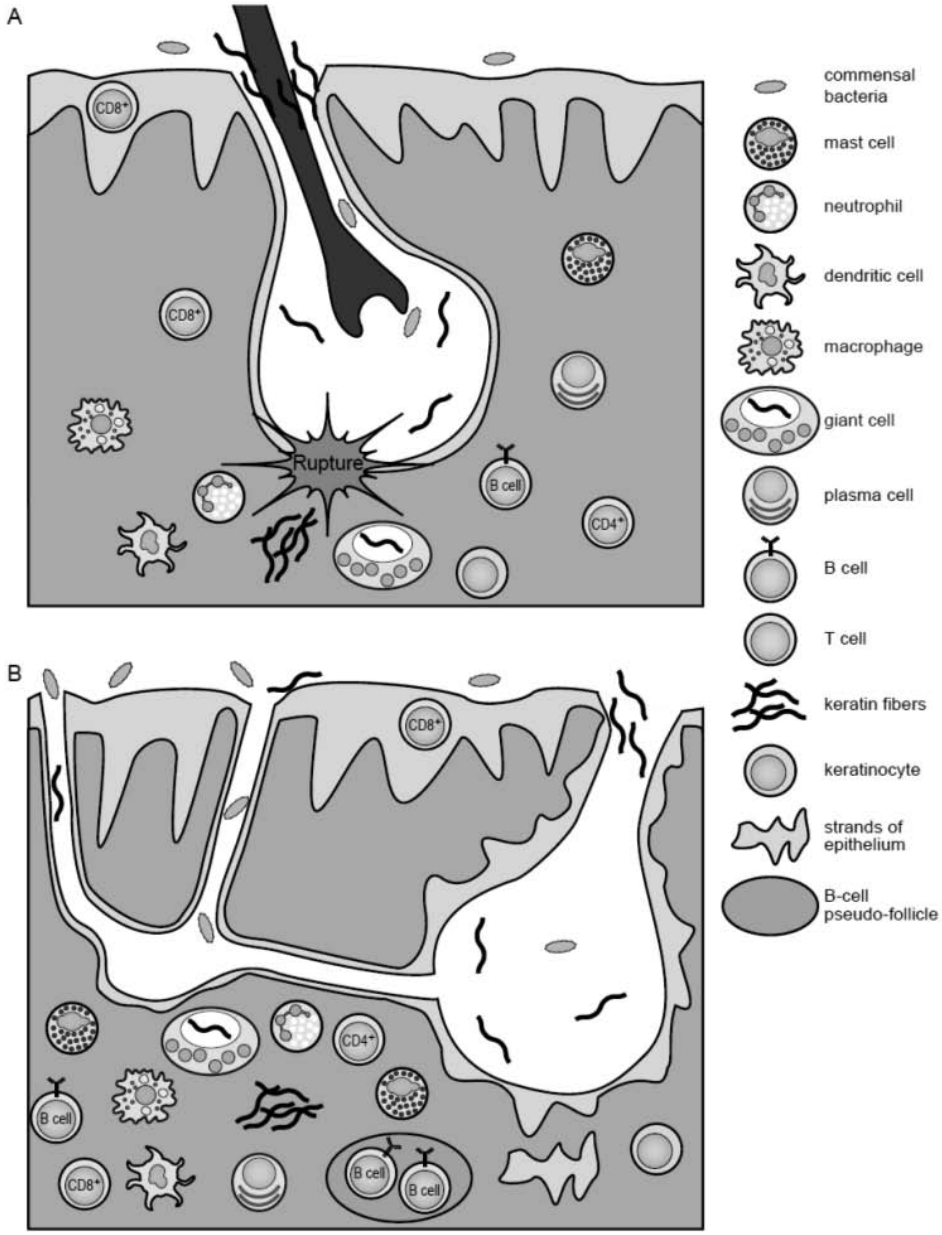
patients with gout[61]. Two patients received 1.5 mg daily after the first month. A limitation of this study was the small sample size and the fact that selection of patients was restricted to patients refractory to other regular therapies. No patient experienced a clinically significant change in HS severity, while three patients experienced some level of nausea and diarrhea as a side effect. The efficacy of an intervention dose of colchicine should be studied in a larger patient cohort. We suggest that colchicine treatment should start with 1 mg followed by 0.5 mg every 2 hours with a maximum of 5 mg cumulative, followed by 1 mg daily. The plasma levels of colchicine should be monitored and the dose should be adjusted accordingly.

Figure 2. Comprehensive hypothetical model of factors influencing HS development, activity and chronicity



In a genetically predisposed individual, commensal skin microbiota triggers a subclinical inflammatory response. This promotes plugging and occlusion of the hair follicles, leading to formation of cysts, which can be amplified by mechanical friction and smoking. These cysts rupture by mechanical friction or pressure spilling their content of highly cross-linked keratins and epithelial strands into the dermis. These subsequently fuel an abscessing inflammation which promotes follicular plugging in the surrounding skin. Epithelial strands are incited to proliferate by cytokines from the inflammatory reaction leading to epithelialized fistulas. These fistulas form an easy passage for bacteria and allow colonization of ectopic areas aggravating inflammation.

Figure 3. Key histological and immunological alterations in early and chronic HS skin (See also Color section, p. 208.)



A Early HS
Commensal bacteria might elicit an inflammatory response with subsequent occlusion of the hair follicle's infundibulum by keratin plugging or epidermal hyperplasia. Or conversely, friction of pressure forces induce occlusion of the hair follicle's infundibulum followed by overgrowth of commensal bacteria, leading to an inflammatory response. The interfollicular epidermis shows mild psoriasiform hyperplasia. HS clinically manifests acutely when a occluded and dilated hair follicle / cyst ruptures, spilling its keratin-rich content into the dermis. Subsequently 'rod-like' keratin fibers are present freely in the dermis and are phagocytosed by multinucleated giant cells, while single keratinocytes derived from the follicle epithelium are launched into the dermis. Increased numbers of mast cells are diffusely spread in the dermis. There is abscess formation with massive neutrophilic infiltration with in addition dendritic cells, macrophages, T cells, some B cells and plasma cells, and $CD8^+$ T cells show modest epidermotropism.
B Chronic severe HS
In chronic severe HS plugging of the follicular infundibulum increases resulting in cyst formation. After rupture, these cysts may be interconnected by epithelialized sinus tracts, draining onto the skin surface through multiple orifices. The epidermal psoriasiform hyperplasia is pronounced and involves the interfollicular epidermis as well as cyst and sinus tract walls. As in early lesions also free keratin fibers are present in the dermis and within multinucleated giant cells. Single detached keratinocytes but also strands of follicular epithelium are ectopically present in the dermis, which may form epithelialized fistulas. The abscessing infiltrate expands, with increasing numbers of T cells, dendritic cells, macrophages, B cells and plasma cells. B cells are diffusely located around abscesses, and in addition demonstrate a patchy distribution in the infiltrate forming pseudo-follicles. The number of mast cells increases and $CD8^+$ cells show modest epitheliotropism.

VIEW ON HS PATHOGENESIS

Based on the results of studies in this thesis and existing literature we postulate the following model and sequence of events in HS pathogenesis Figure 2 and 3.

HS results from a combination of intrinsic and environmental factors in a genetically predisposed individual. The pathogenic process starts prior to the first visible clinical manifestation in response to unknown triggers, which might be the commensal skin microbiota. Pre-clinically a mild influx of mainly T cells but also mast cells occurs which secrete a mixture of chemokines and cytokines including TNF-α, IL-1β and IL-10. This subclinical inflammation results in epidermal psoriasiform hyperplasia and follicular infundibular keratosis. Smoking and mechanical friction contributes in this phase by amplifying the follicular keratosis. The hair follicles become plugged, followed by swelling of the hair follicle, forming cysts. Clinical HS, with abscesses and inflammatory nodules, acutely manifests when the fragile epithelial lining of the dilated hair follicle or cyst ruptures. The characteristic loose structure of the subcutaneous tissue of the axilla and groin skin facilitates vertical and horizontal inward spreading of the spilled keratin content into the dermis. In response to these keratin fibers together with commensal flora, an abscessing neutrophilic inflammatory reaction forms at the site of rupture with an additional massive influx of macrophages, monocytes, T cells, B cells and dendritic cells and the formation of multinucleated giant cells. The latter likely are involved in the clearing of keratin fibres and keratinocyte remnants by phagocytosis. We argue that

the inflammasome plays a key role in HS pathogenesis and is activated by phagocytosing these free keratin fibers. This inflammatory response is boosted by host-derived molecules, indicative of tissue destruction, such as self DNA and RNA, together with AMP, pyroptosis and bacteria. The activated inflammasome generates the bioactive and highly pro-inflammatory IL-1β by cleaving intracellular pro-IL-1 by means of caspase 1.

After this acute phase, HS lesions may become chronic. Secondary bacterial colonisation nourishes inflammation. Furthermore, the multinucleated giant cells fail to phagocytose all keratin fibrils. Strands of epithelial lining and single keratinocytes in the dermis launched from follicular epithelia after rupture proliferate from which epithelialized fistulas are formed. These fistulas form a direct route for bacteria to invade the deep dermis. A vicious circle of inflammation leading to follicular plugging, rupture, and then again an inflammatory reaction occurs. This inflammation may spread the disease into the surrounding healthy tissue causing plugging of hair follicles.

FUTURE PERSPECTIVES

HS is scientifically still an orphan disease, although the number of publications is rising. Our advancing insight gives rise to new questions while still many basic questions remain to be answered. Important questions to be addressed in future studies are discussed below and are presented in Box 2.

Box 2. Outstanding questions

Genetics	**Immunology**
Concordance of HS in monozygotic twins	The role of the inflammasome in HS
Identification of SNP in HS	The role of mast cells in HS pathology
Smoking	Functional studies on inflammatory cell subsets
Effect of smoking cessation on disease severity	**Treatment**
Obesity	Preventive effects of current HS drugs
The effect on HS severity after weight loss in the obese	Efficacy of inflammation modulating drugs
Bacteria	**Clinical**
Bacterial characterisation of HS lesions by 16S ribosomal RNA	Creation of an accepted validated severity score
Mode of action of smoking in HS pathogenesis	Identification of clinical HS subgroups

Diagnosis

The diagnosis of HS is made on the basis of the clinical signs; no additional tests are necessary[62]. But despite its frequent occurrence and straight forward clinical presentation, the reported time until diagnosis is 7 years[63]. This puzzling fact reflects the limited awareness about the disease of many clinicians. Intensification of HS research and the thereby generated publicity might raise the degree of awareness about the disease and the available treatment options

among clinicians. In The Netherlands the medical curriculum does not specifically elaborate on HS. Medical students are likely to encounter HS only once or twice during their internships, most likely during the ER surgery internship. Frequently they are wrongfully educated that HS is an apocrinitis which is treatable with oral antibiotics and incision and drainage. A single lecture incorporated in the future curriculum would be sufficient to teach medical students the basics of HS pathogenesis and treatment. Furthermore, since general practitioners are frequently the first and only medical doctor a HS patient visit, education about HS should be incorporated in the GP traineeship and post-graduate courses as well. Visibility of HS would also be greatly promoted if and when a well–known public figure would manifest as an HS patient in popular media.

Prognosis and pathomechanisms

Data on disease progression and the natural course are limited[27]. Thus it is unpredictable which patients and how rapidly patients will progress to the more severe stages of the disease. It is therefore important to identify predictive parameters. This would ideally be done prospectively in a patient cohort from the first disease manifestation and onward followed without interventions. However, since such a study would not be ethical. The clinical predictive parameters might also be determined in a retrospective study in a large cohort, focusing on a comprehensive set of patient characteristics and detailed data on disease progression.

Genetics

Genetic predisposition evidently contributes to HS development[64], but ill-defined HS populations hamper current genetic research. Whether nature or nurture is more important in HS pathogenesis has yet to be established. The concordance of HS in monozygotic twins might answer this question. To our knowledge, HS in monozygotic twins has yet to be reported. Furthermore, genome wide association studies (GWA) might discover single nucleotide polymorphisms (SNP) that provide guidance into pathogenic mechanisms and HS variants. GWA studies require large cohorts of well-documented HS patients[65]. For such a GWA study specialized HS centres in different countries should combine forces to reach appropriate power in terms of numbers of patients and matched controls.

Smoking

Cigarette smoking is undoubtedly associated with HS[66]. It is, however, unclear which pathogenic mechanisms of smoking contribute to HS disease development and/or its maintenance. Cigarette smoke contains many components which may potentially contribute to HS pathogenesis (reviewed by Smith[67]). Furthermore, it is unknown whether smoking cessation influences disease severity, and whether the quantity of smoking correlates with disease severity. Smoking cessation is a low cost intervention and it would be highly worthwhile studying its effects in patients with HS in a prospective case control study.

Bacteria

The results from bacterial studies in HS are hitherto inconclusive, but an important factor has not been addressed thus far. Before one can speak of an aberrant microbiome in HS, the microbiome of healthy skin should be characterized. Currently, the US National Institutes of Health human microbiome project is characterizing the human skin microbiota using advanced, molecular, deep sequencing techniques with interesting results relevant for HS pathogenesis[68]. First, it is now evident that only a small minority of skin bacterial species are able to thrive in culture[68]. Particularly anaerobic microorganisms in the hair follicles are difficult to isolate using routine culture[68]. It has been estimated that less than 1% of bacterial species can be cultured[68]. This suggests that the culture-based approach used in the past to study HS lesions is unreliable and that no hard conclusions can be drawn from these data. New molecular sequencing techniques can not only identify but also quantify skin microbiota. Genomic characterization of bacteria relies on sequence analysis of the 16S ribosomal RNA. This genomic approach already revealed a much greater diversity of microorganisms on and in the skin than obtained by culture-based methods[68]. Hence, the HS microbiome should be reinvestigated using these new molecular deep sequencing techniques. Such a study might reveal non culturable bacteria causing HS or a microbiome shift to more pathogenic species.

Immunological pathways

We demonstrated that the dense cellular infiltrate in HS contains many leucocyte subsets, but the functional roles and balance between inflammatory cell subsets in HS infiltrates is currently unknown. Further functional and in situ investigations into the inflammatory cell subsets and their cytokine production are highly needed. In this thesis we demonstrated that mast cells are present in vast quantities, not only in clinical HS skin, but also in normal appearing perilesional skin. The role of mast cells in HS pathogenesis is currently unknown. They might play an important role in HS pathogenesis and form a target for further investigations. In this thesis we demonstrate high levels of IL-1β in HS lesions together with the presence of free keratin fibers in the dermis. We therefore proposed that the inflammasome is activated in HS. This pathogenic mechanism could explain the intense inflammatory response characterizing HS. The contribution of individual inflammasome components such as Nlrp3, Nlrp1, Nlrc4, and AIM2 need to be explored in more detail. The Nlrp3 is the most widely studied inflammasome and can be activated by a large number of stimuli. It can be activated by muramyl dipeptide (MDP), bacterial RNA, double stranded RNA and DAMP such as uric acid crystals, all components that are present in HS lesions, but also by amyloid-β and an exogenous compound such as asbestos[30]. Nlrp1 can be activated by MDP and anthrax toxin, Nlrc4 by flagelin and AIM2 by dsDNA[30]. Since inflammasomes not only generates IL-1β but also IL-18, levels of IL-18 should also be investigated additionally in future studies and its natural antagonist IL-18 binding protein.

Therapy and monitoring

HS is notoriously difficult and challenging to treat. The efficacy for many HS treatment modalities is based on case series or trial and error rather than on randomized controlled trials. The lack of evidence-based therapies in patient care is illustrated by the example that failure of immunosuppressive therapy such as systemic corticosteroids is required before anti-TNF-α biologics are reimbursed by health insurers in The Netherlands. However, there is no scientific evidence that systemic corticosteroids improve HS. The presumed efficacy of systemic corticosteroids is solely based on a single report from 1970[69]. Another example is the use of intralaesional corticosteroid injections. Intralaesional corticosteroid injections are used on a regular basis, whereas there is no evidence on the efficacy of this treatment what so ever.

HS patients will benefit from improved clinical care based on evidence-based medicine. But clinical trials in HS have to deal with several challenges. First, there is no accepted uniform HS severity score. The currently used severity scores according to Hurley[70] and Sartorius[71] both have clear limitations. In order to be able to compare the efficacy of different studies, a sensitive and specific validated severity score is warranted. Such a new severity score is currently under development by a European consortium on HS scoring. Second, the heterogeneous results of most drugs in HS may indicate the existence of clinical variants of HS. Attempts should be made at identifying clinical variants of HS, because patients with a specific clinical HS variant may show a positive response to certain therapy even when the HS population as a whole remains unresponsive. Such personalized treatment would greatly improve clinical care. In this thesis we demonstrate subclinical disease activity in perilesional skin. This observation provides a rationale for the use of treatments aimed at disease prevention such as topical or systemic keratolytic and immunomodulating agents, which should preferably be investigated prospectively.

It has been suggested that HS is classifiable as an auto inflammatory disease[72] defined as a disease caused by a dysregulation of the innate immune system[72], and as such many anti-inflammatory and/or immunomodulating drugs available on the market qualify for the treatment of HS. Some of these drugs have already been anecdotally described for the treatment of HS, but most have not. Below we briefly discuss several immunomodulating drugs and summarize them in Table 1.

By demonstrating high levels of IL-1β in HS lesions, we identified the inflammasome pathway as a potential new target for therapy. Anakinra is an IL-1 receptor antagonist and canakinumab is a human monoclonal antibody targeting IL-β. Both these biologics show a marked efficacy in inflammasome-mediated auto inflammatory diseases[73]. It would be worthwhile investigating the efficacy of these IL-1β modulating biologics in randomized controlled clinical trials in HS.

Ustekunimab is a fully human monoclonal antibody directed against interleukin 12 and interleukin 23 (anti-p40). IL-12 is considered a Th1-promoting cytokine and IL-23 a Th17-promoting cytokine. The efficacy of ustekunimab has been demonstrated in psoriasis[74]. Since

Table 1. Immunomodulating drugs with potential use in HS

Immunomodulating drug	Main inflammatory disease indications	Main mechanism of action	Efficacy in HS
Anakinra	RA, cryopyrin-associated periodic syndromes	IL-1 receptor antagonist	?
Canakinumab	RA, cryopyrin-associated periodic syndromes	IL-1β neutralising monoclonal antibody	?
Ustekunimab	Psoriasis	Anti-p40 (IL-12 and IL-23) monoclonal antibody	Improvement in 2/3 patients
Azathioprine	CD, RA	Inhibits lymphocyte proliferation, and decreases immune response	?
Methotrexate (MTX)	Psoriasis, RA, CD	Inhibits TNF-α, IL-6, IL-8, , sIL-2 receptor, blocks IL-1r, promotes IL-10	Limited in 3/3 patients
Cyclosporine	Graft vs host, psoriasis, AD, RA	Inhibits T cells	Improvement in 4/4 patients
Tacrolimus	AD, CD	Inhibits calcineurin	?
Thaladomide / lenalidomide	CD, RA	Inhibits TNF-α, IL-6, IL-1β, IL-12, GM-CSF, ICAM-1, COX-2	?
Rituximab	RA	Depletion of $CD20^+$ B cells	?
HuMab 10F8	Palmoplantar pustulosis	IL-8 neutralizing monoclonal antibody	?

AD: Atopic dermatitis, CD: Crohn's disease, RA: Rheumatoid arthritis

we showed that IL-17 is elevated in HS and a recent paper demonstrated elevated levels of p19 IL-23 mRNA[38] (Th17 cytokines), ustekunimab is a potential new biologic for the treatment of HS. Gulliver et al. reported that in a pilot study ustekunimab improved HS in 2/3 patients[75], but this should obviously be investigated in a larger HS patient population.

Azathioprine is an immune-modulating drug that was originally developed for the control of graft rejection in transplant surgery, but nowadays it is also in use for treatment of a wide range of auto-immune and immune-mediated diseases such as Crohn's disease (CD) and rheumatoid arthritis (RA). Azathioprine is an imidazole derivative of 6-mercaptopurine (6-MP) that interferes with DNA and RNA synthesis, resulting in a significant inhibition of lymphocyte proliferation and a decreased immune response[76]. Since azathioprine is a highly effective drug for Crohn's disease, it constitutes a potential therapeutic for HS which has yet to be tested in clinical trials.

Methotrexate (MTX), a folate antagonist with anti-inflammatory and immunomodulatory properties, is used for treating inflammatory diseases such as psoriasis, RA and CD. MTX inhibits DNA synthesis, the production of the pro-inflammatory cytokines TNF-α, IL-6 and IL-8 and impairs neutrophil chemotaxis. Furthermore secretion of sIL-2 receptor and the IL-1 receptor are inhibited, while production of IL-10 is promoted[77]. The efficacy of MTX has been investigated in 3 patients with HS, but showed minimal therapeutic effects[78].

Cyclosporine is a calcineurin inhibitor that acts selectively on T cells by preventing the phosphorylation of nuclear factor of activated T cells and, therefore the transcription of IL-2[79].

Cyclosporine is used to prevent graft vs. host disease, psoriasis, atopic dermatitis (AD) and other inflammatory diseases. Since we demonstrated increased numbers of T cells in HS lesions, cyclosporine could be a treatment option for HS. Cyclosporine has thus far anecdotally been used in 4 HS patients and showed favorable results[80-82].

Tacrolimus is a macrolide antibiotic with potent immunomodulating properties. It is in use for the prevention of organ-transplant rejection and it is clinically effective in different autoimmune diseases[83]. Tacrolimus can be applied topically as an ointment or systemically. The mechanism of action of tacrolimus in general is comparable with that of cyclosporin, since most effects of tacrolimus are thought to be mediated by the inhibition of calcineurin[83]. However, it has secondary effects on other inflammatory cells that are normally activated by T cell-derived factors. Tacrolimus inhibits expression of IL-2, ICAM-1, NF-κβ, INF-γ and TNF-α[84]. The use of tacrolimus in HS has not yet been reported. Both topical and systemic applications could potentially be effective.

Thalidomide is glutamic acid derivate; lenalomide is an amino substitute of thalidomide and 2000 times more potent in TNF-α inhibition[85]. Thalidomide acts as an immunomodulator, by inhibiting critical inflammatory cytokines/mediators as TNF-α, IL -6, IL-1β, IL-12, GM-CSF, ICAM-1 and COX-2[85]. It has been used with promising efficacy in inflammatory diseases such as for RA, ankylosing spondylarthritis and systemic sclerosis[86]. Thalidomide is highly teratogenic and neurotoxic. One patient with pyoderma gangrenosum with comorbid HS has been described using thalidomide, but the clinical effect on HS severity was not described[87].

With our demonstration of ectopic 'pseudo' B cell follicles in chronic HS lesions, a new field of research in HS pathogenesis has potentially opened. Till to date the function of B cells in HS inflammation remains largely unclear. Therapeutic intervention aimed at depletion of B cells, via treatment with rituximab, a chimeric monoclonal antibody against the protein CD20, requires further in depth investigations into the function of these B cells in HS. Demonstration of their pathogenicity in HS would provide a rationale for rituximab treatment of patients with HS.

Since high numbers of neutrophils are present in abscessing HS lesions, IL-8, as a potent neutrophil chemoattractant, forms a potential therapeutic target. Interleukin-8 is a chemokine that has been implicated in a number of inflammatory diseases involving neutrophil activation. HuMab 10F8 is a novel fully human mAb against IL-8, which effectively neutralizes IL-8-dependent human neutrophil activation and migration[88]. Treatment with HuMab 10F8 for palmoplantar pustulosis showed promising results and could be effective in HS[88].

CONCLUDING REMARKS

HS remains a difficult to treat disease, which is frustrating for patients as well as clinicians. In the past few years our understanding of the pathomechanisms involved has increased

and we gained more insight into the efficacy of multiple treatment options. HS research is still in its infancy and much research as indicated in this chapter remains to be done. Our investigations confirmed the severe burden of the disease, provided support for existing treatments and generated novel insights into disease pathomechanisms with a perspective of new therapies.

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6

Samenvatting

Hoofdstuk 1 geeft een algemene inleiding tot dit proefschrift. Hidradenitis suppurativa (HS) is een recidiverende of chronische, inflammatoire huidziekte uitgaande van de haarzakjes/follikel. HS komt voornamelijk voor in de huidplooien zoals oksels, liezen en billen, terwijl bij vrouwen ook de borsten aangedaan kunnen zijn. De huidafwijkingen (lesies) bestaan uit pijnlijke abcessen en acuut gevormde ontstekingen. Deze ontstekingen kunnen verbindingen met elkaar vormen en op deze manier een netwerk van gangen vormen met uitgangen naar buiten, zogenaamde fistels. Bij langdurige ontsteking kan er ook ernstige verlittekening optreden. De klachten bestaan voornamelijk uit pijn en riekende pus uitvloed die vaak schaamtegevoelens oproept. De prevalentie is recent geschat tussen de 1% en 4%. Ondanks het feit dat HS dus zeker geen zeldzame ziekte is, duurt het gemiddeld 7 jaar tot de diagnose gesteld wordt. Waarschijnlijk komt dit door onbekendheid van artsen met de aandoening. De ziekte ontstaat meestal na de puberteit, tussen het 20ste en 30ste levensjaar, en komt vaker voor bij vrouwen dan bij mannen met een ratio van 3:1. Op het eerste gezicht lijkt HS een besmettelijke (infectieuze) ziekte, maar vaak worden geen of alleen normale huidbacteriën gekweekt. Anders dan de naam doet vermoeden, hidradenitis=zweetklierontsteking, is HS een aandoening van verstopte haarzakjes. Factoren die een rol in het ziekte proces lijken te spelen, zijn genetische aanleg, roken, wrijvingskrachten, zwaarlijvigheid, kolonisatie door bacteriën, een disbalans van het immuunsysteem en hormonen. HS is erg moeilijk te behandelen, de meeste behandelingen geven slechts een gedeeltelijke verbetering of slechts een verbetering van korte duur. Behandelingen voor HS bestaan uit antibiotica kuren, immuunsysteem modulerende middelen zoals anti-TNF-α biologics, anti-hormonale middelen, licht- of lasertherapie, retinoïden en chirurgische ingrepen. Chirurgische behandelingen kunnen weer onderverdeeld worden in kleine, lokale ingrepen en grote waarbij de gehele haardragende huid van een gebied verwijderd wordt. Er is wetenschappelijk gezien relatief weinig bekend over HS. Zowel over de ontstaanswijze als de daaraan ten grondslag liggende mechanismen, als de effectiviteit van behandelingen, bestaat veel onduidelijkheid. Daarom is het onderwerp van dit proefschrift niet beperkt tot één enkele factor van de aandoening, maar worden zowel kwaliteit van leven en pathogenese als de therapeutische mogelijkheden bestudeerd.

In **hoofdstuk 2.1** beschrijven wij de resultaten van ons onderzoek naar het voorkomen (prevalentie) van depressie en de kwaliteit van leven. In totaal onderzochten wij 211 HS patiënten en vergeleken deze met 233 patiënten met een andere dermatologische aandoening. Voor het onderzoek naar depressie gebruikten wij de MDI vragenlijst en voor kwaliteit van leven de DLQI vragenlijst. De DLQI score was significant hoger voor HS patiënten dan de controle patiënten en stond in verband met de ziekte ernst. De MDI score was ook significant hoger voor HS patiënten. Klinisch gedefinieerde depressie (volgens het ICD-10 model) was met 9% bij HS patiënten niet duidelijk hoger dan bij de controle patiënten (6%). Wij concluderen dat HS een grote impact heeft op de kwaliteit van leven en dat de ernst van HS hiermee

gecorreleerd is. De depressie vragenlijst kan ook gebruikt worden om de kwaliteit van leven te bepalen, en om de effectiviteit van behandelingen te meten. Verder vinden wij dat artsen bij HS patiënten alert moeten zijn op symptomen van depressie en, indien nodig, patiënten moeten doorverwijzen voor gespecialiseerde hulp.

In **hoofdstuk 2.2** onderzoeken wij of HS vaak voorkomt bij patiënten met chronisch inflammatoire darmziekten (IBD). IBD bestaat uit de ziekte van Crohn en colitis ulcerosa (UC). Onze hypothese was dat als IBD en HS vaker samen voorkomen, dit zou wijzen op gelijksoortige ontstaans- en werkingsmechanismen in het ziekte proces. Tijdens een IBD patiënten informatiedag vroegen wij 158 IBD patiënten (65% Crohn en 35% UC) naar klachten die op HS wijzen en lieten voorbeeldfoto's zien van HS lesies. Op grond van deze enquête concludeerden wij dat 16% van de IBD patiënten zeer waarschijnlijk HS hebben (17% Crohn, 14% UC). Hiermee lieten wij voor het eerst zien dat HS relatief frequent voorkomt bij patiënten met Crohn of UC. Mogelijk kunnen geneesmiddelen voor IBD ook gebruikt worden voor de behandeling van HS.

In **hoofdstuk 2.3** beschrijven wij een patiënt met een onderbeenprothese die HS-achtige huidafwijkingen ontwikkelde enkel op zijn beenstomp. Deze casus suggereert dat HS kan ontstaan op locaties met toegenomen wrijving en een warm vochtig milieu, en niet alleen op plaatsen die apocriene zweetklieren bevatten, zoals algemeen wordt verondersteld.

In **hoofdstuk 3.1** wordt onderzocht welke ontstekingsfactoren (cytokinen) verhoogd zijn in chronisch aangedane HS lesies en in normaal ogende huid naast een HS ontsteking (perilesionaal). Deze waarden werden vergeleken met die verkregen zijn uit psoriasis lesies. Deze huidbiopten werden kortdurend gekweekt en de uitgescheiden ontstekingsfactoren in het kweekmedium bepaald. De ontstekingscytokinen IL-1β en TNF-α, maar ook het anti-inflammatoire cytokine IL-10, bleken niet alleen significant verhoogd in HS huid, maar ook in de perilesionale huid. Deze cytokines waren in HS lesies duidelijk sterker verhoogd dan in psoriasis lesies. De cytokinen IL-2, IL-4, IL-5 en IFN-γ waren daarentegen vaak onmeetbaar laag. Deze resultaten suggereren dat geneesmiddelen gericht tegen TNF-α en IL-1β effectief zouden kunnen zijn voor behandeling van HS.

In **hoofdstuk 3.2** onderzoeken wij of behandeling met de anti-TNF-α biologic adalimumab veranderingen in cytokinen en het ontstekingsinfiltraat in HS huid teweeg brengt. Wij verbreedden onze kennis door het meten van verschillende cytokinen in HS huid van de betreffende patiënten. Biopten van HS lesies werden afgenomen van 9 HS patiënten, voor start van de behandeling, en na 16 weken behandeling met adalimumab. Behandeling met adalimumab verlaagde voornamelijk de cytokinen IL-1β, CXCL9 en BLC, evenals het aantal

dendritische cellen, monocyten en macrofagen. Ook vond tijdens adalimumab behandeling afname plaats van het aantal T cellen en B cellen in de betreffende biopten.

In **hoofdstuk 3.3** wordt de aanwezigheid onderzocht van ontstekingscellen in normaal ogende perilesionale huid, net-ontstane HS ontstekingen, en chronische HS ontstekingen. Perilesionale HS huid laat verschillende afwijkingen zien, onder andere verstopping van de haarzakjes, psoriatiforme verbreding van de opperhuid en een toegenomen aantal ontstekingscellen. Nieuw gevormde HS ontstekingen worden gekarakteriseerd door een abcederende neutrofiele ontsteking met een aanzienlijke toename van macrofagen, monocyten en dendritische cellen. In de chronische fase neemt de grootte van de ontsteking toe en wordt in het bijzonder een toegenomen aantal B cellen en plasmacellen gezien. In alle HS stadia wordt een toegenomen aantal mestcellen gezien. Ook zien wij keratine resten van de opperhuid diep in de huid en in reuscellen, als reactie hierop kan mogelijk een ontstekingsreactie plaatsvinden. Daarnaast zien wij losse keratinocyten en strengen van vitaal epitheel diep in de lederhuid, waar die normaal niet voorkomen, die mogelijk de basis zijn voor fistelvorming.

In **hoofdstuk 4.1** wordt de effectiviteit van een combinatietherapie bestaande uit de antibiotica clindamycine en rifampicine met verschillende duur en doseringen onderzocht. In dit kader werden 34 HS patiënten door ons behandeld met deze therapie. In 82% van de patiënten resulteerde deze behandeling in een verbetering van de aandoening en in 47% was zelfs geen andere behandeling meer nodig. Het maximale effect van de behandeling werd bereikt binnen 10 weken. Echter, in 62% van de patiënten, die geen andere behandeling nodig hadden, verergerde de ziekte weer na een periode van 5 maanden. Patiënten, die geen verbetering lieten zien, hadden over het algemeen een meer ernstige vorm van HS. Aangezien wij HS niet als een ziekte beschouwen die wordt veroorzaakt door bacteriën, denken wij dat de werking van deze antibiotica meer berust op ontsteking beperkende mechanismen dan bacteriedodende mechanismen. Uit deze resultaten concluderen wij dat deze combinatietherapie een veelbelovende therapie is voor HS. De optimale duur en dosering moeten nog nader worden onderzocht.

In **hoofdstuk 4.2** onderzoeken wij de effectiviteit van de chirurgische methode deroofing. Deroofing is een huidsparende chirurgische techniek waarbij het dak van de ontsteking wordt verwijderd en alle fistels worden opgezocht met een sonde. 88 HS lesies werden op deze manier behandeld bij 44 patiënten. Slechts 17% van de plekken kwamen terug na 4,6 maanden, wat voor HS een goed resultaat is. Patiënten waren tevreden met de procedure en beoordeelden deze met een 8 uit 10. 90% van hen zou deroofing aanraden aan andere patiënten. Wij stellen dat deroofing een effectieve, gemakkelijk uit te voeren chirurgische behandeling is voor milde tot matig ernstige HS.

In **hoofdstuk 4.3** onderzoeken wij de effectiviteit van colchicine in HS. Colchicine wordt gebruikt voor behandelingen van andere ontstekingsziekten, waarbij, net als in HS, het cytokine IL-1β verhoogd is. Acht HS patiënten kregen 0.5 mg colchicine 2 maal daags gedurende 1 tot 4 maanden. Gebruik van colchicine liet geen duidelijke verbetering in HS ernst zien, terwijl 3 patiënten misselijkheid als bijwerking meldden.

Hoofdstuk 5 beschrijft de belangrijkste bevindingen en conclusies uit **hoofdstukken 2 t/m 4** in de context van de beschikbare literatuur. Wij bespreken de mogelijkheid van een diermodel voor HS onderzoek, presenteren onze visie over hoe HS ontstaat, en zich ontwikkelt tot een chronische aandoening. Het hoofdstuk wordt afgesloten met suggesties voor toekomstig onderzoek naar HS.

Abbreviations

6-MP	6-mercaptopurine
AD	Atopic dermatitis
AhR	Aryl hydrocarbon receptor
ALA PDT	Aminolevulinic acid photodynamic therapy
ALS	Amyotrophic lateral sclerosis
AMP	Anti-microbial peptide
APH1	Anterior pharynx defective 1
BID	Twice a day
BLC	B-lymphocyte chemoattractant
BMI	Body mass index
Ca	Cyproterone acetate
CARD15	Caspase recruitment domain family, member 15
CD	Crohn's disease
CES-D	Center for epidemiologic studies depression scale
CI	Confidence interval
DAMP	Damage associated molecular pattern
DC	Dendritic cell
DLQI	Dermatology life quality index
DSM-IV	Diagnostic and statistical manual of mental disorders version 4
EAE	Experimental autoimmune encephalomyelitis
EE	Ethinylestradiol
EOW	Every other week
FDA	Food and drug administration
FMF	Familial Mediterranean fever
GSP	German shepherd dog pyoderma
GWA	Genome wide association
HLA	Human leukocyte antigen
HS	Hidradenitis suppurativa
IBD	Inflammatory bowel disease
ICD-10	International classification of diseases-10
IFN	Interferon
IL	Interleukin
IMDM	Iscove's modified Dulbecco's medium
LHS	Lesional hidradenitis suppurativa
LPS	Lipopolysaccharide
MDI	Major Depression Inventory
MDP	Muramyl dipeptide

MIG	CXCL9
MMP	Matrix metalloproteinase
NAMPT	Nicotinamide phosphoribosyltransferase
NCT	Nicastrin
Nd-Yag laser	Neodymium-doped yttrium aluminium garnet laser
NK	Natural killer
NLR	NOD-like receptor
NOD	Nucleotide-binding oligomerization domain
OR	Odds ratio
PAMP	Pathogen-associated molecular pattern
PCOS	Hyperandrogenic polycystic ovarian syndrome
PEN2	Presenilin enhancer 2
PGA	Physician's global assessment
PGP9.5	protein gene product 9.5
PHS	Perilesional HS
PP	Palmoplantar pustulosis
RA	Rheumatoid arthritis
RBP4	Retinol binding protein 4
RCT	Randomized controlled trials
SNP	Single nucleotide polymorphism
Th	T helper
TLR	Toll like receptor
TNF	Tumor necrosis factor
UC	Ulcerative colitis
VEGF	Vascular endothelial growth factor
QD	Once a day
QoL	Quality of life

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Curriculumv itae

Hindrik Hessel van der Zee werd op 23 november 1979 geboren als zoon van Siebe en Marijke van der Zee-van Schie te Leiderdorp. Hij groeide op met een zus in Hazerswoude-Dorp, Nijkerk en vanaf 1990 in Hillegom. In 1997 behaalde hij het HAVO diploma en in 1999 het VWO diploma aan het Fioretti college te Lisse. Dit zelfde jaar werd hij uitgeloot voor de studie geneeskunde en koos hij om bewegingswetenschappen te studeren aan de Vrije Universiteit te Amsterdam. In 2000 kon hij alsnog met de studie geneeskunde starten aan de Rijks Universiteit Groningen. Na het behalen van het doctoraal diploma in 2006 verhuisde hij naar Deventer waar hij zijn coschappen liep in het Deventer Ziekenhuis. Hier leerde hij Dermatoloog dr. Jurr Boer kennen die hem enthousiasmeerde voor de dermatologie en de ziekte hidradenitis suppurativa in het bijzonder. In 2008 liep hij zijn keuze-coschap dermatologie in het Erasmus MC, Rotterdam en verhuisde naar Den Haag. In datzelfde jaar deed hij zijn artsexamen en ging aansluitend werken als arts-assistent op de spoedeisende hulp in het Ruwaard van Putten Ziekenhuis te Spijkenisse. In april 2009 keerde hij terug in het Erasmus MC als arts-onderzoeker op de afdelingen Immunologie en Dermatologie en startte hij onder supervisie van profs. E.P. Prens J.D. Laman en R. Benner met het huidige proefschrift. Hij trouwde met Leonie van Wulften op 9 september 2010 en verhuisde naar Voorschoten. In januari 2012 zal hij starten met de opleiding tot dermatoloog in het Erasmus MC te Rotterdam. Zijn vrije tijd besteedt hij het liefst op de Waddeneilanden waar hij twee van zijn 3 hobby's kan beoefenen, wedstrijdzeilen en sportvissen. Zijn andere hobby behelst de sportwagen Corvette.

Publications

1. **van der Zee HH**, van de Scheur MR. Een man met verspreide gele bultjes. *Ned Tijdschr Geneeskd* 2007; **15**: 862.
2. **van der Zee HH**, Boer J, Prens EP, Jemec GB. The effect of combined treatment with oral clindamycin and oral rifampicin in patients with hidradenitis suppurativa. *Dermatology* 2009; **219**: 143-7.
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4. **van der Zee HH,** Hidradenitis suppurativa. *Derma Novum* 2010; **9**: 9-12.
5. **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.
6. **van der Zee HH**, de Ruiter L, van den Broecke DG, Dik WA, Laman JD, Prens EP. Elevated levels of TNF-α, IL-1β and IL-10 in hidradenitis suppurativa skin; a rationale for targeting TNF-α and IL-1β. *Br J Dermatol* 2011; **164**: 1292-8.
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10. Onderdijk AJ, **van der Zee HH**, Esmann S, Lophaven S, Dufour DN, Jemec GB, J. Boer J. depression in patients with hidradenitis suppurativa. Provisionally accepted.
11. **van der Zee HH**, Laman JD, de Ruiter L, Dik WA, Prens EP. Adalimumab (anti-TNF-α) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. Provisionally accepted.
12. Kimball AB, Kerdel F, Adams D, Mrowietz U, Gelfand JM, Gniadecki R, Prens EP, Schlessinger J, Zouboulis CC, **van der Zee HH**, Rosenfeld M, Mulani P, Gu Y, Paulson S, Okun M, Jemec GBE. Efficacy and safety of adalimumab for the treatment of moderate to severe hidradenitis suppurativa: results from a phase II, placebo-controlled, double-blind study. Submitted.
13. de Winter K, **van der Zee HH**, Prens EP. Hidradenitis suppurativa-like lesions induced by wearing a leg prosthesis: evidence for mechanical stress as pathogenic factor in hidradenitis suppurativa. Submitted.
14. **van der Zee HH**, Boer J, Prens EP. Hidradenitis suppurativa: a review and viewpoint on its pathogenesis. Submitted.

PhD portfolio

Name PhD student: H.H. van der Zee	PhD period: April 2008 till December 2011
Erasmus MC Departments of Immunology and Dermatology	Promotor(s): E.P Prens, R. Benner, J.D. Laman
Research School: MolMed	Supervisor: E.P Prens

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Biomedical english writing and communication	2009	4 ECTS
- Writing successful grant proposals	2009	9 hours
- BROK Cursus (Good clinical practice)	2009	20 hours
- Cursus management voor promovendi en postdocs (NIBI)	2009	24 hours
Research skills		
- Biostatistics for clinicians	2009	1 ECTS
- Introduction to clinical research	2009	1 ECTS
- SPSS course	2010	16 hours
In-depth courses (e.g. Research school, Medical Training)		
- Molmed molecular diagnostics III	2008	16 hours
- Course molecular medicine	2008	32 hours
- Molmed molecular microbiology of infectious disease	2008	8 hours
- Molmed annual course	2008	8 hours
- 3rd symposium and master classes on mucosal immunology	2008	16 hours
- Immunologie cursus Lunteren	2008	8 hours
- Cursus medische immunologie	2008	16 hours
- Biomedical research techniques 7	2009	24 hours
Presentations (oral)		
- Problems of the current HS scoring systems, European Academy of Dermatology and Venereology, Paris, France	2008	1 ECTS
- HS studies in Rotterdam, Skintermezzo, Rotterdam, The Netherlands	2008	1 ECTS
- Werkbespreking Immunologie, Rotterdam, The Netherlands	2008	1 ECTS
- De behandeling van HS, Skintermezzo, Rotterdam, The Netherlands	2009	1 ECTS
- HS scoring system, American Academy of Dermatology AD, San Francisco, USA	2009	1 ECTS
- How to help your HS patients, 5th Aegean Dermatology Symposium, Bodrum, Turkey	2009	1 ECTS
- Clindamycin and rifampicin for HS, Wetenschapdag, Deventer Ziekenhuis, Deventer, The Netherlands	2009	1 ECTS
- Werkbespreking Immunologie, Rotterdam, The Netherlands	2010	1 ECTS
- Deroofing for HS, skintermezzo, Rotterdam, The Netherlands	2010	1 ECTS
- HS treatment, hidradenitis suppurativa Vereniging, Utrecht, The Netherlands	2010	1 ECTS
- A new HS score, European Academy of Dermatology and Venereology, Gothenburg, Sweden	2010	1 ECTS
- Deroofing for HS, Dept. Surgery, Rotterdam, The Netherlands	2010	1 ECTS
- Werkbespreking Immunologie, Rotterdam, The Netherlands	2011	1 ECTS
- IL-1b, TNF-a and Il-10 elevated in HS, NVED, Lunteren, The Netherlands	2011	1 ECTS
- Deroofing for HS, Jozefprijs, Deventer Ziekenhuis, Deventer, The Netherlands	2011	1 ECTS
- Introduction and immunopathogenic mechanisms in HS pathology, EADV, Lissabon, Spain	2011	1 ECTS
- Werkbespreking Immunologie, Rotterdam, The Netherlands	2011	1 ECTS
Presentations (poster)		
- Poster NVED IL-1b elevated in HS lesions, NVED, Lunteren, The Netherlands	2011	1 ECTS
- Immunohistological alterations in peri, early and chronic hidradenitis suppurativa, ESDR, Barcelona, Spain	2011	1 ECTS

International conferences		
- European Academy of Dermatology and Venereology, Paris, France	2008	1 ECTS
- American Academy of Dermatology, San Francisco, USA	2009	1 ECTS
- 5th Aegean Dermatology Symposium, Bodrum, Turkey	2009	1 ECTS
- European Academy of Dermatology and Venereology, Berlin, Germany	2009	1 ECTS
- European Academy of Dermatology and Venereology, Gothenborg, Sweden	2010	1 ECTS
- European Academy of Dermatology and Venereology, Lissabon, Portugal	2011	1 ECTS
Seminars and workshops		
- Gata-3 in T-cell development and effector function, Rotterdam, The Netherlands	2008	5 hours
- Auto immunity to neural antigens in MS, Rotterdam, The Netherlands	2009	5 hours
- Interleukin-17 skin disorders, Rotterdam, The Netherlands	2009	5 hours
- Transcriptional control of B-cell development, Rotterdam, The Netherlands	2009	5 hours
- Ontstekingsziekten, Rotterdam, The Netherlands	2010	5 hours
Didactic skills		
- Teach the teacher	2008	16 hours
Other		
- Research meeting, Kopenhagen, Denmark	2008	20 hours
- Research meeting, Dessau, Germany	2009	10 hours
- Research meeting, Brussel, Belgium	2010	8 hours
- Research meeting, Brussel, Belgium	2011	8 hours
- HS session chair, European Academy of Dermatology and Venereology, Lissabon, Portugal	2011	
Occasional Reviewer for:		
- Dermatology	2009	4 hours
- Ned Tijdschrift voor Geneeskunde	2010	4 hours
- Journal American Academy Dermatology	2010	4 hours
- Dermatology Treatment	2010	4 hours
- Case Reports in Dermatology	2010	4 hours
- Experimental Dermatology	2011	4 hours
- Dermatologic Surgery	2011	4 hours

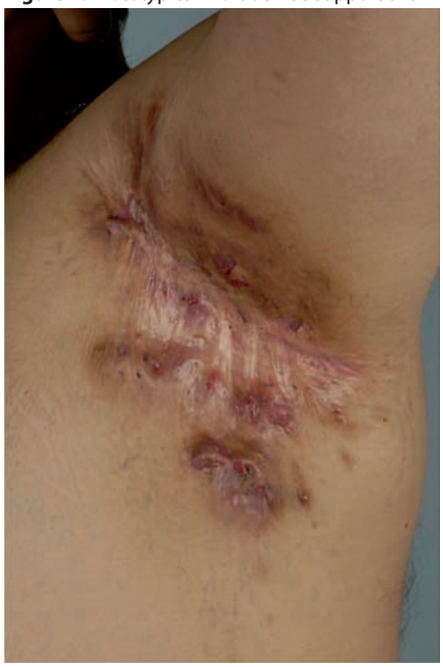
2. Teaching activities

	Year	Workload (Hours/ ECTS)
Lecturing		
- Immunological lymediated skin diseases	2010-2011	10 hours
Supervising practicals and excursions		
- Casuistiek onderwijs 2de jaars geneeskunde studenten	2009-2011	100 hours
Supervising Master's theses		
- Research thesis medical student Lisette de Ruiter	2010	
- Research thesis medical student Kim de Winter	2011	

Colors ection

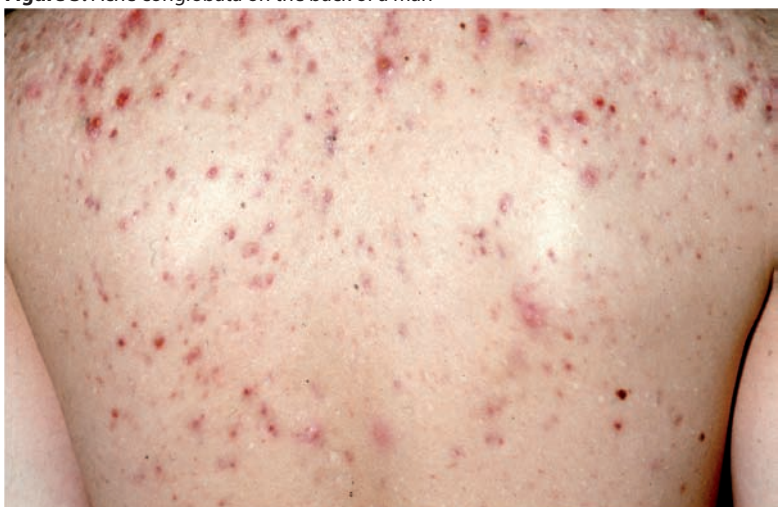
CHAPTER 1

Figure 1. Prototypical hidradenitis suppurativa



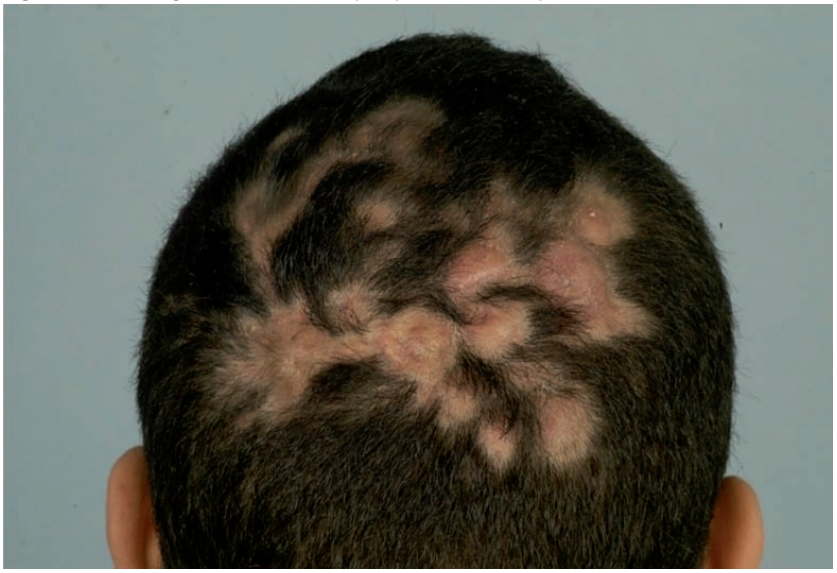
Axillary HS in a man (source Dept. Dermatology, Erasmus MC).

Figure 3. Acne conglobata on the back of a man



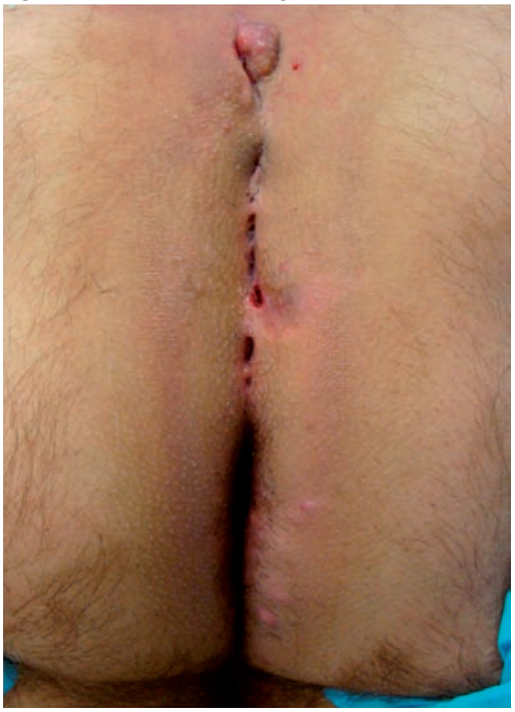
Acne conglobata is characterized by severe eruptive nodulocystic acne without systemic manifestations (source Dept. Dermatology, Erasmus MC).

Figure 4. Dissecting cellulitis of the scalp or perifolliculitis capitis et suffodiens



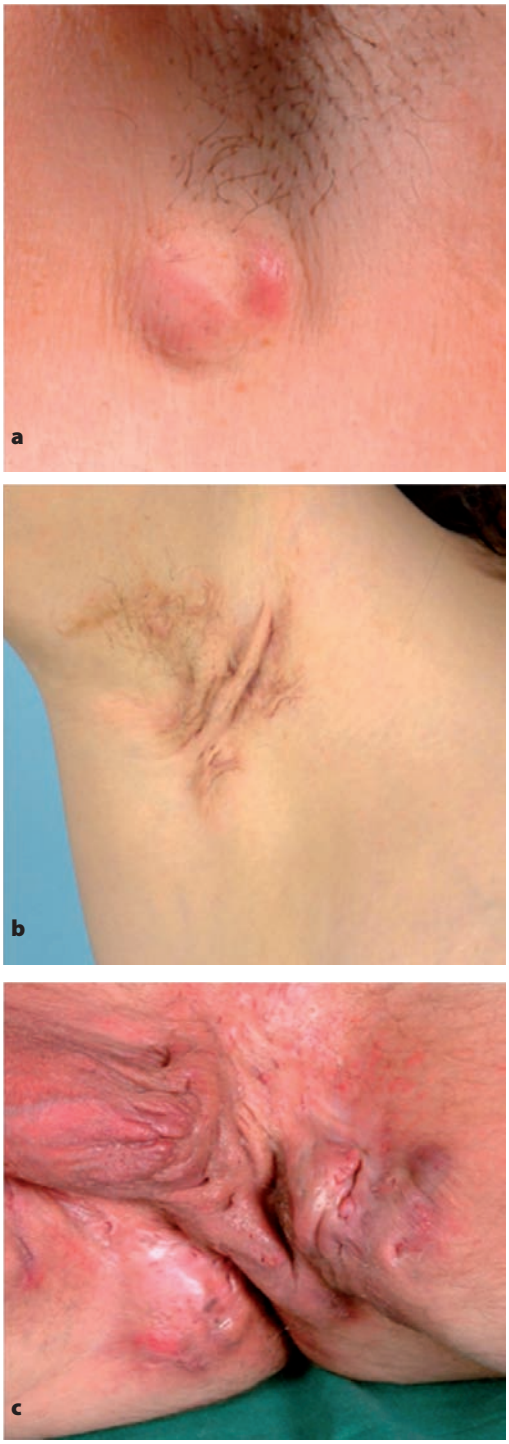
Dissecting cellulitis of the scalp is characterized by scarring alopecia with suppurating nodules and fistulas (source Dept. Dermatology, Erasmus MC).

Figure 5. Pilonidal sinus at the gluteal cleft



Pilonidal sinus characterized by inflammatory nodules and fistulas caused by ingrown hairs (source Dept. Dermatology, Erasmus MC).

Figure 6. Examples of HS by Hurley stage

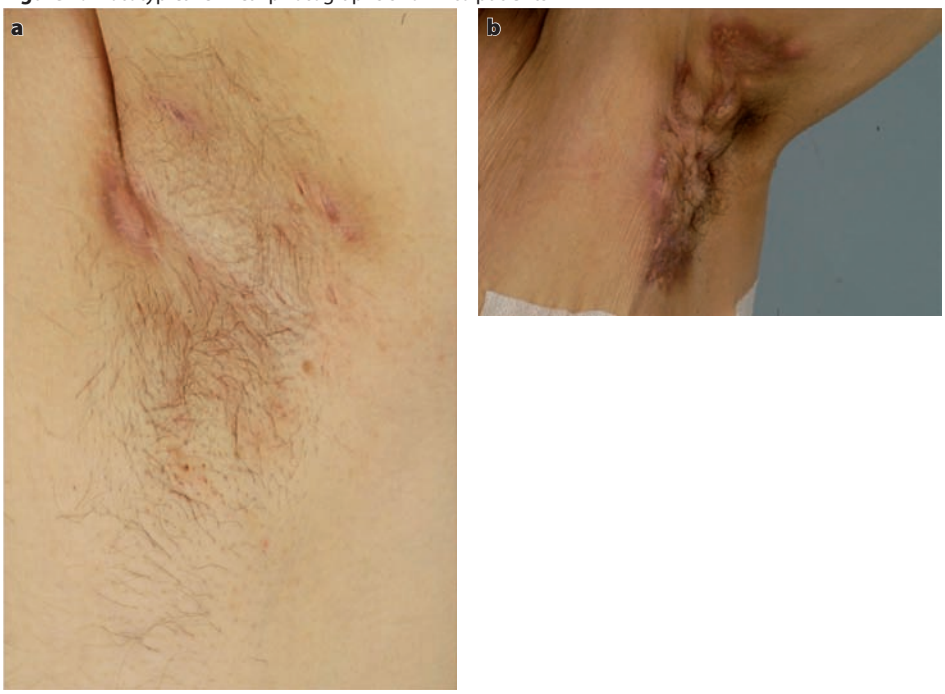


a) Hurley stage I HS in the axilla of a woman
b) Hurley stage II HS in the axilla of a woman
c) Hurley stage III HS in the groin of a man

All images: source Dr. J. Boer, Deventer Hospital, The Netherlands.

CHAPTER 2.2

Figure 1. Prototypical clinical photographs shown to patients



a) Mild hidradenitis suppurativa in the axilla.
b) Severe hidradenitis suppurativa in the axilla.

CHAPTER 2.3

Figure 1. Left lower leg stump with fistulas and follicular keratotic papules

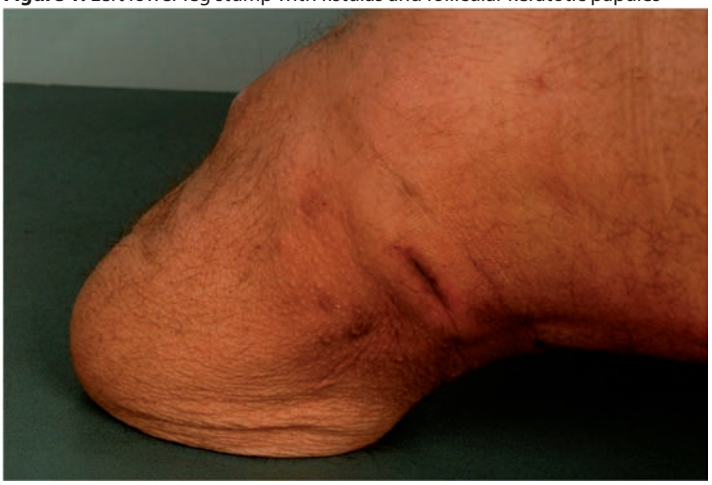


Figure 2. Detail of follicular keratotic papules on the medial side of the knee

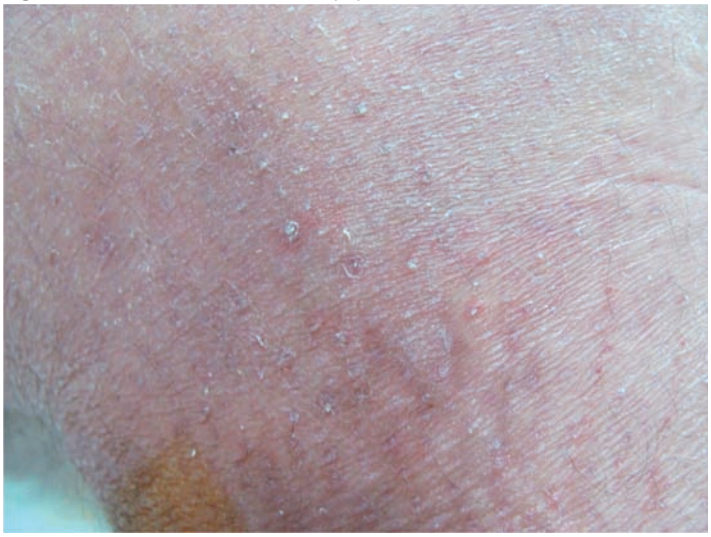


Figure 3. Probed fistula

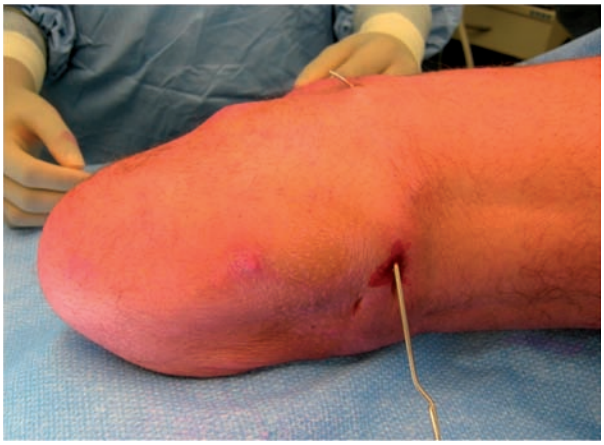
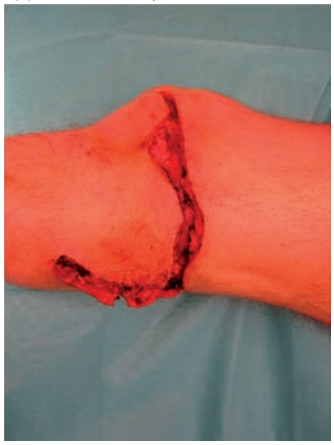
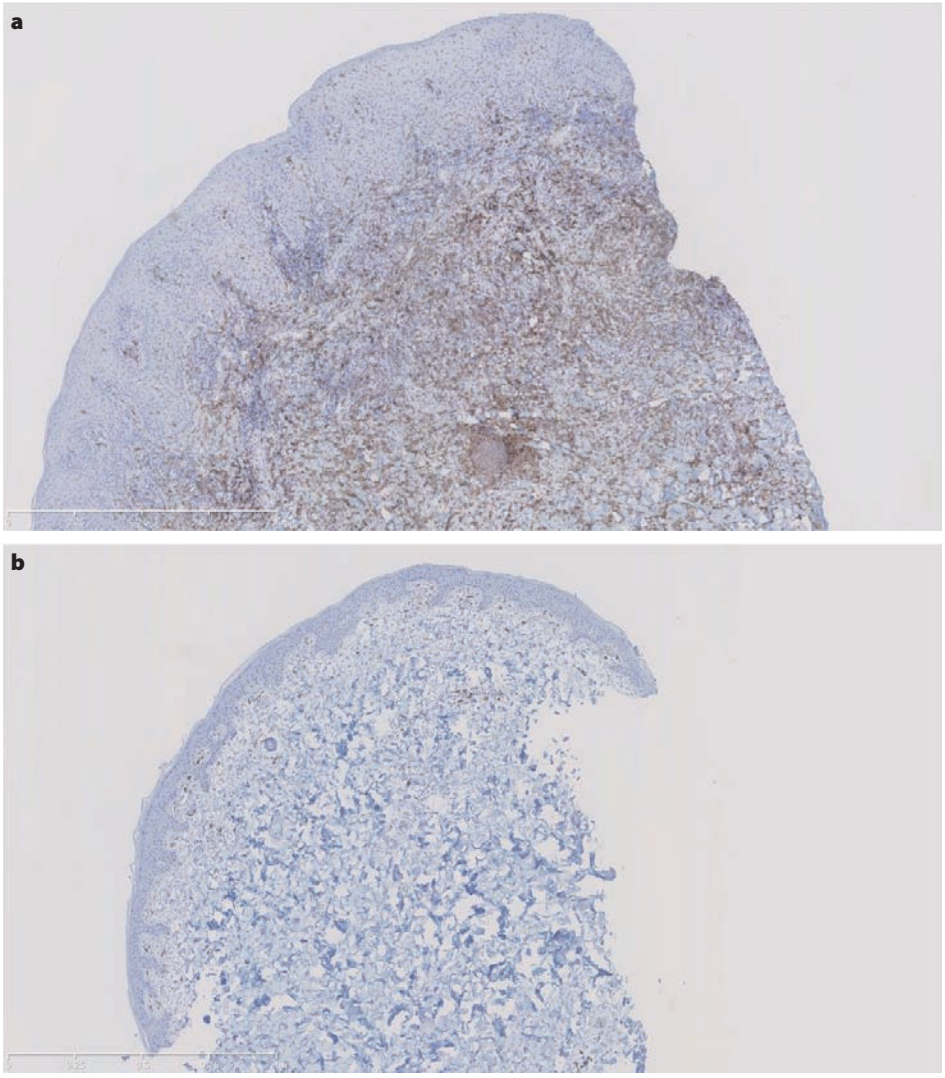


Figure 4. Extent of fistulas after surgical deroofing and slight approximation by sutures



CHAPTER 3.2

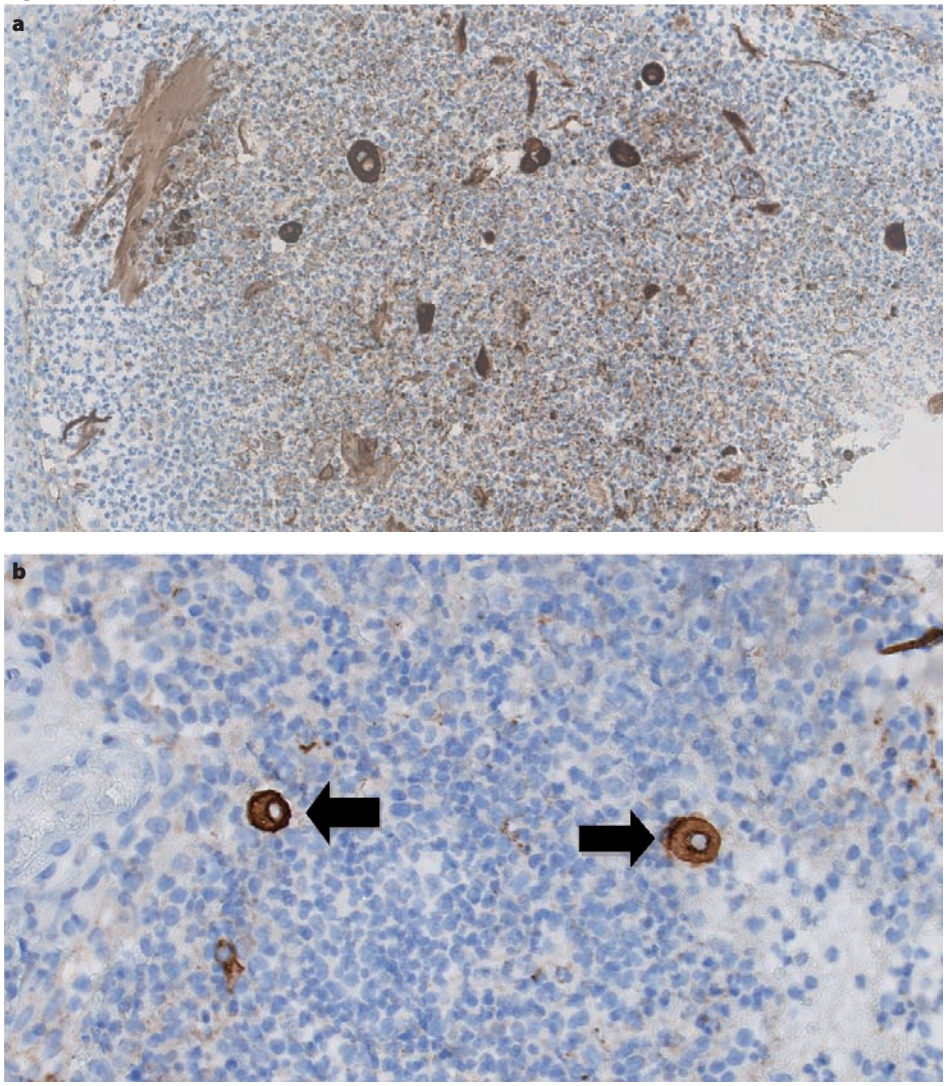
Figure 3. Immunohistochemical staining of CD11c$^+$ dendritic cells in the clincally improved patient 6 in group 2: adalimumab EOW.



a) Week 0 **b)** Week 16.

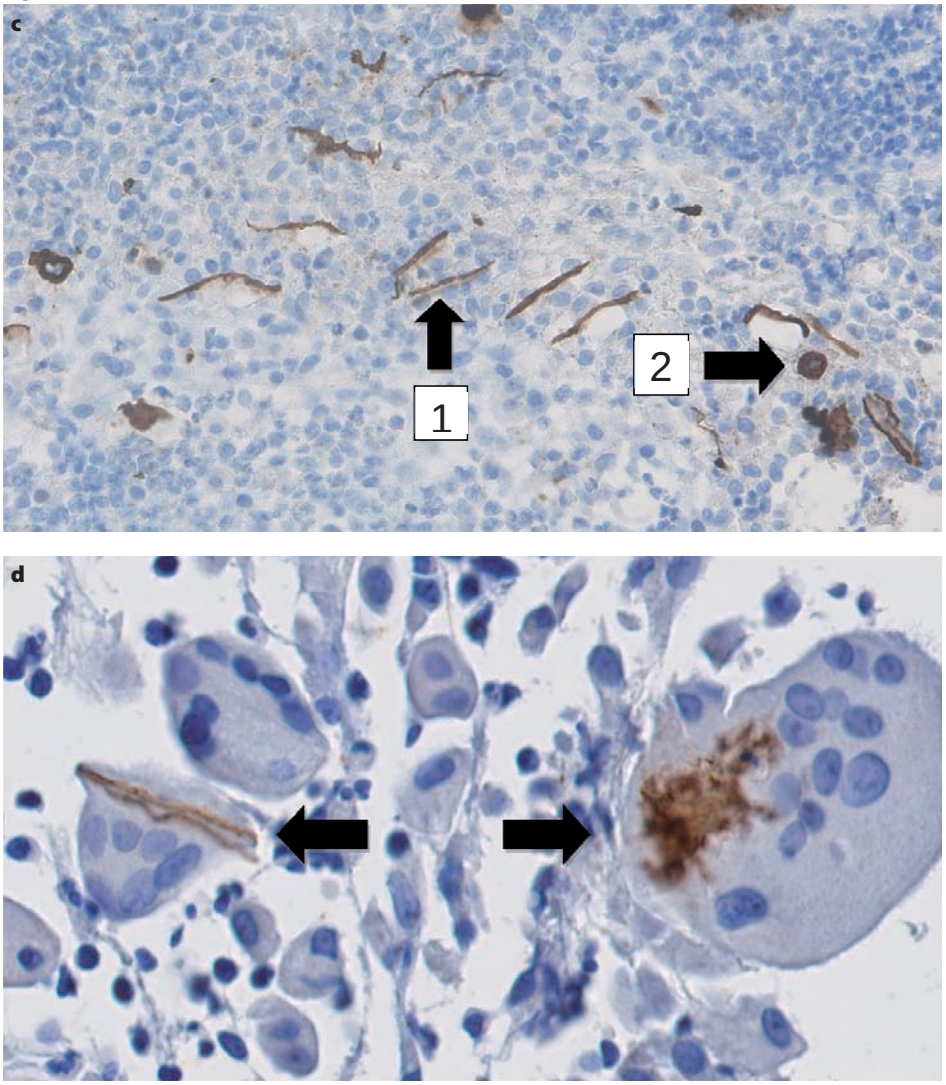
CHAPTER 3.3

Figure 2. Expression and distribution of keratin in HS lesions



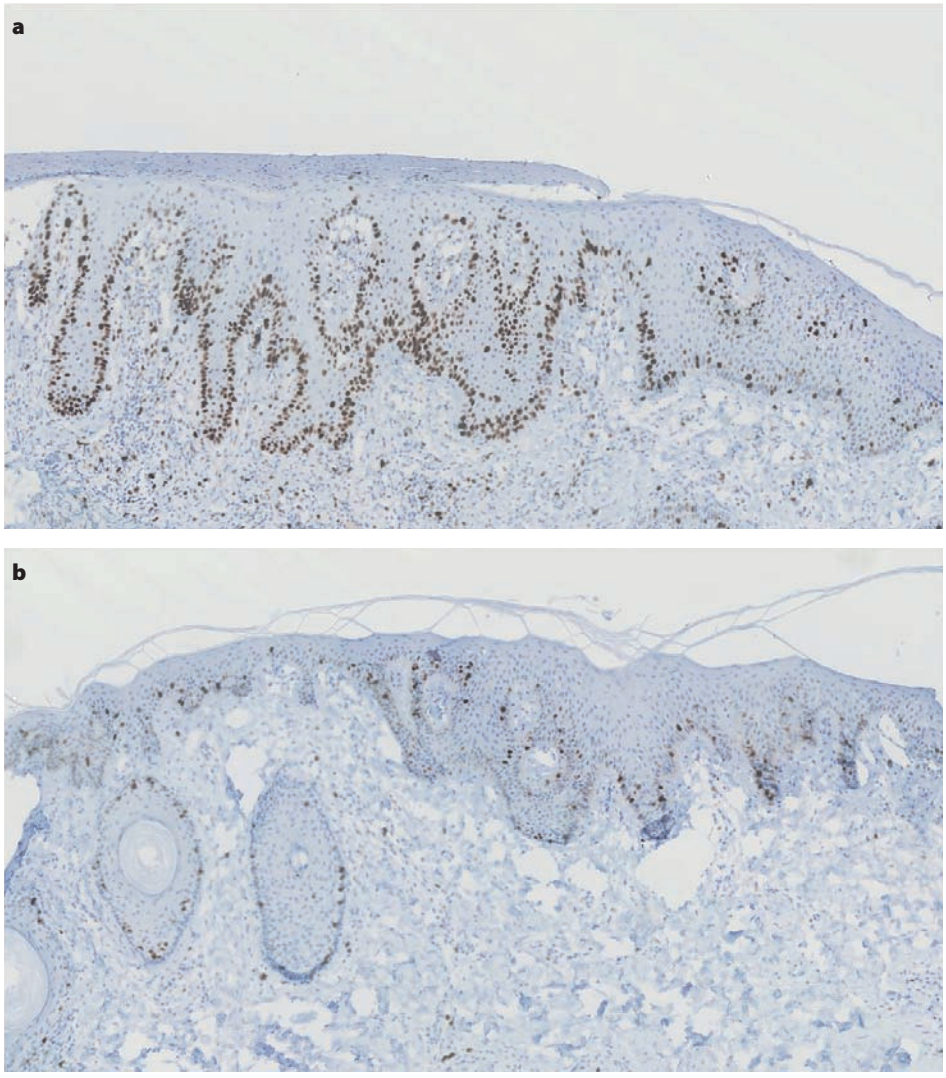
a) Keratin fibers and isolated detached keratinocytes (brown staining) in an abscess in a chronic HS lesion.
b) Single detached keratinocytes in the dermis (arrows) in a chronic HS lesion.

Figure 2. (continued)



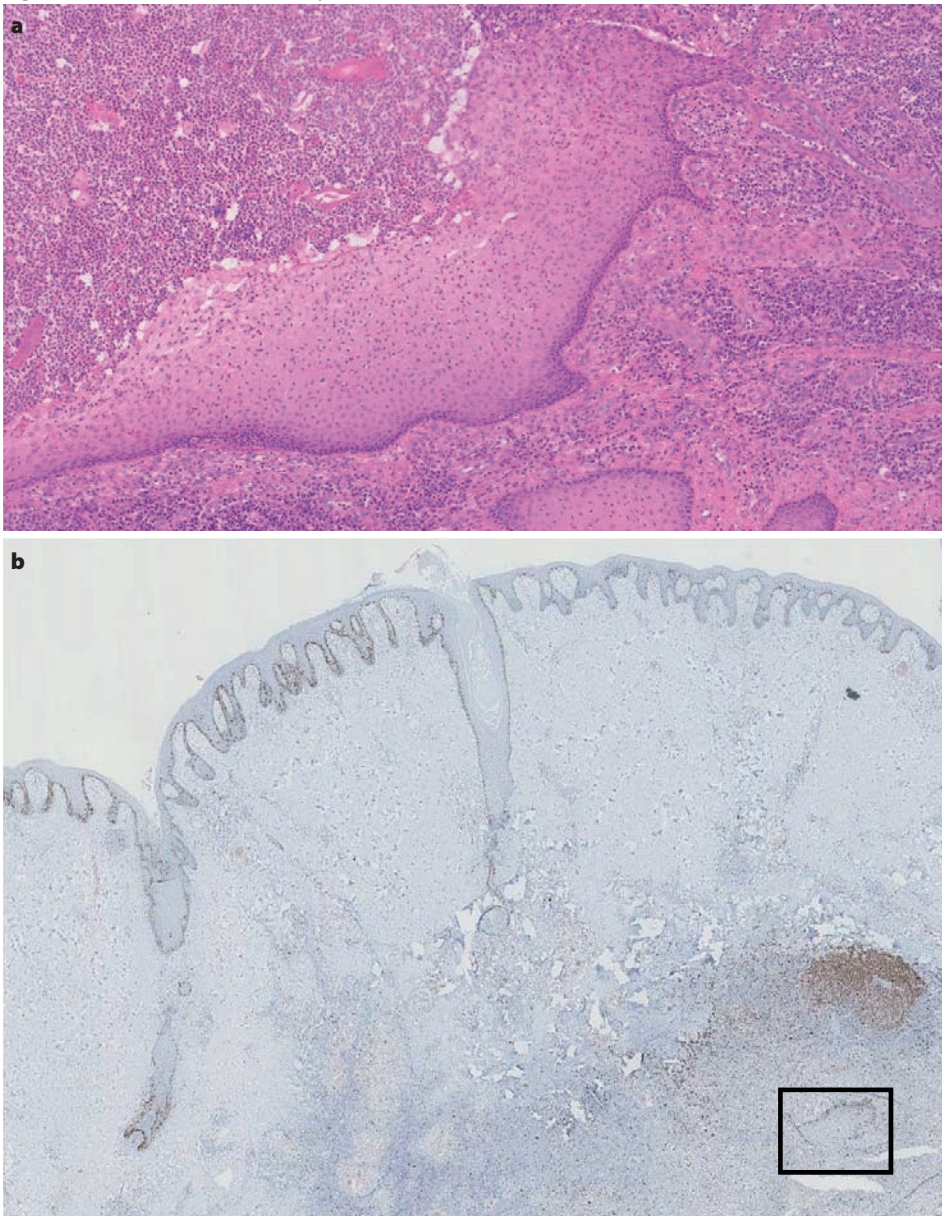
c) Free keratin fibers (arrow 1) and detached keratinocytes (arrow 2) in the dermis in an early lesion.
d) Phagocytosed keratin fibers and remnants in multinucleated giant cells, in an early lesion (indicated with arrows).

Figure 3. Psoriasiform epidermal hyperplasia



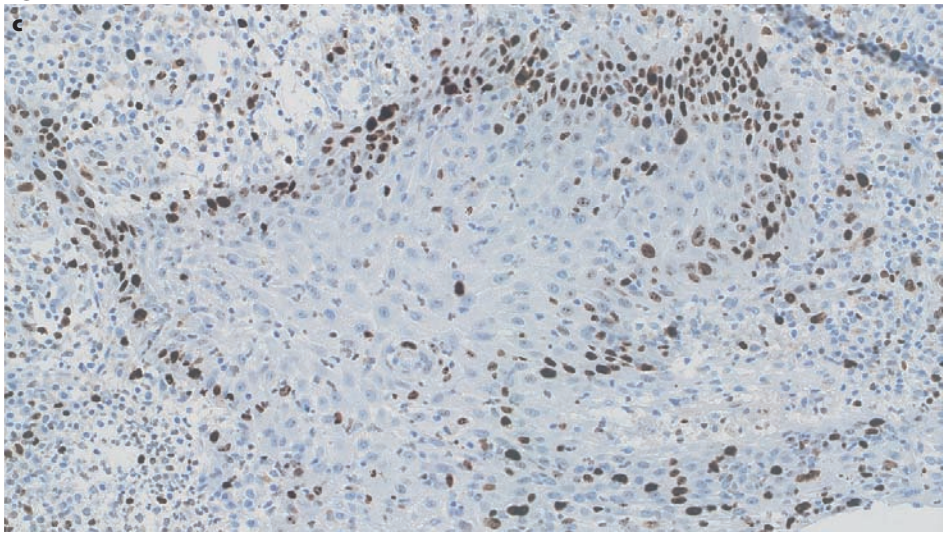
a) Psoriasiform epidermal hyperplasia with parakeratosis overlying an infiltrate in a chronic HS lesion. Basal epidermal keratinocytes frequently express Ki67, indicating proliferation.
b) The same sample adjacent to the infiltrate shows less pronounced psoriasiform epidermal hyperplasia and fewer Ki67 positive keratinocytes.

Figure 4. Strands of Ki67$^+$ keratinocytes in the dermis in a chronic HS lesion



a) Keratinocytes, in an epidermal strand in the dermis in a chronic lesion (H&E stained).
b) Overview of the same HS lesion, Ki67 stained, with an epidermal strand in the dermis indicated by the square.

Figure 4. (continued)



c) Higher magnification of this epidermal strand showing multiple Ki67+ keratinocytes.

Figure 5. Scattered CD20 positive B cells, and a prototypical patch, creating 'pseudo' lymphoid follicles in a chronic HS lesion

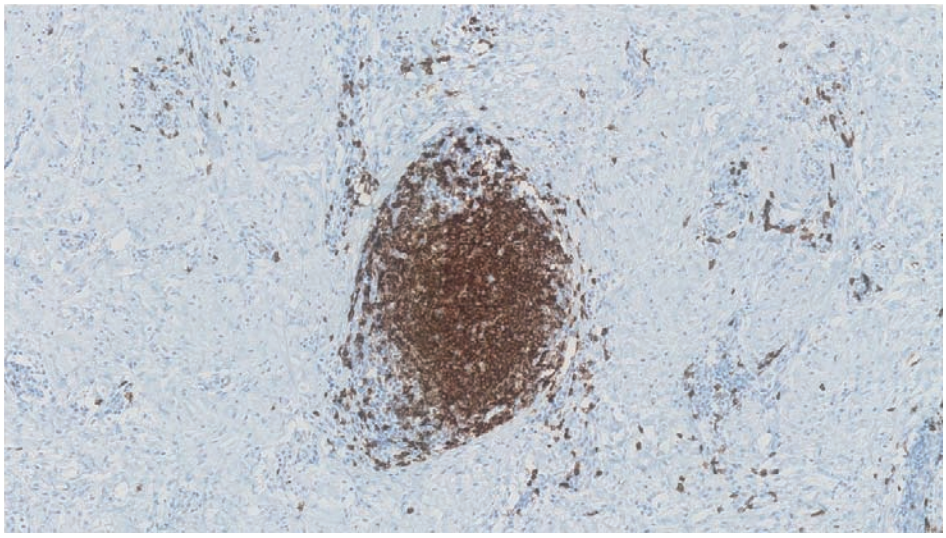
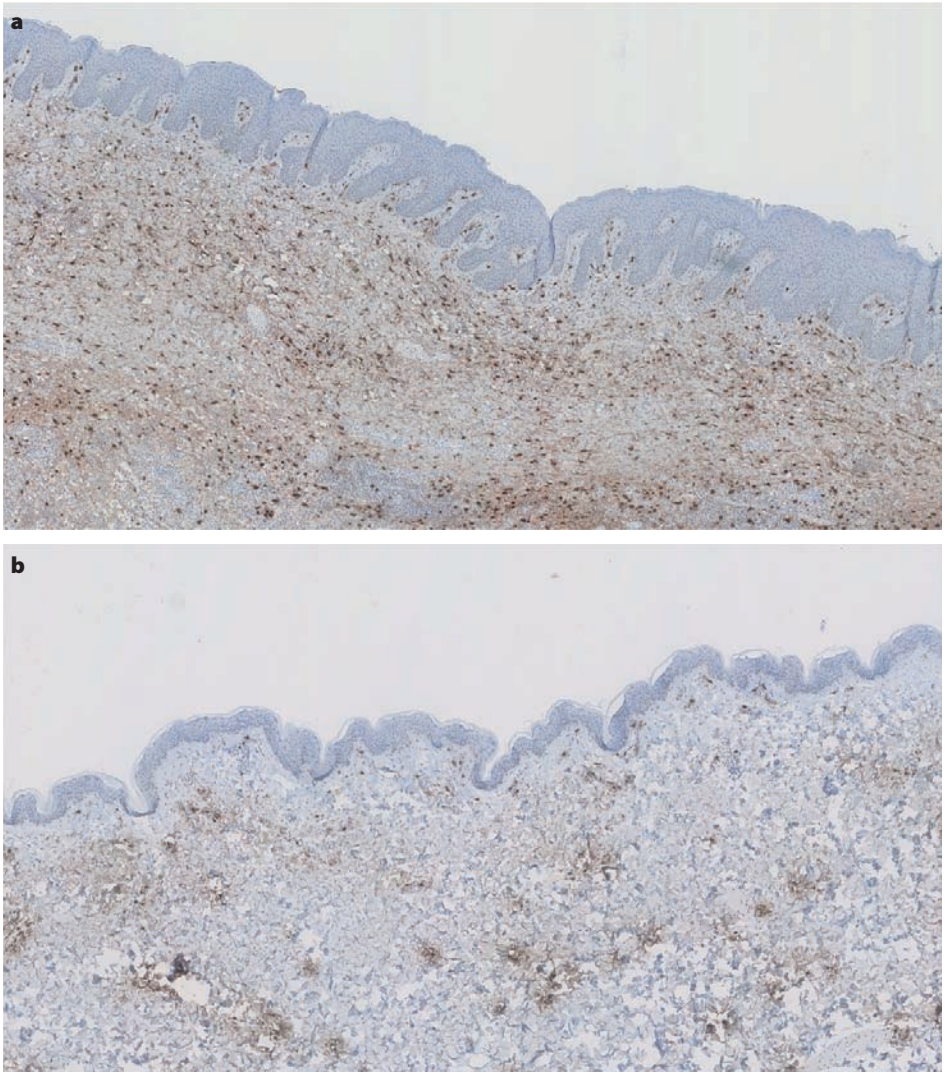


Figure 6. Increased number of tryptase positive mast cells in a chronic HS lesion



a) Tryptase positive mast cells in a chronic HS lesion.
b) Tryptase positive mast cells in healthy control skin.

CHAPTER 4.2

Figure 1. Draining HS nodule, located in groin (Patient 1)

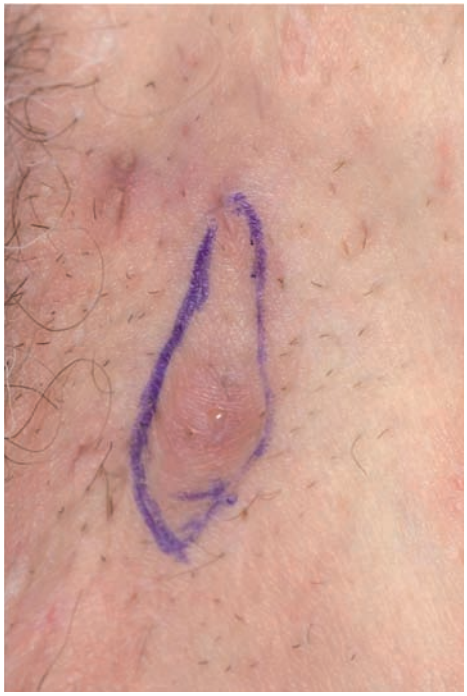


Figure 2. Blunt probe is inserted to explore extent of lesion (Patient 1)

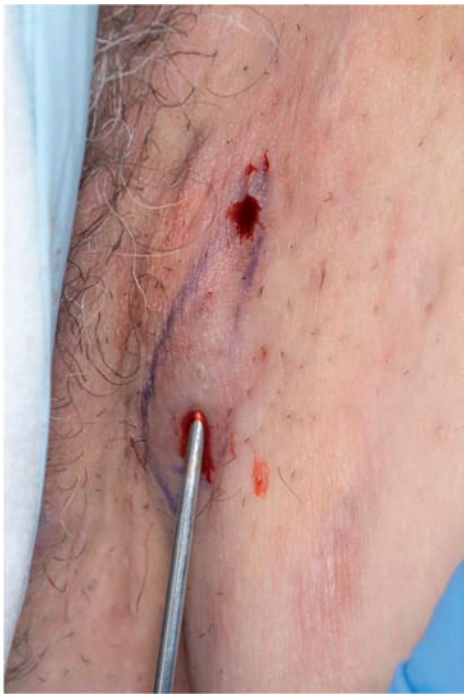


Figure 3. Sinus tracts can be unexpectedly long (Patient 2)

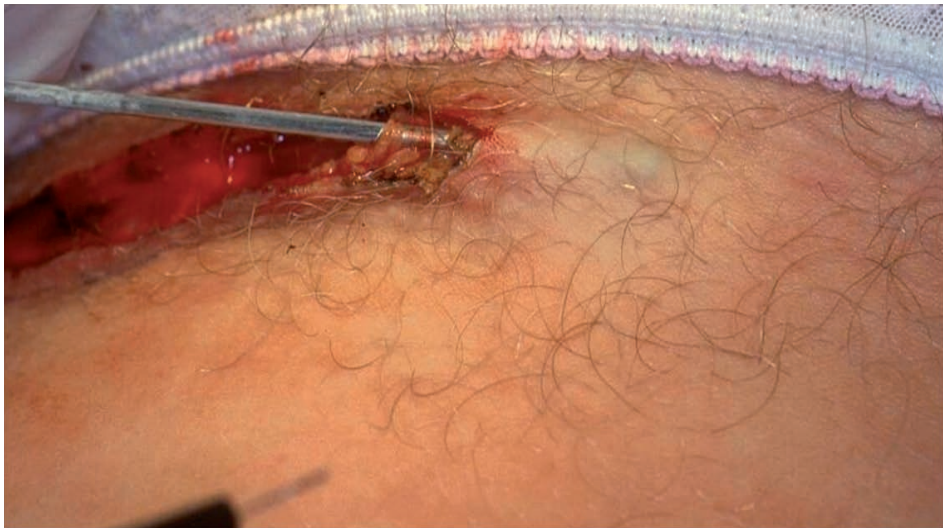


Figure 4. The roof of the lesion is removed and left for healing by second intention, after removal of debris by curette (Patient 2)

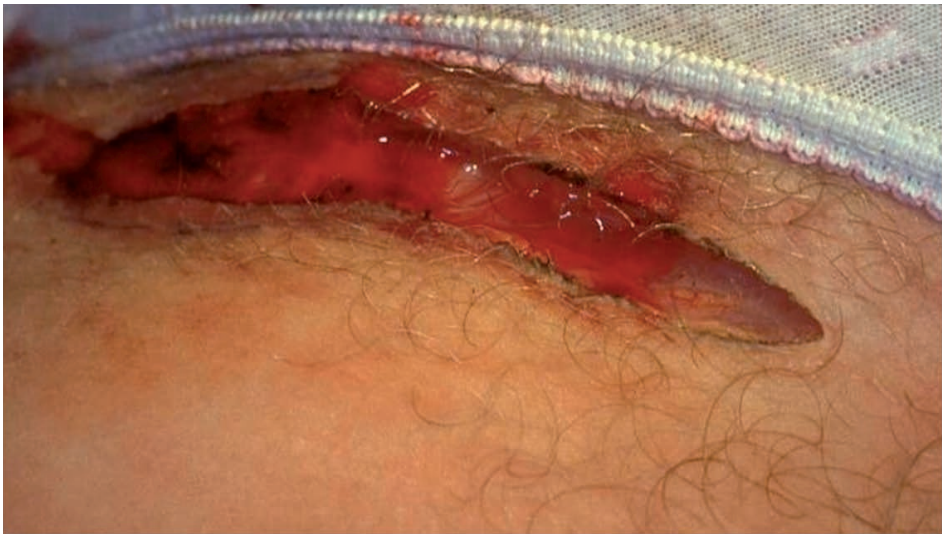
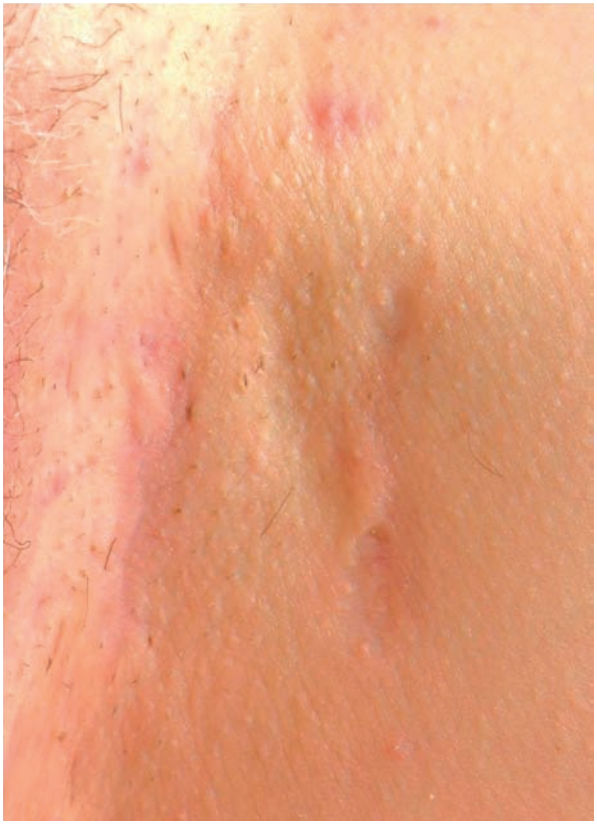
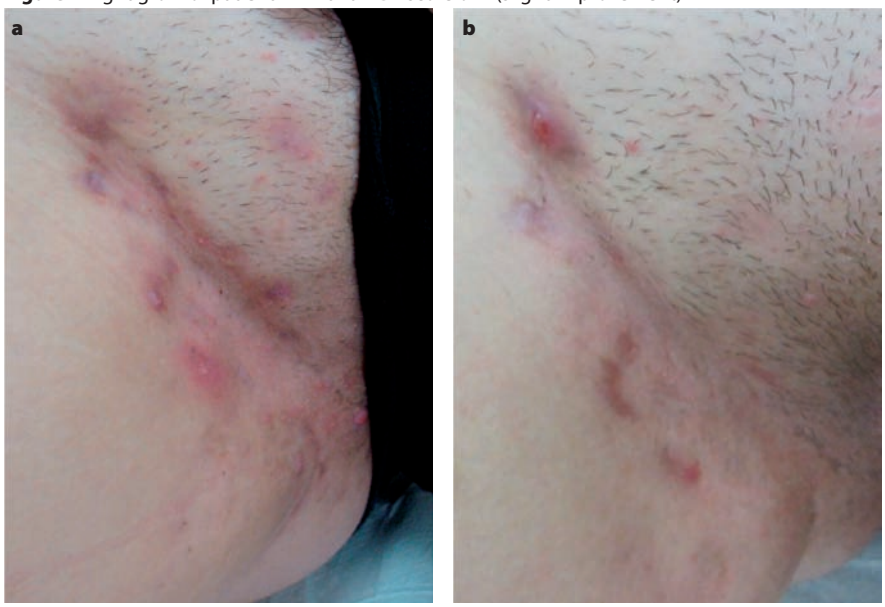


Figure 5. Lesion healed into a cosmetically acceptable scar. Result after 1 year (patient 1)



CHAPTER 4.3

Figure 1 Right groin of patient #1 with a PGA score of 1 (slight improvement)



a) at baseline; **b**) at 4 months

CHAPTER 5

Figure 1. The back of a German shepherd dog with GSP (source Dr. M.A. Wisselink)

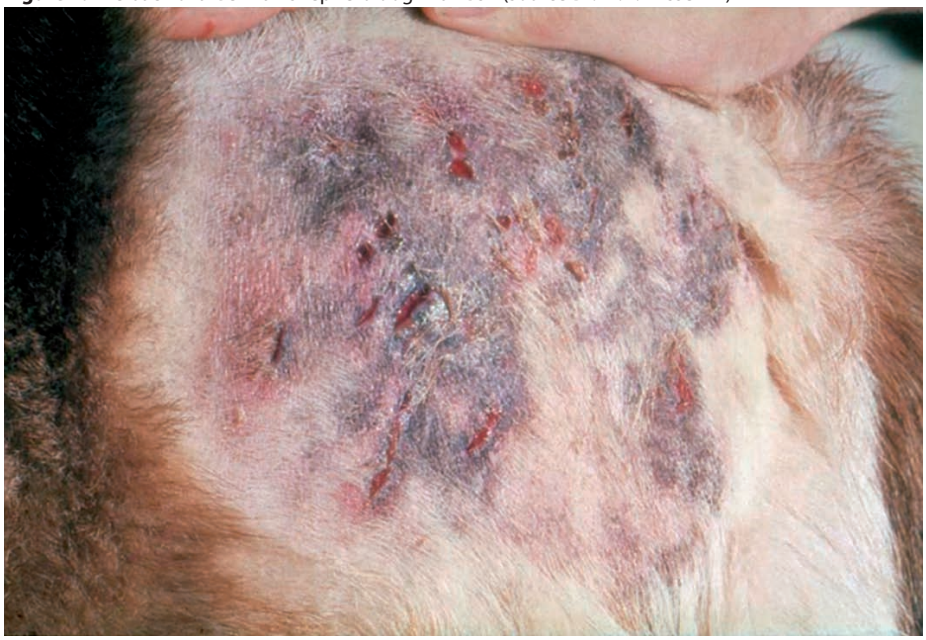


Figure 3. Key histological and immunological alterations in early and chronic HS skin