HIDRADENITIS SUPPURATIVA

clinical studies with focus on evaluation

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Stockholm 2010
To my family
ABSTRACT

Hidradenitis suppurativa (HS) is a chronic inflammatory disease of mainly unknown aetiology which in most cases involves axillae or groins and that can last for decades. In this thesis different clinical aspects of HS are studied: development of a clinical scoring system, relation to smoking and obesity, outcome of laser surgery, bacteraemia in HS patients and distribution of the neuroendocrine marker protein gene product (PGP) 9.5 in HS skin biopsies.

In Paper I the objective is to evaluate the modified Hidradenitis Suppurativa Score (HSS) and to study the impact of BMI and smoking habits on disease severity. Altogether 246 HS patients completed the Dermatology Life Quality Index (DLQI) questionnaire and 115 were scored by HSS. Points were given for regions, types of lesion (nodules, fistulas), total area involved, and whether lesions were separated by normal skin. A positive correlation of fair degree between HSS and DLQI was found, as well as significant higher median scores in more advanced HS, in smokers compared to non smokers and in obese women compared to those of normal weight, respectively. The results indicate that the HSS reflects disease severity, and may be a relevant outcome measure in clinical trials.

In Paper II scanner-assisted carbon dioxide laser treatment with subsequent healing by secondary intention is evaluated in 34 HS patients, with in total 67 operating sites. They were interviewed by telephone about recurrences and end results, after a mean follow-up time of 34.5 (range 7–87) months. Four patients had had recurrences in one of the treated areas. In twelve cases, lesions had developed separated from the initial surgical site by >5 cm. Twenty-five patients had flare-ups of HS lesions in another anatomical region. Eight had no symptoms of HS at all during the follow-up period. Mean healing time was 4 (range 3–5) weeks. The conclusion is that scanner-assisted carbon dioxide laser treatment of HS is an efficient treatment, well accepted by the patients.

In Paper III the objective is to determine the number and type of bacteria circulating in the bloodstream in HS patients undergoing carbon dioxide laser surgery. Blood samples were taken before, during and after surgery in 21 patients with HS Hurley stage II, and from five healthy controls. Bacterial growth in the first blood sample was found in nine patients, from the second in ten and from the third in six. In one patient, bacteria were detected in all the three samples. The dominating bacteria were coagulase-negative staphylococci, of which most were subtyped as *S. warneri*. In six patients all samples were negative, which may indicate that the method of surgery itself caused no spread of bacteria.

In Paper IV the presence and distribution of the nerve fibre-marker PGP 9.5 is investigated by immunohistochemistry. Biopsies were taken from the groin or axilla of 16 HS patients and 12 healthy controls. The median number of PGP 9.5 positive profiles was decreased in lesional epidermis, yet statistically significant only in the groin. A similar difference was found in lesional dermis of the axilla, whereas in the lesional upper dermis of the groin the median number of profiles was increased. Cells with strong PGP 9.5 immunofluorescence were few or absent in epidermis, but significantly increased in lesional dermal skin of the groin. It is possible that PGP 9.5 positive nerve fibres and cells have pathological roles in HS, but further investigations are needed.
LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


RELATED PUBLICATIONS


### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CGRP</td>
<td>Calcitonin gene-related peptide</td>
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<td>CO₂ (laser)</td>
<td>Carbon dioxide (laser)</td>
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<td>CoNS</td>
<td>Coagulase-negative staphylococci</td>
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<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<tr>
<td>DLQI</td>
<td>Dermatology life quality index</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<td>HS</td>
<td>Hidradenitis suppurativa</td>
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<td>HSS</td>
<td>Hidradenitis suppurativa score</td>
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<td>IQR</td>
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<td>NGF</td>
<td>Nerve growth factor</td>
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<td>p</td>
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<td>PGP (9.5)</td>
<td>Protein gene product (9.5)</td>
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<tr>
<td>R (Spearman)</td>
<td>Spearman's rho, rank correlation coefficient</td>
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<td>RCT</td>
<td>Randomized controlled trial</td>
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<td>SCC</td>
<td>Squamous cell carcinoma</td>
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<td>SCF</td>
<td>Stem cell factor</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SP</td>
<td>Substance P</td>
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<td>Sp</td>
<td>Species (singular)</td>
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<td>Spp</td>
<td>Species (plural)</td>
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<tr>
<td>STD</td>
<td>Sexually transmitted diseases</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha</td>
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<td>VAS</td>
<td>Visual analogue scale</td>
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1 INTRODUCTION
Hidradenitis suppurativa (HS, syn. acne inversa) is 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 (Dessau consensus definition). HS causes substantial negative impact on quality of life due to the burden of purulent discharge, odour and pain. The disease was described already in the 19th century by two French surgeons; in 1833 by Velpeau, who wrote about an axillary phlegmon with slowly and sometimes painful inflammation that usually ended in suppuration, and in 1854 by Verneuil, who localized the abscesses to the sweat glands and named the disease “hidrosadénite phlegmoneuse” in 1864. From the beginning of the 20th century it was considered a disorder of the apocrine glands, but more recent studies have described HS as a disorder of follicular rather than apocrine tissue, and have suggested that apocrine involvement rather is secondary to the process that involves the terminal hair follicle.

1.1 CLINICAL FEATURES
The predilected HS-regions are inverse areas such axillae and groins, but the gluteal, perineal or breast region are often affected as well, with differences between women and men. Genito-femoral lesions are more common in women while perianal lesions as well as buttocks are more common in men. Axillary involvement is common in both genders. HS is a clinical diagnosis based on three main features: deep seated nodules and or fibrosis, typical localisations and relapses and chronicity (Figure 1 and 2). Differential diagnoses include furunculosis and other staphylococcal skin infections, epidermoid cysts and in perianal cases sometimes Crohn’s disease. The 1-year prevalence of HS has been estimated to 1% in the general population in several studies, and a point prevalence of 4.1% was found among younger people attending a STD-clinic in Denmark. Onset of the disease is principally after puberty with a reported mean age of 21.8 years in a British study and median age at onset 20 years in a French study of 302 HS patients. There are some case reports of earlier onset, however. Women are more commonly affected than men with an estimated ratio of 2–5:1. In women, HS may persist to the climacteric, and onset after menopause is uncommon. About one third of the patients report a positive family history of similar symptoms in a first-degree relative. A familial form of HS with findings that support the concept of autosomal dominant inheritance has been described in Great Britain, as well as one disease gene locus in a Chinese family. The condition may remain relatively mild, but nevertheless distressing, ranging from a few but recalcitrant suppurating lesions to an advanced, widespread and disabling disease that lasts for years or decades. In a study of 110 patients with mean age 40.1 years, the reported mean duration of HS was 18.8 years, and the majority still had experienced active disease within the last year.

1.2 AGGRAVATING FACTORS
Smoking and obesity are frequent among HS patients and are considered plausible risk factors. König et al. found in a case-control study of 84 HS patients a rate of active cigarette smokers of 88.9% whereas 6.4% had never smoked.
An epidemiological study showed a strong association between HS prevalence and current smoking as well as with high body mass index (BMI).\textsuperscript{15} A correlation between increased BMI and HS severity has also been described.\textsuperscript{10} Aggravating factors mentioned by patients include sweating or heat, stress or fatigue and tight clothing or friction.\textsuperscript{16} So far it has not been proved that skin irritation, patterns in use of cosmetics, use of oral contraceptives or clinical signs of androgenisation would play a significant role in HS development.\textsuperscript{3, 24} However, aggravating factors in HS may be highly individual.

1.3 SCORING AND CLASSIFICATION

The best measures for clinical medicine should be acceptable to use in clinical or research settings and generate interpretable results. To use results of clinical trials to improve the quality of care, valid and interpretable severity scores are needed. Validity testing is a challenge when no “gold” standard exists and the variables are complex or multifactorial. Another issue of particular importance in dermatology is that patients with the same clinical picture and disease severity often experience their illness with varying impact on the well-being.\textsuperscript{25} For subjective measurement of the disease burden in dermatological conditions, the Dermatology Life Quality Index (DLQI) developed by Finlay and Khan,\textsuperscript{26} is the most established life quality instrument. HS causes a high degree of morbidity, with the highest scores obtained for the pain caused by the disease, von der Werth and Jemec concluded in a study that included 114 patients.\textsuperscript{5} The mean DLQI score for HS was higher than for previously studied skin diseases, and correlated with disease intensity, as expressed by lesions per month.\textsuperscript{5}

![Figure 1. An inflamed nodule in a mild case of HS (Hurley I).](image)

**Staging of HS according to Hurley**

Traditionally, the Hurley clinical grading system, with three stages that ranges from localised inflammation to fulminant disease, has been used.\textsuperscript{27} Hurley stage I consists of one or more abscesses with no sinus tract or cicatrisation and stage II consists of one or more widely separated recurrent abscesses, with a tract and scarring. The most severe cases (stage III) are described as having multiple interconnected tracts and abscesses throughout the entire affected area (Figure 1 and 2).\textsuperscript{27} Hurley clinical grading is well suited for classification or staging, and may serve as base for choice of treatment.
However, for clinical trials that require a more detailed measurement of changes it is important to have a more dynamic and precise scoring system, by adding clinical details to the staging. This led to the development that started a few years ago, of a proposed scoring system. This system has since then been continuously upgraded.

Figure 2 (a-c). Axillary lesions including nodules, scars, comedones and fistulas in three patients with (a, b) moderate (Hurley II) and (c) severe (Hurley III) hidradenitis suppurativa.

1.4 PATHOPHYSIOLOGICAL ASPECTS

HS has been known as a clinical entity since more than 150 years, but the etiopathogenesis is still unclear to a large extent, even though there are many hypotheses concerning infection, genetics, immunity, hormones, tobacco et cetera that are raised and discussed. Histopathological studies have shown that an early change in HS is hyperkeratosis of the infundibulum of the terminal hair follicle, which in turn give rise to horny filling, subsequent occlusion and dilation. Inflammatory changes occur, leading to perifolliculitis. The cellular infiltrate consists of neutrophils, lymphocytes and histiocytes. Another early inflammatory event is a segmental rupture of the follicular epithelium, followed by spilling of foreign body material, such as cornocytes, bacteria, sebum products, and hairs into the dermis. The dumping of foreign products initiates an inflammatory response provoking foreign body granuloma, and epithelial strands encapsulate the necrotic tissue. Yu and Cook observed squamous epithelium-lined structures that probably represent abnormal dilated hair follicles, which was a more constant diagnostic feature in hidradenitis suppurativa than inflammation of apocrine glands which appeared to be a secondary phenomenon. Attanoos et al. have concluded that follicular occlusion by keratinous material, with subsequent active folliculitis and secondary destruction of the skin adnexae and subcutis, occur as an integral step in the pathogenesis of HS. In chronic lesions an infiltrate, granulation tissue, giant cells subcutaneous abscesses and sinus tracts may be
found. One recently published histopathological investigation found, in addition to follicular hyperkeratosis, hyperplasia of follicular epithelium and perifolliculitis, also epidermal psoriasiform hyperplasia and a subepidermal interfollicular inflammatory infiltrate, where some of the lymphocytes showed epitheliotropism.

Sinus tracts, which are a hallmark of HS, represent a phenomenon where non-malignant keratinocytes proliferates to infiltrative growth. Epithelial lining in sinus tracts is heterogeneous and comprises different phenotypes of pathological squamous cell epithelium that expresses various cytokeratins and desmosomal proteins that differ from normal epithelium. Gniadecki and Jemec hypothesised that sinus formation could represent an aberrant epidermal repair response executed by an activated special subpopulation of stem cell like keratinocytes capable of non-malignant infiltrative growth in the dermis and subcutis.

Although the apocrine gland has been regarded to be affected as a secondary event, it may be hypothesised that there are factors, for instance an abnormal apocrine secretion as suggested by Revuz, which could be a triggering factor of HS. Another potential component of the pathogenesis could be the anti-microbial peptides, which in other conditions are known to exert both pro- and anti-inflammatory functions and induce keratinocyte proliferation. Smoking is a suspected risk factor, but without evidence of causality. In an experimental study of non-neuronal acetylcholine and its receptor in organotypic cultures and HS skin a strong immunoreactivity around follicular infundibulum has been shown. This could indicate a mechanism by which tobacco promotes infundibular epithelial hyperplasia and thus follicular plugging, and provide an explanation of why smokers are mostly affected by HS. It would also fit well with findings of microcomedones as an early pathogenic event as proposed by Sellheyer and Krahl. HS has some morphological similarities to acne, but in contrast to acne no significant abnormality in sebaceous gland activity has been shown.

1.4.1 Microbiology

The clinical relevance of bacterial findings in hidradenitis suppurativa (HS) is controversial. The bacteria are sometimes considered contaminants from the normal skin flora or as secondary infection in a previously sterile process. Interpretation of the results of bacteriological examinations from the surface of HS lesions is obscured by the possible contamination of resident skin bacteria. Bacterial cultures from HS lesions are often polymicrobial and have a predominance of anaerobic bacteria. In a study of axillary HS, the aerobic bacteria found were *S. aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The most frequently isolated anaerobes were *Peptostreptococcus* spp and *Prevotella* spp. Another study has shown that coagulase-negative staphylococci (CoNS) were the most common bacteria found in the deeper portions of chronic HS-lesions, and *Staphylococcus aureus* was the second. *Peptostreptococcus* was the most common anaerobic organism.

The normal microflora of the skin varies significantly depending on amount of sebaceous glands as well as if the areas are moist or dry. The prevalence of propionibacteria increases with age and the activation of the apocrine sweat glands at puberty plays an important role in the colonisation process. This may theoretically contribute to the age of onset of HS. Propionibacteria produce propionic acid and other enzymes that may contribute to tissue damage. Although *Propionibacterium* species are rarely regarded as
pathogens, these organisms cause serious infections in some cases.\textsuperscript{42} \textit{P. acnes} has been associated with late postoperative infections of implants such as prosthetic heart valves, intraocular lenses and shunts.\textsuperscript{43} Among the CoNS, \textit{Staphylococcus epidermidis} is an important resident, colonizing moist areas of the skin, preferentially in the upper part if the body.\textsuperscript{38} It is also commonly isolated from implants. The ability of these bacteria to colonise polymer surfaces and form a multi-layered biofilm is of particular importance in the pathogenesis of foreign body-associated infections. Other potential virulence factors of \textit{S. epidermidis} and \textit{S. warneri} include the formation of extracellular enzymes and toxins.\textsuperscript{44-46}

Simple infection is not the main cause of HS, but lesions may be worsened by secondary infection. In the later stages of the disease, bacterial infection seems to be a risk factor for destructive scarring and extension of the lesions.\textsuperscript{47}

1.4.2 The neuroendocrine system

Various inflammatory skin diseases such as atopic dermatitis, psoriasis, seborrhoeic eczema, prurigo nodularis and lichen planus have long been recognized by dermatologists to worsen in response to psychological stress.\textsuperscript{48} The underlying mechanism is not fully understood, but in recent years knowledge about some of the mechanisms behind the so-called neuroendocrine, or neuroimmunoendocrine system, has increased.\textsuperscript{49-55} The “brain-skin connection” includes neurohormones, neurotrophins, neurotransmitters and neuropeptides, which in response to stress can interact with the vascular, metabolic and immune system, with immunological changes that range from immunosuppression to inflammation.\textsuperscript{49} Between the nervous system, via activation of the hypothalamic-pituitary-adrenal (HPA)-axis with hormone release, upregulation of substance P (SP) and calcitonin gene-related peptide (CGRP), and the skin, which acts as both a target for and a producer of several of the substances, there is a cross-talk.\textsuperscript{49, 53, 56} These systems are part of the allostatics, a concept where physiologic systems within the body fluctuate to meet demands from external forces, e.g. stress.\textsuperscript{57} Among various stress mediators, CRH (corticotropin-releasing hormone), ACTH (adrenocorticotropic hormone), SP, CGRP, SCF (stem-cell factor) and NGF (nerve growth factor) activate mast-cells in the skin. In addition, the neuropeptides SP and NGF trigger mast-cell degranulation, with secretion of proinflammatory mediators and vasoactive substances, contributing to vasodilatation and inflammation.\textsuperscript{49}

Knowledge is sparse regarding involvement of the neuroendocrine system in HS, but in the light of the knowledge that certain neuropeptides play an important role in the pathogenesis of inflammatory skin diseases, it could be assumed that they are engaged in HS as well. There are several markers for the neuroendocrine system.\textsuperscript{53, 54, 58} One is the general neuronal marker protein gene product (PGP) 9.5,\textsuperscript{59} which is an ubiquitin C-terminal hydroxylase found in neurons and nerve fibers of central and peripheral nervous system, many neuroendocrine cells, renal tubules, spermatogonia, Leydig cells of testis and ova.\textsuperscript{60} The presence and distribution of PGP 9.5 has previously been studied in various dermatoses,\textsuperscript{61-69} but not in HS.

1.5 THERAPY

A number of treatments exist, based on whether HS is regarded as an infection, a type of acne or a separate inflammatory disease. Treatments with immunosuppressive
agents, anti-inflammatory drugs, antibiotics and oestrogens are of value in some patients. Currently, curative treatment consists mainly of surgery, but adjuvant medical treatment is often needed, and is in many cases appropriate as single therapy. The selection of treatment modality depends on several factors, including HS stage, anatomical location, frequency of exacerbation and the patient’s condition and preference.

1.5.1 Medical treatment

Several different types of topical therapy have been tried in HS, although there are few randomized controlled clinical trials. Topical treatment can be effective in milder cases and as adjunct therapy in more advanced disease, to reduce inflammation and suppuration and for maintenance of therapeutic results. The only topical treatment that so far has been studied in randomized controlled trials (RCT) is clindamycin, which has been shown superior to placebo. Clemmensen evaluated 27 patients that were treated with 1% clindamycin or placebo twice daily, with significant improvement of abscesses and pustules but not of inflammatory nodules at 1 and 2 months. Topical clindamycin has in another RCT been compared to systemic tetracycline in a double blind study of 46 HS patients. Both treatments showed a progressive improvement in patient and physician overall assessment during the three months; abscesses were reduced after three months, but the soreness score reported by the patients did not change. There were no significant differences between the two treatments. Azelaic acid, topical antibiotics other than clindamycin and corticosteroids have also been tested, but await formal studies. Intralesional steroid (triamcinolone) injection, which is effective in cystic acne, is sometimes used with effect on inflamed lesions.

Systemic treatment can be divided into antibiotics, retinoids, hormonal modulators and immunosuppressants. Tetracycline, as mentioned above, is effective in treatment for 3 months or longer. Long-term treatment with rifampicin and clindamycin in combination has been used, and recent publications report effect on severe HS. Unlike the results in treatment of severe acne, the effect of systemic retinoids, in particular isotretinoin, has not been satisfactory in HS, but still successful in some patients. A long-term follow-up of 68 patients treated with isotretinoin showed that 23.5% of the HS patients cleared during therapy and 16.2% maintained their clearance during the follow-up period of in mean 46 months. The generally unsatisfactory therapeutic effect of retinoids could at least partly be explained by the fact that sebum excretion is not increased in HS. Case reports and one RCT that evaluated combination therapy with cyproterone acetate have suggested that antiandrogen therapy may be of value in women with HS. A more recent study of 64 female HS patients found in a retrospective analysis that the response to antiandrogen therapy was superior to antibiotics; 55% vs. 26% of treated patients responded to the given therapy. Finasteride has also reported efficacy in small case series. Immunosuppressive therapies have been used with varying results. While methotrexate was of limited value in three patients, two cyclosporine-treated HS patients were improved, in line with two earlier case reports. Improvement was noted within 4–12 weeks in five patients treated with dapsone at doses ranging between 25 and 150 mg/day. All patients required maintenance therapy to sustain their disease control. Short courses of antibiotics or systemic steroids are sometimes used in periods of acute exacerbation. Interestingly, in a study of the natural history of HS, it was observed that the mean time for an acute boil to resolve was
6.9 days, which roughly equals a short antibiotic course. In recent years several publications of off-label treatment with tumour necrosis factor-alpha (TNF-α) inhibitors (infliximab, etanercept, adalimumab) describe varying, but mostly positive effect on HS. Initially, patients with Crohn’s disease with concomitant HS showed improvement when treated with infliximab. All medical treatment options mentioned have potential adverse effects and contraindications that need to be taken into consideration, in addition to the patient’s circumstances and disease burden.

1.5.2 Surgery

For early disease stages, smaller local excisions can be performed, but for more widespread disease wide excisions, beyond the clinical borders of activity, are needed, regardless of the location. It has been shown that the extent of surgery affects the recurrence rates and the disease-free interval after surgery. HS recurred in patients who had undergone drainage procedures only, and incision by lancing should therefore be avoided. After limited excision and radical wide excision the recurrence rates were lower. Kagan et al reported a surgical treatment algorithm based on the extent of involvement, chronicity, and comorbid conditions, where excision and primary closure was used for localized disease; wide excision with or without skin grafting was used for diffuse disease. After wide excision the skin grafting and flaps sometimes brings less good cosmetic outcome and discomfort including the donor site, and healing by granulation (secondary intention) would be preferred, as shown in some publications. However, the wound healing time may be very long, up to three months after large excision areas. Treated patients have in another study of secondary intention healing reported minimal inconvenience, analgesic requirements, or interruption of daily activities.

1.5.3 Carbon dioxide laser surgery

The use of carbon dioxide laser treatment followed by secondary intention healing in HS was first described by Dalrymple and Monaghan, then by several authors during the past two decades. The CO2-laser emits a beam at a wavelength of 10600 nm that is absorbed by biological tissues since the target of interaction is water. Small lymphatic vessels as well as blood vessels less than approximately 0.5 cm in diameter are sealed with the laser, resulting in an almost bloodless operating field and potentially less postoperative edema. It can be used with a small-diameter spot size to cut or a broad-diameter spot to vaporise tissue. In HS cases the aim is to achieve complete radical ablation of diseased tissue combined with preservation of healthy tissue. Previously, a free-hand technique was used, but nowadays the procedure is facilitated and also safer by using a carbon dioxide laser with a microprocessor-controlled flash scanner that ablates the tissue in an even and controlled manner. Also, thermal diffusion is time dependent and can be reduced if the laser energy is delivered to the tissue for less than the thermal relaxation time, by scanning the laser beam rapidly over the tissue area in a spiral type pattern that maintains laser exposure of any spot to less than a millisecond, and therefore reducing the risk of necrosis or thermal injury of adjacent tissue. Two recent publications evaluate CO2-laser treatment in cutting mode in nine cases of recalcitrant disease, and in 61 patients using the laser excision and marsupialisation technique, with low recurrence rates and high degree of patient satisfaction.
Healing time by secondary intention after CO\textsubscript{2}-laser of HS has been reported to 3–8 weeks\textsuperscript{98, 99} although some cases of delayed healing may occur.

1.6 ASSOCIATED DISEASES

HS has been associated with various dermatologic disorders including Dowling Degos disease and the follicular occlusion tetrad, but also other conditions, for example arthritis and Crohn’s disease.\textsuperscript{104} The follicular occlusion tetrad consists of acne conglobata, hidradenitis suppurativa, dissecting folliculitis of the scalp and pilonidal sinus.\textsuperscript{2} Scrotal or pubic lymph edema or elephantiasis may arise as a complication of chronic inflammation and repeated episodes of cellulitis. Association between HS and Crohn’s disease have been recognized in several case reports,\textsuperscript{105} and in a recent a pilot study 158 patients with inflammatory bowel disease (IBD) were interviewed about recurrent painful boils in the axillae and/or groin.\textsuperscript{106} Twenty-five people (16\%) responded that they had experienced painful boils in the axillae and/or groin, of whom 17 were patients with Crohn’s disease (17\%) and eight had ulcerous colitis (14\%).\textsuperscript{106}

Longstanding cases of HS may be complicated by squamous cell cancer (SCC), secondary to chronic inflammation.\textsuperscript{3, 16, 107, 108} In a Swedish study it was found that the risk of developing any cancer increased by 50\% (relative risk of 1.5) in a cohort of 2119 patients that had been hospitalized for HS, compared with the age- and sex-matched general population during the time of the study, based on 73 observed cases.\textsuperscript{107} Statistically significant risk elevations were observed for squamous cell carcinoma (SCC) of the skin (5 cases), buccal cancer (5 cases), and primary liver cancer (3 cases), but the latter two associations should be interpreted cautiously because of multiple significance testing and few observed cases that may have generated chance findings.\textsuperscript{107} Almost all HS associated SCC are located in the gluteal area, and are mostly seen in men.\textsuperscript{11} Constantinou et al.\textsuperscript{108} reviewed in 2008 forty-three published cases of SCC as a complication to HS. Thirty-six cases had perianal location and mean duration of HS before development of SCC was 24 years. It was in that review concluded that although HS is a common condition, it rarely is associated with SCC.\textsuperscript{108}
2 AIMS

In recent years more attention has been paid to hidradenitis suppurativa (HS) and new therapies are discussed, which requires tools to measure disease activity and treatment outcome. The general aim of this project was to develop an objective scoring system for HS, in order to evaluate disease severity and treatment methods including carbon dioxide (CO<sub>2</sub>)-laser. The aim was also to study some aspects of potentially pathogenic events in HS.

The specific aims of the individual papers were:

**Paper I:** To evaluate the modified Hidradenitis Suppurativa Score (HSS) and to study the impact of body mass index (BMI) and smoking habits on disease severity. In addition life quality, measured by the Dermatology Life Quality Index (DLQI), in HS patients was investigated and correlated to HSS.

**Paper II:** To evaluate a surgical method for treatment of Hurley stage II hidradenitis suppurativa; vaporisation by CO<sub>2</sub>-laser with micro-processor controlled scanner in continuous mode, with subsequent healing by secondary intention.

**Paper III:** To determine the number and type of bacteria circulating in the bloodstream in patients with HS undergoing surgical treatment with the carbon dioxide laser vaporisation technique.

**Paper IV:** To investigate the presence and density of the nerve fibre-marker protein gene product 9.5 (PGP 9.5), by immunohistochemistry, in skin biopsies from patients with hidradenitis suppurativa.
3 MATERIALS AND METHODS

3.1 SUBJECTS

The patients were selected as consecutive clinical cases referred to a centre with special interest in the disease at Department of Dermatology, Karolinska University Hospital, Huddinge, Sweden. The diagnosis of HS was based on history and clinical presentation at the examination. The study patients and control subjects gave informed consent and the local ethics committee had approved the study protocols.

**Paper I:** The patients were selected as consecutive clinical cases referred during seven years (January 2001 – April 2008). 251 patients were included, of which 246 completed the DLQI questionnaire, and 115 were assessed by HSS (2004 and onwards). The comparison of DLQI and HSS is based on the 110 patients that were evaluated both by HSS and DLQI. 11 patients were excluded because of psychiatric disorder, mental retardation or language difficulties.

**Paper II:** Thirty-four patients were evaluated after treatment; 31 women and 3 men, with a mean age of 33.9 (range 15–55) years, who had had HS for a mean of 13.4 (range 1–35) years and more than 3 recurrences of suppurating lesions in the year before inclusion in the study. All lesions had been classified as Hurley stage II. The mean follow-up time after carbon dioxide laser surgery was 34.5 (range 7–87) months.

**Paper III:** A total of twenty-one HS patients (20 females and 1 male) with a mean age of 36 (range 20–55) years, were included in the study. The patients were undergoing carbon dioxide laser surgery. Five healthy persons, all females, with a mean age of 36 (range 23–48) years and not undergoing any kind of operation, were used as controls.

**Paper IV:** Sixteen HS patients (14 females and 2 males) with a mean age of 41 (range 25–61) years were included in the study. All patients had had one or more active suppurating lesion in the groin or axilla during the last 12 months. The patients were selected as consecutive clinical cases, December 2005 – January 2007. Twelve healthy persons (9 females and 3 males) with a mean age of 41.5 (range 29–59) years were used as controls.

3.2 METHODS

3.2.1 Clinical procedures

Hidradenitis Suppurativa Score (HSS) (Paper I)

The HSS is a revised version of a former suggestion for uniform variables when evaluating treatment outcome in HS. The following is recorded by the dermatologist: the anatomical regions involved; axilla, groin, gluteal (left/right) or other region, 3 points per region. The number and scores of lesions (nodule 1, fistula 6 points) are calculated for each region. The longest distance between two relevant lesions (or size of lesion if single) in each region: less than 5 cm; 1, 5–10 cm; 3, more than 10 cm; 9 points. Are all lesions separated by normal skin? Yes: 0, No (= Hurley III): 9 points. Regional scores are added, resulting in the patient’s total score, which has an open upper limit of the scale. The HSS protocol is shown in Figure 3.
A supplementary subjective scoring by the patient is also recommended; soreness or pain of the most symptomatic lesion at the time of consultation, by using a visual analogue scale (VAS), graded 0–10. This value is not included in the calculation of the score.

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<th>Parameter</th>
<th>Right Axilla</th>
<th>Left Axilla</th>
<th>Total sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>noduli &amp; fistulae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>longest distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurley III no/yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Groin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>noduli &amp; fistulae</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>longest distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurley III no/yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right Gluteal Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>noduli &amp; fistulae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>longest distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurley III no/yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>noduli &amp; fistulae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>longest distance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hurley III no/yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patient Report
(not included in the score):

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Right Axilla</th>
<th>Left Groin</th>
<th>Right Gluteal Region</th>
<th>Other Region</th>
<th>Total sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of boils during the latest month:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soreness of most symptomatic lesion: VAS (0 - 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend to Hidradenitis Suppurativa

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Points/parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Number of regions</td>
<td>3 points per region</td>
</tr>
<tr>
<td>2. Number and severity of lesions</td>
<td>noduli 1 fistulae 6</td>
</tr>
<tr>
<td>3. Longest distance between two relevant lesions</td>
<td>&lt; 5 cm 1 5 - 10 cm 3 &gt; 10 cm 9</td>
</tr>
<tr>
<td>4. Lesions clearly separated by normal skin?</td>
<td>yes 0 no (Hurley III) 9</td>
</tr>
</tbody>
</table>
**Dermatology Life Quality Index (DLQI) (Paper I)**

The index consists of ten questions regarding the impact of the skin disease on feelings and different aspects of daily life activities during the latest week. Each question is scored by the patient from 0 (not at all) to 3 (very much). A total of 30 points is the maximum score, where 0–1 is regarded as no effect, 2–5 small, 6–10 moderate, 11–20 very large and 21–30 as extremely large effect on the patient’s life. An authorized Swedish translation of DLQI was used in the study.

**Anaesthesia (Paper II, III and IV)**

The affected area was outlined with ink. After cleaning of the skin with 0.05 mg/ml chlorhexidine solution, the area was anaesthetised by injecting lidocaine 0.5–1.0 mg/ml and epinephrine (Xylocaine®adrenalin, AstraZeneca, Södertälje, Sweden). The solution was injected and infiltrated around and not directly into the affected site to avoid direct contact with inflamed tissue and injection into the abscess. In some cases a lidocaine prilocaine cream (EMLA®, AstraZeneca) was applied an hour before the injections, to reduce pain.

**Scanner-assisted carbon dioxide laser surgery (Paper II and III)**

A Sharplan carbon dioxide laser 1030® (Lumenis Inc., New York, NY, USA) with a focusing hand-piece attached to the miniature optomechanical flash scanner delivery system, SilkTouch® (Lumenis Inc.) has been used. Since 2005 we have also used a Unilas®10600 carbon dioxide laser (Limmer Laser, GmbH, Berlin, Germany) with Surgiscan® scanner, in mode Powerscan. Figure 4 shows the two lasers. The scanner device generates a focal spot which rapidly and homogeneously spiral scans and covers a round area of tissue in the focal plane. The area selected is ablated with the laser beam by passing it over the tissues and this is followed by repeated ablations in the same manner after removing devitalised tissues by cleaning the surface with a swab soaked in 0.9 % sodium chloride. The depth of each level of vaporisation is controlled by the selection of power, focal length, scanner-controlled spot-size and the velocity of the movements of the hand-held scanner. We have used 20–50 W, 3–6 mm spot-size and 12.5 or 18 cm focal length setting. The vaporisation procedure has been repeated downwards and outwards until fresh yellow adipose tissue was exposed in the deep and relatively thin and anatomically normal skin margins laterally, with no remaining dense or discoloured tissue. The vaporisation usually extended to the deep subcutaneous fat or fascia.

**Postoperative care (Paper II and III)**

The patients remained in the department for 3 hours after the procedure to discover any bleeding before leaving. The dressings (hydrofibre dressing, Aquacel®, ConvaTec Ltd, Deeside, UK) were initially left on for 2 or 3 days without changing, to avoid early bleeding. Thereafter, the wound was cleaned, rinsed with tap-water and the bandage changed as often as necessary, sometimes daily, pending complete healing. The wound was inspected after one week and then, until it healed, if there were signs of complications. Routinely, it was also examined at six weeks and at six months.
3.2.2 Sampling of specimens and laboratory procedures

This section is an overview of the methods used in papers III and IV. More detailed descriptions are given in the respective “Materials and Methods” sections of the individual papers.

Blood culture technique and identification of microorganisms (Paper III)

One blood sample was taken from the patients before surgery, one during the operation and finally one 10 minutes post-op. The samples were immediately transported to the lab, where the blood was cultured by a lysis-filtration technique which had been shown to be very sensitive. The number of bacteria in the samples could be determined since a filter catches the microorganisms, and colonies are formed during culturing.
A number of bacterial strains were subject to molecular typing by DNA sequence analysis. Susceptibility to some antibiotics was tested for two of the CoNS found.

**Punch biopsies, immunohistochemical staining and microscopy (Paper IV)**

Punch biopsies were taken from HS lesions and clinically non-involved skin in the groin or axilla from patients and from groin or axilla from matched controls. The specimens were put in fixative solution and transported to the lab. Serial cryostat sections were obtained from the biopsies, and stained for indirect immunofluorescence according to standard protocols. Coded slides were observed and photographed in the microscope and PGP 9.5 positive nerve fibre profiles as well as PGP 9.5 positive cells were counted.

3.3 **STATISTICS**

**Paper I:**

Statistical calculations were made with Statistica 8 software (StatSoft Inc). For the analyses of correlation and comparison of groups, non parametric methods were chosen; Spearman rank order correlation test, Mann-Whitney test, Kruskal-Wallis ANOVA and median test. Correlations were interpreted according to Colton as: <0.25; weak/little and 0.25–0.50 fair correlation. For descriptive statistics of the HSS results median values with lower and upper quartiles were chosen and described as “median (IQR)” in the text and the figures. Basic facts about patients as well as DLQI results in the first section are described by mean values with standard deviation (SD).

**Paper II and III:**

Basic facts and background characteristics such as age, disease duration et cetera are described as mean values or mean values with standard deviation (SD).

**Paper IV:**

Nonparametric statistical analyses were performed, using the Mann-Whitney test for comparison between patients and controls, and Wilcoxon matched pairs test for involved – non-involved skin in patients. For descriptive statistics of the results, median values with interquartile range (IQR) were used.
4 RESULTS AND DISCUSSION

4.1 HSS REFLECTING SMOKING HABITS AND OBESITY (PAPER I)

A total of 251 consecutive HS patients were included in the study. Basic characteristics of the study group are summarised in Table 1. The reported mean ± SD duration of HS was 15.5 ± 10.1 (range 1–47) years, and age at onset was 22.1 ± 9.8 (range 9–57) years. Ninety-two patients (38%) reported similar symptoms or HS diagnosis in a first-degree relative. 70% of the patients were active smokers, 15% former smokers and 16% non-smokers. Only one male (of 35) was a non-smoker, compared to 38 out of 177 women. In average, the patients reported overweight, with a mean ± SD body mass index (BMI) of 28.3 ± 6.5. The patients were divided in three BMI classes, defined as 18.5 ≤ BMI <25 kg/m² normal; 25 ≤ BMI <30 kg/m² overweight and BMI ≥30 kg/m² obese. They were relatively evenly distributed with one third of the patients under- to normal weight, one third overweight and one third obese. Mean ± SD number of boils per month reported by the patients were 2.5 ± 4.0, and soreness of the most symptomatic lesion 2.1 ± 2.7, measured by VAS with maximum score 10. Of the 115 patients that were evaluated by HSS, eight patients (7 females, 1 male) had Hurley stage I, and 12 (8 females, 4 males) had Hurley stage III hidradenitis suppurativa. The majority (95 patients) had Hurley stage II disease.

Table 1. Characteristics of the patients (plus-minus values, when given, are means ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Total</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.)</td>
<td>251</td>
<td>37.5 ± 11.4</td>
<td>36.9 ± 11.1</td>
<td>41.6 ± 12.1</td>
</tr>
<tr>
<td>Gender</td>
<td>251</td>
<td>251</td>
<td>216 (86%)</td>
<td>35 (14%)</td>
</tr>
<tr>
<td>Age at onset (yrs.)</td>
<td>247</td>
<td>22.1 ± 9.8</td>
<td>21.3 ± 9.3</td>
<td>27.0 ± 11.3</td>
</tr>
<tr>
<td>Disease duration (yrs.)</td>
<td>247</td>
<td>15.5 ± 10.1</td>
<td>15.7 ± 9.9</td>
<td>14.7 ± 11.1</td>
</tr>
<tr>
<td>Heredity: Yes</td>
<td>244</td>
<td>92 (38%)</td>
<td>87 (42%)</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>152 (62%)</td>
<td>122 (58%)</td>
<td>30 (86%)</td>
</tr>
<tr>
<td>Smoker: Yes</td>
<td>249</td>
<td>173 (70%)</td>
<td>147 (68%)</td>
<td>26 (76%)</td>
</tr>
<tr>
<td>Former</td>
<td></td>
<td>37 (15%)</td>
<td>30 (14%)</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Non</td>
<td></td>
<td>39 (16%)</td>
<td>38 (18%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>235</td>
<td>28.3 ± 6.5</td>
<td>28.2 ± 6.6</td>
<td>29.1 ± 5.5</td>
</tr>
</tbody>
</table>
Hidradenitis suppurativa score (HSS)

The median (IQR) total HSS recorded was 38 (18–66), range 5–175. There was no significant difference in median (IQR) HSS between men; 37 (19–51) and women; 38 (18–71). The highest median regional score was recorded in the axillary region for the whole group of patients, as well as for the women, while the highest median regional score among the men was recorded in the groin. The HSS values differed significantly between the three patient groups according to Hurley grade, with higher values for stage II and III, respectively (Figure 5). Representative photographs of a patient with HS Hurley stage II are shown together with the HSS of each region in Figure 6.

Figure 5. Hidradenitis Suppurativa Score (HSS) versus Hurley stage for women and men \((n = 115)\). The difference between the three groups was statistically significant \((p < 0.001, \text{Kruskal-Wallis test})\).

Figure 6 (a-d). A 45-year-old woman with HS in the axillae, with a total HSS of 42 points. (a,b) Regional score right side 20 points; 2 fistulas, 2 nodules, distance 5-10 cm. (c,d) Regional score left side 22 points; 2 fistulas, 4 nodules, distance 5-10 cm.
Further, in order to analyse HSS according to smoking habits, the study population was divided in three groups: non smokers, former smokers and smokers. Figure 7 shows a statistically significant difference in median (IQR) HSS between the non smokers; 22 (10–57), and the smokers; 41 (22–75). The median HSS of former smokers compared to the other two groups was not significantly different. The women showed the same pattern as for the whole group with higher HSS in non smokers versus smokers, but the men did not show the same result, maybe due to a small number, with only one non-smoker among the scored males.

![Figure 7](image)

Figure 7. Hidradenitis Suppurativa Score (HSS) versus smoking category for women and men \((n = 115)\). The difference between non smokers and smokers was statistically significant \((p = 0.032, \text{Kruskal-Wallis test})\), but not significant for former smokers compared to the other groups.

There was a weak positive correlation between BMI and HSS (Figure 8), and non-significant differences in HSS between the BMI groups for the whole group of patients; with median (IQR) 32 (12–54) for normal, 44 (22–56) for overweight and 50 (18–86) for obese patients. The median HSS was significantly lower for the normal weight women compared to obese (Figure 9), which was not true for the men.
Figure 8. Hidradenitis Suppurativa Score (HSS) versus BMI ($n = 107$). Correlation coefficient according to Spearman $R = 0.202$, a weak correlation according to definitions by Colton.

Figure 9. Hidradenitis Suppurativa Score (HSS) versus BMI category (defined as $18.5 \leq \text{BMI} < 25 \text{ kg/m}^2$: normal, $25 \leq \text{BMI} < 30 \text{ kg/m}^2$: overweight and $\text{BMI} \geq 30 \text{ kg/m}^2$: obese) for women ($n = 86$). It shows a statistically significant difference between the normal and the obese group ($p = 0.013$, Kruskal-Wallis test), but not significant for the overweight group compared to the other groups.
Figure 10. Hidradenitis Suppurativa Score (HSS) versus DLQI ($n = 110$). Correlation coefficient according to Spearman $R = 0.342$, a fair correlation according to definitions by Colton.

**Dermatology life quality index (DLQI)**

Two hundred and forty-six patients completed the DLQI questionnaire. Mean ± SD DLQI for the whole group of patients was 10.3 ± 7.5, and median (IQR) 9 (4–12), range 0–30. Men scored higher (median 11) compared to women (median 9). The highest scores were recorded for question 1 (soreness, pain), 4 (clothing) and 2 (embarrassment, self consciousness). No statistically significant differences in DLQI score between the subgroups according to smoking habits or BMI was found. The HSS was positively correlated to DLQI of a fair degree, based on the 110 patients that both were scored and had completed the DLQI questionnaire (Figure 10).

HS causes a high degree of morbidity, which is reflected as high DLQI scores in a study by von der Werth and Jemec, with a mean DLQI score of 8.9 in 114 patients. Wolkenstein et al found probably a similar degree of morbidity in 61 consecutive HS patients, although their result is not directly comparable since VQ-Dermato, a French quality of life questionnaire for skin diseases, was used. However, according to the literature, quality of life scores correlate poorly with objective disease assessment. An objective scoring system should be able to reflect disease activity and treatment effect in a local region, even if another region has developed in another way. Another important aspect is the need for a more detailed score, since the majority of HS patients seeking help from dermatologists are cases graded as Hurley II, and within this group there is a wide variation of clinical findings and symptoms. Milder cases with comparatively small problems are in this group as well as the more severe cases that may have debilitating symptoms.
The earlier proposed version\textsuperscript{28} of the HSS has been used in at least three clinical treatment studies with TNF-alpha inhibitors. Fardet et al. evaluated seven severe HS-patients prior to and after infliximab treatment.\textsuperscript{117} The score decreased by at least 40% in three patients. Ten HS patients were in another study treated with three infusions of infliximab and followed for one year.\textsuperscript{118} According to the authors, the HSS seems to be suitable for monitoring effects of treatment. They also found a good correlation between skin score and laboratory values and patient’s perception of disease activity. Further, they concluded that the score probably is too time-consuming for the routine patients but of value for research purposes or to decide whether a patient has severe enough HS to consider treatment with TNF-alpha inhibitors.\textsuperscript{118} In the third study, 10 HS patients were treated with etanercept for 12 weeks.\textsuperscript{119} The disease activity was positively correlated with the HSS and more than 50% score improvement was found in six patients at week 12 and in seven patients at week 24.\textsuperscript{119} Negative experiences with HSS have also been reported. Brunasso et al. found “…the scoring system proposed by Sartorius et al which is very difficult to apply in severe HS as the lesions (comedones, nodules, fistulas and scars) tend to coalesce, making the numbers impossible to determine”.\textsuperscript{120}

In Paper I it was also attempted to use the score to illustrate a possible relationship between disease severity and tobacco smoking and obesity, respectively. Accumulating evidence indicates that smoking has negative effects on HS.\textsuperscript{1, 23} The group of non-smoking patients in this study had significantly lower median HSS compared to the smokers, with the group who had stopped smoking in between. The DLQI did in that respect not discriminate the three groups. Overweight is another proposed risk factor.\textsuperscript{1-19} In this material, BMI was positively, but weakly, correlated to the HSS, and when divided in BMI-classes the obese group had higher median score values compared to the overweight patients, who in turn had higher medians compared to the normal weight patients. This was a trend for the material as a whole, as well as for the females, but not true for the men, perhaps due a rather small number of males included. As for smoking, the DLQI did not reveal any significant differences between the three weight groups, which suggest that a new scoring system is needed for such purposes.

\section*{4.2 SCANNER-ASSISTED CO\textsubscript{2}-LASER SURGERY (PAPER II)}

The outcome of carbon dioxide (CO\textsubscript{2}) laser surgery with healing by secondary intention was retrospectively evaluated in 34 patients (31 women, 3 men) with mean age 33.9 (range 15–55) years. One patient was lost to follow-up. The mean age at onset of disease was 22.3 (range 5–37) years. The areas treated varied from 5 to 50 cm\textsuperscript{2} area in a total of 67 operating sites; one in 16, two in 10, three in two, four in four and five in two cases. A typical case is shown in Figure 11–12. The mean follow-up time was 34.5 (range 7–87) months. The mean healing time was 4 (range 3–5) weeks. Four cases had had recurrence in a scar of one of the treated sites. The remaining patients had no recurrence in the treated regions, but in 12 patients new lesions or recurrences >5 cm away from the operation scars developed more than 8 months after surgery. Twenty-five patients had had recurrences of their hidradenitis suppurativa in an anatomical region different from the one that had been operated. Eight patients had had no apparent symptoms from their hidradenitis suppurativa during the follow up period (Table 2).
Figure 11 (a, b). CO₂-laser treatment in the axilla of a patient with Hurley stage II HS. (a) Local anaesthesia is administered after delineating the lesions with ink. (b) CO₂-laser vaporisation.

Table 2. Number of reported recurrences at the time for follow-up (mean 34.5, range 7–87 months) after scanner-assisted carbon dioxide laser treatment of 34 patients with hidradenitis suppurativa.

<table>
<thead>
<tr>
<th>Number of recurrences</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
</tr>
<tr>
<td>In operated areas</td>
<td>4</td>
</tr>
<tr>
<td>In the same region but &gt;5 cm from operated area</td>
<td>12</td>
</tr>
<tr>
<td>In another anatomical area</td>
<td>25</td>
</tr>
</tbody>
</table>

Postoperative course

On a scale from 0 to 3, four patients classified their pain in the postoperative period as 3 (made other activities impossible), fifteen as 2 (interfered with daily activities) nine as 1 (did not interfere with daily activities) and six as 0. In our experience, the pain during care of the wound – i.e. changing the dressings – was the most difficult aspect of the postoperative course for these patients. After healing, at the time of interview, 31 patients considered that their condition was better than before surgery. Two patients considered that their illness had hardly changed and one patient stated it worse. The treated areas, which prior to surgery had been the most troublesome, had become asymptomatic, except in four cases. Although there was much postoperative scarring, the patients’ general and local functions were not impaired. All but one of them were pleased with the appearance of their scars after complete wound healing. One inguinal scar in a female patient was hypertrophic and had a 10 mm ulceration that healed after a single intrallesional injection of steroids. Hospitalisation was not needed, but professional medical assistance for wound care was necessary in four cases. Only minor bleeding during cleaning of the wound was reported. There were no secondary infections. One had an episode of paresthesia in the arm lasting for 6 months after axillary surgery, and then subsided. Twenty patients were able to resume their regular job in less than 3 days. Four had to stop work for 4–8 days and ten for 2–3 weeks. Patients who had undergone other surgical methods for this condition preferred the
laser method and all patients except one would have accepted the same type of laser surgery again, if necessary.

Carbon dioxide laser has previously been used in hidradenitis suppurativa surgery. Before scanner devices became available, a carbon dioxide laser operating at 30 W with a manually-controlled hand-piece was used that made the vapourisation very dependent of the surgeon’s experience. The micro-processed optomechanical scanner system uses parallel mirrors to produce a fine spiral beam with an extremely short laser exposure time, ablating tissue with a minimum of thermal injury. When set at the continuous mode, the scanned laser beam drills downwards into the tissues and removes the lesion rapidly in an even and controlled manner. This is providing an almost blood-less operating field which permits macroscopic examination of pathology of the tissue, early detection and minimal unintended damage of healthy or vascular tissues. Still, special caution is recommended in patients who have a low ratio of diseased skin/underlying healthy subcutaneous tissue, where the procedure is limited by muscular fascia or vital parts, i.e. nerves and blood vessels. The main goal is to maintain a majority of the uninvolved tissue with appropriate surgical margins for radicality and thus provide a good chance of cure and yet leave a defect as small as possible.

Figure 12 (a-d). Healing by secondary intention after carbon dioxide laser treatment (same patient as in Figure 11). (a) Immediately after, (b) 1 week, (c) 3 weeks, and (d) 3 months after CO₂-laser surgery.

The hypothesis underlying this surgical method is that radical removal and vapourisation of macroscopically active HS tissue will prevent recurrences. It is thought that if the epithelial sinuses, producing keratin and harbouring debris and bacteria, survive the treatment they can be the locus of recurrence. The scanner device has made the treatment safer and less surgeon-dependent. Since 2006 a new laser (Unilas®10600 with SurgiScan®) that permits higher effect has been used in most cases, which permits faster treatment sessions. The clinical post-operative routine controls have in recent years been reduced to a wound control after one week and thereafter only when necessary.

There are limitations that should be noted in the interpretation of our study. Firstly, the out-patient files were analysed retrospectively and, in the follow-up, all patients were interviewed by telephone using a questionnaire. This information was therefore reported by the patient. Secondly, our patients had been referred to us by other specialists, who had treated them unsuccessfully with other methods. Therefore we may have se-
lected HS patients with a relatively more therapy resistant type of HS. Finally, our patients had Hurley stage II disease, which we regarded as operable with this method. Patients classified as Hurley III were referred to plastic surgeons for wide excision and reconstructive surgery.

The carbon dioxide laser has also been used to excise HS. Excision techniques are essential in cases requiring pathological anatomic examination, e.g. to exclude suspected squamous cell carcinoma. This method is also preferred in removal of larger amount of tissue in HS lesion and for Hurley stage III HS. Most patients seen in our Department of Dermatology have Hurley stage II and are not candidates for advanced major plastic surgery, although they suffer from a chronic disabling disease. Our results accord with those using other surgical methods, and the need to repeat the treatment must be considered. Development of new abscesses outside symptomatic skin area cannot be prevented by any known surgical method and seem to indicate activity of the underlying disease rather than a shortcoming of the method. In recurrent disease after carbon dioxide laser surgery, it should be treated again, if necessary, in conjunction with supplementary medical therapies. Recently, Hazen and Hazen described the outcome of CO₂-laser treatment of 61 patients with long-standing HS and, in most cases healing by secondary intention. A total of 185 areas were treated, in 154 sessions (all but three of them in local anaesthesia), follow up after 1–19 years showed acceptable to excellent healing and only two recurrences in the 185 operated areas. The authors concluded that CO₂-laser treatment appears to be an effective therapy for persistent HS, with good patient comfort, good healing and minimal risk of recurrence within treated areas.

4.3 BACTERAEMIA DURING CO₂-LASER SURGERY OF HS (PAPER III)

Blood samples for bacterial culture were obtained from 21 patients undergoing carbon dioxide laser surgery of HS; before, during and 10 minutes after operation. The total incidence of bacteraemia was 71%, i.e. bacteria in at least one sample (Table 3). Bacterial growth in the first blood sample was found in nine patients, from the second in ten and from the third in six. Bacteria were detected in three samples from one patient. No microorganism was found in six of the 21 patients.

Table 3. Incidence of bacteraemia before, during and 10 minutes after carbon dioxide laser surgery of hidradenitis suppurativa.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total number of bacteraemic patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>9 (43)</td>
</tr>
<tr>
<td>During</td>
<td>10 (48)</td>
</tr>
<tr>
<td>10 min after</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (71)*</td>
</tr>
</tbody>
</table>

*Bacteria in at least one sample

In total, 75 isolates were obtained from the samples collected before, during and after the surgical procedure. At least 12 bacterial species belonging to nine genera were identified (Table 4). The dominating group of bacteria was CoNS (29 isolates). Of
these, the species level of 18 strains was difficult to determine by conventional methods and these were therefore subjected to molecular typing. DNA sequence analysis showed that of the unidentified isolates 15 were *Staphylococcus warneri* and three were *S. epidermidis/caprae*. The other facultative anaerobic bacteria were alpha-streptococci and *Enterococcus faecalis*. Among the anaerobic isolates, *Propionibacterium acnes* and *Propionibacterium granulosum* were the most common bacteria. A few other anaerobes, like peptostreptococci, *Eubacterium* sp. and *Veillonella*, were also seen. Facultative anaerobic strains were more frequently isolated than anaerobic ones.

Table 4. Microorganisms obtained from blood samples collected before, during and 10 minutes after carbon dioxide laser surgery for hidradenitis suppurativa.

<table>
<thead>
<tr>
<th>Bacteria isolated</th>
<th>No. of patients from whom species were isolated in relation to the time of surgery (total colony count of species)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (38a)</td>
</tr>
<tr>
<td>Facultative anaerobic bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus warneri</em></td>
<td>4 (16)</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Staphylococcus</em> sp.</td>
<td>2 (4)</td>
</tr>
<tr>
<td><em>Micrococcus</em> sp.</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Streptococcus</em> sp.</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Corynebacterium</em> sp.</td>
<td></td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td></td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>4 (7)</td>
</tr>
<tr>
<td><em>Propionibacterium granulosum</em></td>
<td>2 (5)</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> sp.</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Veillonella</em> sp.</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Eubacterium</em> sp.</td>
<td></td>
</tr>
</tbody>
</table>

a Total colony count, all species

In the literature, postoperative transient bacteraemia vary due to differences in the type of surgery and the method used for isolation of bacteria from blood. In the present study, the lysis-filtration technique with subsequent anaerobic incubation was used. The incidence and severity of bacteraemia were higher than those reported for other surgical procedures, which accords with previous studies of bacteraemia in various dental surgical procedures, and supports the view that the combination of lysis-filtration and brain-heart infusion agar has very high sensitivity. Certain microorganisms requiring special conditions may not grow or survive in many common blood culture systems. Asymptomatic bacteraemia and a high frequency of contamination have been found with the use of blood culture systems based on the lysis of blood. Therefore, preoperative blood samples have to be taken to estimate the true postoperative bacteraemia.

In six patients, all samples were negative which may suggest that the surgical method itself did not cause a spread of bacteria from the lesions. If it had, we assume that an
increase in the number of isolated microorganisms in the second sample, followed by a marked decrease of bacteria in the third, would have been found. Halpern et al. studied the incidence of transient bacteraemia in 45 patients undergoing skin surgery on the sebaceous-rich areas of the head and neck. All their baseline blood culture results were negative, but three of them developed transient bacteraemia within the first 15 minutes after the start of the procedures.\textsuperscript{125}

The interpretation of blood cultures that are positive for CoNS or propionibacteria is often difficult. In the present study a surprisingly high number of positive preoperative samples were found, which may indicate a high frequency of contamination, compared to other studies using the same method in various oral surgical procedures.\textsuperscript{110, 111} On the other hand, the samples from healthy controls were negative, and all samples and cultures were handled carefully under strict aseptic conditions to avoid contamination. It cannot be excluded that the surgical procedure itself may have had an effect on the patients which was not comparable to the conditions in the controls and this may have affected the contamination. Another possibility is that bacteria from the chronic lesions continually leak into the bloodstream of a subgroup of HS patients, and that they are detected with this very sensitive method of culture. This was an unexpected finding and more studies are needed for verification and to determine the clinical relevance. Our findings must be interpreted with caution; the number of patients was low and the patients were referred to us by other specialists, which may have selected patients with a relatively therapy-resistant HS. Further, our patients had Hurley stage II disease, and we have no data on bacteraemia in milder or more severe cases. Finally, the incidence of bacteria in the blood samples taken before surgery was high, which was the most surprising result of this study.

The significance of bacteria in HS is disputed. Interpretation of the results of bacteriological cultures from the surface of HS lesions is difficult because of possible contamination by the skin flora. The bacterial species found accorded with those of a previous study\textsuperscript{39} of deep cultures from HS lesions taken during CO\textsubscript{2}-laser vaporisation of the affected tissue to thereby minimise contamination, since the carbon dioxide laser permits heat sterilization.\textsuperscript{126-128} In that study, \textit{S. aureus} and CoNS were the most frequent, while \textit{Peptostreptococcus} species and \textit{P. acnes} were common as well.\textsuperscript{39} Maybe the findings in Paper III indicate a need to re-evaluate the significance of bacteria in the development of HS, and also the question if CoNS are true pathogens. It is known that foreign bodies increase the virulence of CoNS in surgical implants. A milieu which resembles that produced by a foreign body, as found in chronic HS tissue, could increase the pathogenic properties of CoNS. In the present study, it is noteworthy that molecular typing of CoNS showed that 15 of 29 staphylococci isolates were \textit{S. warneri}, which has been regarded as a contaminant, but more recently has become associated with significant morbidity in infants at intensive care, where it has been isolated from subdural empyemas, urinary tract infections, osteomyelitis and bacteraemias.\textsuperscript{129, 130} Moreover, and perhaps of more relevance to HS; \textit{S. warneri} has been found in chronic conditions associated with a foreign body.\textsuperscript{129, 130} These conditions resemble certain features of HS, as discussed above, but in our experience of almost 300 treated patients and from the literature, HS does not spread to other organs. However, it is customary to give prophylactic preoperative antibiotic treatment regularly to HS patients with concomitant heart valve defects or immunodeficiency, but not to otherwise healthy ones.
4.4 PGP 9.5 DISTRIBUTION PATTERNS IN HS (PAPER IV)

Skin biopsies from 16 HS patients of which 10 had inguinal and 6 had axillary involvement, were investigated both from lesions and perilesional location, and compared to biopsies from 12 healthy matched controls. The intraepidermal nerve fibre profiles were distributed through the epidermis and most of them ran perpendicularly to the skin surface. In lesional skin there were a lower number of profiles in epidermis compared to controls. In the upper dermis, some PGP 9.5 positive nerve bundles were observed parallel to the epidermal-dermal junctional zone. In the middle and lower dermis, abundant nerves surrounded blood vessels, sweat glands, and hair follicles. Single PGP 9.5 positive cells were seen in the upper dermis in the healthy subjects whereas there were a substantial number of strongly fluorescent, spindle-shaped PGP 9.5 positive cells in the dermal parts of the sections from lesional skin (Figure 13).

4.4.1 Groin

PGP 9.5 immunoreactive nerve fibre profiles

The median (IQR) number of profiles/mm² in epidermis in patients was significantly lower than in controls, Table 5. On the contrary, the median (IQR) number of profiles in upper dermis was significantly higher in HS patients than in controls. In the middle and lower parts of dermis there were no significant differences in the number of nerve fibre profiles between patients and control subjects. In clinically non-involved skin from HS patients there was significant lower median number of profiles in epidermis, whereas in deep dermis there were a higher median number of profiles, compared to healthy controls. Comparison of lesional and non-involved skin from HS patients showed only one significant difference; a smaller median number of profiles from lesional skin in lower dermis (Table 5).

Figure 13 (a, b). (a) Overview and (b) high magnification micrograph, of lesional skin from the groin of a HS patient, showing few nerve fibre profiles (arrows) in the epidermis, but a high number of PGP 9.5 positive cells (arrowhead) throughout the dermis. Bar equals 100μm.
Table 5. PGP 9.5 positive nerve fibre profiles/mm² of the projected skin surface in lesional and clinically non-involved skin of the groin from HS patients, and groin skin from healthy controls. Median values with interquartile range (IQR). Statistically significant differences from controls, defined as $p$-values < 0.05 (Mann-Whitney U-test), in bold.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Control ($n = 8$)</th>
<th>Lesional ($n = 10$)</th>
<th>$p$-value</th>
<th>Non-involved ($n = 9$)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>181.5 (132–276)</td>
<td>45.5 (13–65)</td>
<td>0.0014</td>
<td>33 (8–53)</td>
<td>0.0079</td>
</tr>
<tr>
<td>Upper dermis</td>
<td>174 (127–203)</td>
<td>802 (246–1439)</td>
<td>0.0434</td>
<td>420 (275–611)</td>
<td>0.0592</td>
</tr>
<tr>
<td>Mid dermis</td>
<td>15.5 (6.5–126.5)</td>
<td>69.5 (13–169)</td>
<td>0.4598</td>
<td>90 (50–306)</td>
<td>0.0745</td>
</tr>
<tr>
<td>Deep dermis</td>
<td>0 (0–26.5)</td>
<td>11.5 (0–26)*</td>
<td>0.3599</td>
<td>109 (49–225)*</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

*Statistically significant difference lesional – non-involved skin in HS patients, $p = 0.0357$ (Wilcoxon matched pairs test)

**PGP 9.5 immunoreactive cells**

In epidermis there were no or few cells, without difference between patients and controls. In all three dermal layers there were statistically significant higher median numbers of PGP 9.5 immunoreactive cells in lesional skin from hidradenitis suppurativa patients compared to the healthy controls (Table 6 and Figure 13). There was no significant difference regarding median number of cells between non-involved skin from HS patients and controls. There was no difference between lesional and non-involved skin from HS patients (Table 6).

Table 6. PGP 9.5 positive cells/mm² of the projected skin surface in lesional and clinically non-involved skin of the groin from HS patients, and groin skin from healthy controls. Median values with interquartile range (IQR). Statistically significant differences from controls, defined as $p$-values < 0.05 (Mann-Whitney U-test), in bold.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Control ($n = 8$)</th>
<th>Lesional ($n = 10$)</th>
<th>$p$-value</th>
<th>Non-involved ($n = 9$)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>0 (0–0.5)</td>
<td>0 (0–1)</td>
<td>0.6334</td>
<td>5 (0–20)</td>
<td>0.1672</td>
</tr>
<tr>
<td>Upper dermis</td>
<td>269.5 (207–362.5)</td>
<td>749 (394–1281)</td>
<td>0.0014</td>
<td>260 (113–557)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mid dermis</td>
<td>98.5 (59.5–126.5)</td>
<td>513.5 (282–1825)</td>
<td>0.0009</td>
<td>178 (42–350)</td>
<td>0.6058</td>
</tr>
<tr>
<td>Deep dermis</td>
<td>23 (2–75.5)</td>
<td>339 (84–716)</td>
<td>0.0085</td>
<td>100 (28–550)</td>
<td>0.0745</td>
</tr>
</tbody>
</table>

**4.4.2 Axilla**

**PGP 9.5 immunoreactive nerve fibre profiles**

Consistent with the findings in specimens from the groin, the median number of profiles/mm² in epidermis was lower in lesional skin from HS patients than in control skin, although the difference was not statistically significant. In all dermal layers there were significantly less profiles in lesional HS skin compared to control skin (Table 7, Figure 14). Clinically non-involved perilesional skin from HS patients showed the same trend
as lesional skin compared to controls, however only significant in mid dermis. There was no difference between lesional and non-involved skin from HS patients (Table 7).

Table 7. PGP 9.5 positive nerve fibre profiles/mm² of the projected skin surface in lesional and clinically non-involved skin of the axilla from HS patients, and axillary skin from healthy controls. Median values with interquartile range (IQR). Statistically significant differences from controls, defined as \( p \)-values < 0.05 (Mann-Whitney U-test), in bold.

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 4))</th>
<th>Lesional ((n = 6))</th>
<th>( p )-value</th>
<th>Non-involved ((n = 6))</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>98 (86–148)</td>
<td>4.5 (1–120)</td>
<td>0.2571</td>
<td>31 (1–114)</td>
<td>0.2571</td>
</tr>
<tr>
<td>Upper dermis</td>
<td>475 (285–645)</td>
<td>133 (87–266)</td>
<td>\textbf{0.0381}</td>
<td>138.5 (73–191)</td>
<td>0.1714</td>
</tr>
<tr>
<td>Mid dermis</td>
<td>196 (137.5–293.5)</td>
<td>12 (2–30)</td>
<td>\textbf{0.0095}</td>
<td>59 (25–86)</td>
<td>\textbf{0.0381}</td>
</tr>
<tr>
<td>Deep dermis</td>
<td>128 (80.5–331.5)</td>
<td>8.5 (0–59)</td>
<td>\textbf{0.0381}</td>
<td>114 (81–124)</td>
<td>0.7619</td>
</tr>
</tbody>
</table>

PGP 9.5 immunoreactive cells

There were no statistically significant differences either between lesional skin and controls, or between non-involved skin in HS patients and controls, regarding median number of PGP 9.5 positive cells in specimens from the axilla (Table 8). There were no or few PGP 9.5 positive cells in epidermis in lesional, clinically non-involved perilesional skin from HS patients and control skin (Figure 14). In dermis the trend was higher median numbers of PGP 9.5 positive cells in lesional skin compared to control skin specimens. There was only one significant difference between lesional and non-involved skin from HS-patients; a higher median number of cells in mid dermis in lesional skin.

Table 8. PGP 9.5 positive cells/mm² of the projected skin surface in lesional and clinically non-involved skin of the axilla from HS patients, and axillary skin from healthy controls. Median values with interquartile range (IQR). There were no statistically significant differences between patients and controls, defined as \( p \)-values < 0.05 (Mann-Whitney U-test).

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 4))</th>
<th>Lesional ((n = 6))</th>
<th>( p )-value</th>
<th>Non-involved ((n = 6))</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>0.9143</td>
<td>0 (0–0)</td>
<td>0.7619</td>
</tr>
<tr>
<td>Upper dermis</td>
<td>238.5 (91.5–430)</td>
<td>451.5 (353–1570)</td>
<td>0.2571</td>
<td>202 (175–555)</td>
<td>0.6095</td>
</tr>
<tr>
<td>Mid dermis</td>
<td>134.5 (119.5–732)</td>
<td>1528 (193–2102)*</td>
<td>0.2571</td>
<td>213 (66–582)*</td>
<td>0.6095</td>
</tr>
<tr>
<td>Deep dermis</td>
<td>55 (21.5–336)</td>
<td>1084 (59–1965)</td>
<td>0.1714</td>
<td>269.5 (77–490)</td>
<td>0.6095</td>
</tr>
</tbody>
</table>

*Statistically significant difference lesional – non-involved skin in HS patients, \( p = 0.028 \) (Wilcoxon matched pairs test)
Figure 14 (a,b). (a) Skin from the axilla of a healthy control, with vertical nerve fibre profiles (arrow) in the epidermis and an overall low number of PGP 9.5 positive cells. (b) Lesional skin from the axilla of a HS patient, with few nerve fibre profiles (arrows) in the epidermis, median number of profiles in dermis and some PGP 9.5 positive cells (arrowheads) in dermis. Bar equals 100 µm.

Indirectly, the neuroendocrine involvement in HS could be studied through investigations of therapeutic effects. However, only two studies were found in the literature of treatment for HS-patients with the primary focus to influence the neuroendocrine system. First, a fertile woman with severe familial HS was given a combination of dexamethasone and a synthetic gonadotropin releasing hormone agonist. The patient was reported to improve at the same time as her adrenals and ovarians were suppressed, as indicated by blood level changes of testosterone, dehydroepiandrosterone sulphate, androstendione and estradiol. Second, Bogers et al. described a HS-patient with premenstrual exacerbations, who was improved after hysterectomy and bilateral salpingo-oophorectomy combined with estrogen substitution. Prior to ectomias, some improvement was noticed after treatment with a gonadotropin-releasing hormone agonist followed by a gonadotropin-releasing hormone agonist together with estrogen substitution.

A number of studies performed on other inflammatory skin diseases, e.g. atopic dermatitis, contact dermatitis and psoriasis, show increased as well as decreased levels of neuroendocrine markers, including PGP 9.5. In particular, a recent study found a decreased number of epidermal PGP 9.5 immunoreactive nerve fibres in involved psoriatic skin during exacerbation. To our knowledge, this is the first study of PGP 9.5 distribution in HS.

In both the groin and axilla it was noticed that the median number of profiles in epidermis from patients was lower, however not significant in the axilla, compared to normal skin. Contradictory findings were obtained regarding profiles in dermis, where there was an increased number of profiles in upper dermis of the groin in the patients, whereas in the axilla the lesional dermal skin contained significantly fewer profiles compared to healthy controls. Although the number of study subjects was limited, the biopsies were analysed separately according to localisation (groin and axilla, respectively), since previous studies have shown difference in innervation density between certain human skin areas body parts.
Further, the number of PGP 9.5 positive cells was quantified, but only cells with strong fluorescence were counted, while melanocytes and other weak fluorescent cells in epidermis were not. In normal skin sparse, single, fluorescent dendritic cells were seen in upper dermis. In lesional skin, a large number of PGP 9.5 positive cells, mostly spindle-shaped, were noted throughout the dermis although not statistically significant in the axilla, maybe due to a too small number of samples. The origin of the PGP 9.5 positive cells was not further investigated in this study, but there are several candidates including fibroblasts, macrophages, and dendritic cells. The study has several limitations. One is that the biopsies were taken from HS patients with probably different duration of the lesions. Another is that due to the small number of study subjects, no analysis of subgroups, e.g. gender, smoking habits, body weight, et cetera could be done. No investigation of the stress level was performed. Biopsies from patients were taken from clinically non-involved skin as well as involved, but for practical reasons the non-involved skin biopsies were taken relatively close to the lesions (approximately 2 cm perilesionally). We have not been able to explain the reasons behind the discrepancy and opposing results for dermal PGP 9.5 profiles in the axilla compared to the groin, in connection with lesional skin versus control skin. However, different results of nerve fibre density are often found in skin from various anatomical regions, especially when the regions are as far apart as the axilla and the groin, although in our clinical experience, HS has the same characteristics and behaviour in both regions.
5 CONCLUSIONS

The main findings in Paper I were that HSS was positively correlated to Hurley stage, to smoking and obesity and fairly good correlated to DLQI. This indicates that the HSS reflects disease severity and brings out relevant information from a HS patient material. HSS has after modification and simplification of the preliminary version, become more practical to perform, and a patient can be scored in about 5 minutes, depending on the extent of the disease. The HSS is primarily designed for clinicians especially interested in HS. HSS may be suitable as a scoring method in clinical trials since it correlates with existing scores and potential risk factors, suggesting that it reflects a valid estimation of disease severity.

The results in Paper II indicate that in patients with chronic HS of Hurley grade II, macroscopically controlled, tissue selective and skin preserving scanner-assisted carbon dioxide laser treatment of selected affected areas followed by secondary intention healing, is a safe and rapid surgical method with satisfactory cosmetic and functional results and low recurrence rate, suitable for out-patients.

In Paper III, it was concluded that the carbon dioxide laser vaporisation treatment of HS did not seem to cause any additional spread of bacteria into the bloodstream. The evaluation of cultures containing microorganisms from normal skin flora is always controversial. Since the bacteria detected in this study accord with those obtained previously in cultures from deeper parts of HS lesions, they seem to be relevant. The results should be interpreted with caution; however, growth of bacteria in the first blood sample taken before surgery could indicate that some of these patients have bacteria continuously circulating in the blood. Therefore more and larger studies should be done in HS patients.

An imbalance of the PGP 9.5 immunoreactive nerve fibre profiles and PGP 9.5 positive cells by immunohistochemical staining, compared to controls, is demonstrated in Paper IV. In conclusion, despite several study limitations, the findings indicate that PGP 9.5 positive nerve fibres could be involved in the pathogenesis of HS. The functions of the nerve fibres and the PGP 9.5 positive cells are not known. Both regarding the profiles and the cells, further studies must be made to show if these differences are primary events, or secondary to, for example, chronic inflammation, which is considered a major issue of HS. In addition, the origin of the cells needs to be further investigated.

HS patients have a high degree of morbidity as measured by the DLQI. To evaluate this in an objective manner, in addition to the established Hurley staging system, a new clinical score has been proposed and is shown to correlate both to other scores and to HS risk factors. Treatment by carbon dioxide laser seems to give satisfactory results and is not shown to spread bacteria during treatment sessions. Involvement of bacteria in HS is disputed, and the knowledge is limited. This is true also for other pathogenic mechanisms. The presence and role of neuroendocrine-immune system elements such as nerve fibre profiles and cells remain to be further mapped and investigated in HS.
6 PERSPECTIVES

6.1 BACTERIOLOGY

The presence of bacteria, especially CoNS, in deeper parts of chronic HS lesions investigated in a previous study, indicate pathogenic relevance, but whether a primary or secondary event is not known. In another study it was found that the patients in whom *Staphylococcus aureus* was found had a shorter duration of disease, why it was suggested that *S. aureus* may play a temporary role in the early phase of the disease. This issue is investigated in an ongoing study, where we examine and treat patients with acute nodules or abscesses, and bacterial culture is performed before and during carbon dioxide laser vaporisation. Samples are taken from the skin surface and then from the deeper layer of the lesions. At each level two samples are taken, one with a punch biopsy technique and one by pressing a soft agar gel against the skin.

6.2 THE NEUROENDOCRINE SYSTEM

The skin is obviously an easily accessible organ system for investigating the peripheral effects and production of stress mediators, and subsequently gives insights into neuroendocrine-immune dysfunctions. From the growing knowledge about the brain-skin connection, it is known that numerous neurotransmitters, neuropeptides, neurotrophins and neurohormones are present locally and act as both major targets and effector messengers of the stress response. Evidence of neuroendocrine involvement in HS is as yet sparse, but there are parallels to other inflammatory skin diseases, where various immunoreactive cells are known to be active, and from which hypotheses of HS pathogenesis can be generated.

Other conditions where PGP 9.5 have been investigated include cutaneous squamous cell cancer and keratoacanthomas, where cytoplasmic PGP 9.5 staining was associated with tumour aggressiveness. In another recent publication, investigation of intraepidermal nerve fibre density at wrist level in diabetic and non-diabetic patients with mild neuropathy showed no difference between diabetic and non-diabetic patients, but higher nerve fibre density in hairy skin compared with glabrous skin and higher in females than in males. In one study, the effect of acute stress on Langerhans’cells (LC) and cutaneous nerve fibre expression of PGP 9.5 and calcitonin-gene related peptide (CGRP) measured in healthy volunteers, showed reduction of LC and CGRP, while PGP 9.5 was increased, after the stress exposure.

The next step of our team in the investigation of neuropeptides in HS is to employ double-staining for PGP 9.5 and various neuro-immune markers; including nerve growth factor (NGF), substance P (SP), calcitonin gene related peptide (CGRP) and tryptase.

6.3 TREATMENT

It would appear that definitive treatment consists mainly of surgery, while pharmaceuticals mainly serve as adjunct therapy, although recently both tumour necrosis factor-alpha inhibitors and the combination of rifampicin and clindamycin have been shown to induce long-term remission in open case-series. Recently a RCT of infliximab has shown the drug to have significant effect in HS, which is in good agree-
ment with 34 earlier publications reporting generally positive outcomes in cases or open series, with altogether 105 HS patients treated with tumour necrosis factor-alpha inhibitors. Tierney et al. evaluated the effect of an epilating Nd:YAG-laser in a randomised controlled study of 22 HS patients with a significant improvement compared to control treatment (topical antibiotic). The results are in line with the follicular pathogenic hypothesis.

The main potential side effect of long-term antibiotic treatment is that the equilibrium between host and microorganisms may be disturbed by the use of antibiotic therapy. Antimicrobial resistance is a growing problem and is associated with clinical use of antibiotics, since a balanced microflora prevents establishment of resistant strains of bacteria. Antibiotic treatment for three months or more seems to be necessary in HS. Long-term treatment with antibiotics can develop, for example, resistant Propionibacterium acnes and CoNS of the skin. Topical Clindamycin could increase carriage of resistant P. acnes and Staphylococcus epidermidis, and there could be a risk of transfer to other pathogenic bacteria like Staphylococcus aureus and Streptococci. For acne cases it is recommended to combine antibiotics with benzoyl peroxide to reduce the risk of resistant strains of especially P. acnes.

A target for topical therapy, preferably in the early phase, is the follicular hyperkeratinisation, and empirically different acne preparations have been tried. Although not formally studied in HS, azelaic acid cream has been used, in monotherapy or together with topical clindamycin, with improvement, especially in mild cases. Azelaic acid cream can also serve as adjunct therapy when surgery is planned. A personal observation is that some of the patients even achieve control over the disease after long term use, perhaps in addition to having reduced or stopped smoking. This observation needs to be investigated systematically.

At our department, CO2-laser vaporisation has been the treatment of choice in patients with Hurley stage II HS. If the area that needs to be removed is large, a combination technique where the body of the lesion is debulked by cutting electrosurgery, with subsequent vaporisation of the margins to achieve radicality, is used. CO2-laser treatment can sometimes be a complement to advanced plastic surgery in cases where a smaller loco regional recurrence develops in the excision margins. Finally, since the scanner-assisted CO2-laser vaporisation is a safe and relatively fast treatment method suitable for outpatients, it could be considered a first line treatment for acute exacerbations of inflamed nodules as a definitive alternative to short-term solutions such as lancing.

6.4 ASSOCIATED CONDITIONS

6.4.1 Obesity

It is speculated that overweight and obesity may aggravate HS. Analogies from psoriasis, where a higher prevalence of obesity, hypertension and dyslipidemia has been shown in patients, could suggest a potentially causal relationship between parts of the metabolic syndrome and chronic cutaneous inflammation, which could be of importance in the pathogenesis of HS. In our material as well as in other studies, HS patients are on average overweight, but not all of them are and little is known about other parts of the metabolic syndrome in HS. Potentially, HS patients may benefit from additional metabolic interventions, and increased knowledge in this field could contribute to development of targeted therapy. Participation in a Nordic multicentre study of HS...
patients and controls regarding the prevalence of obesity and the metabolic syndrome is in the planning stage.

6.4.2 Smoking

Accumulating observations of the fact that smokers are overrepresented in HS can still not determine if this is a causal relationship or a parallel phenomenon. This needs to be further explored, for instance through studies of the nicotinic receptor in skin biopsies, in analogy to the imbalance found in pustulosis palmoplantaris, and in the above mentioned study of acetylcholine receptors in HS. Anecdotally, a number of patients have reported improvement after having reduced or stopped smoking, and in a recent publication, remission of HS is reported in two cases. A clinical interventional study of tobacco cessation would also be of great importance.

6.4.3 Psychological morbidity

It is well known that HS causes a high degree of morbidity, and that the patients experience lower life quality, but so far the number of publications exploring the psychological load and the prevalence of psychiatric diseases like depression in association with HS are few. Therefore it would add valuable information to set up studies; both large epidemiological and more elaborate investigations of psychic health in smaller series of individual cases. Collaboration with the Psychology Department of Stockholm University is in progress, where HS patients are invited to participate in a detailed interview that intends to depict the patient’s perception of the disease, and where appropriate psychological tests of stress, anxiety or depression can be made.

6.5 SCORING

6.5.1 Reliability

The new system for scoring disease severity, HSS, is shown to correlate with other scores and suspected risk factors, in Paper I as well as in a study by Canou-Poitrine et al., who used the by Revuz modified HSS in 302 French HS patients. It was concluded that the score, although not formally validated, was highly correlated with Hurley stage and intensity and duration of pain as well as suppuration. Another important issue is the reliability of the tool, which can be investigated by measuring the interobserver variability when scoring the same set of patients. This has recently been performed in 23 HS patients, by four observers, and showed a high factor of concordance (0.95) and low interobserver variability (Sartorius et al., in press).

6.5.2 HSS used in clinical studies

The earlier proposed version and modifications of the HSS have been used in a number of studies which are summarized in Table 9. The majority of these studies were published independently of our group. Therapies that have been evaluated include TNF-α inhibitor therapy, photodynamic therapy, RCT of neodymium-doped yttrium aluminium garnet laser, and clindamycin and rifampicin in combination therapy.
Table 9. Published studies where HSS including modified versions has been used

<table>
<thead>
<tr>
<th>First author (year)</th>
<th>n</th>
<th>Pre-score (\text{a}^{\text{b}})</th>
<th>Post-score (\text{a}^{\text{b}})</th>
<th>Treatment</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sartorius (2003)(^{28})</td>
<td>34</td>
<td>30.5 ± 24.2</td>
<td>12.0 ± 17.2</td>
<td>CO(_2)-laser</td>
<td>Total score, (n = 29) for evaluation</td>
</tr>
<tr>
<td>Fardet (2007)(^{117})</td>
<td>7</td>
<td>82 ± 30</td>
<td>NA</td>
<td>Infliximab</td>
<td>Score decreased 40% in 3 patients</td>
</tr>
<tr>
<td>Mekkes (2008)(^{118})</td>
<td>10</td>
<td>164 ± 50</td>
<td>89 ± 49</td>
<td>Infliximab</td>
<td>1 year</td>
</tr>
<tr>
<td>Giamarellos-Bourboulis (2008)(^{119})</td>
<td>10</td>
<td>278(^{b})</td>
<td>114(^{b})</td>
<td>Etanercept</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Pelekanou (2009)(^{153})</td>
<td>10</td>
<td>124.8(^{c})</td>
<td>NA</td>
<td>Etanercept</td>
<td>Long-term follow up of study(^{33}) above</td>
</tr>
<tr>
<td>Sotiriou (2009)(^{156})</td>
<td>5</td>
<td>18.8</td>
<td>17.2</td>
<td>PDT</td>
<td>Regional score, 2 months</td>
</tr>
<tr>
<td>Sotiriou (2009)(^{154})</td>
<td>4</td>
<td>25(^{d})</td>
<td>18(^{d})</td>
<td>Etanercept</td>
<td>3 months after treatment</td>
</tr>
<tr>
<td>Sotiriou (2009)(^{155})</td>
<td>3</td>
<td>24.3</td>
<td>16.6</td>
<td>Adalilumab</td>
<td>3 months after treatment</td>
</tr>
<tr>
<td>Gener (2009)(^{74})</td>
<td>116</td>
<td>29 (14.5)</td>
<td>14.5 (11)</td>
<td>Clindamycin + Rifampicin</td>
<td>(n = 70) for evaluation</td>
</tr>
<tr>
<td>Tierney (2009)(^{45}) Control</td>
<td>22</td>
<td>29.8 ± 13.5</td>
<td>9.7 ± 6.2</td>
<td>Nd:YAG-laser</td>
<td>All sites combined. Modificied score(^{2})</td>
</tr>
<tr>
<td><strong>Other studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wolkenstein (2007)(^{4})</td>
<td>61</td>
<td>25.1 ± 18.2</td>
<td>NA</td>
<td>-</td>
<td>Quality of life study</td>
</tr>
<tr>
<td>Canou-Poitrine (2009)(^{10})</td>
<td>302</td>
<td>17 (16)</td>
<td>NA</td>
<td>-</td>
<td>Clinical characteristics and risk factors</td>
</tr>
<tr>
<td>Sartorius (2009, Paper I)</td>
<td>115</td>
<td>38 (18–66)</td>
<td>NA</td>
<td>-</td>
<td>Clinical characteristics and risk factors</td>
</tr>
<tr>
<td>Sartorius (2010, accepted)</td>
<td>61</td>
<td>40 (18–73)</td>
<td>NA</td>
<td>-</td>
<td>Interobserver variability (n = 23)</td>
</tr>
</tbody>
</table>

\(\text{a}^{\text{b}}\) before and after treatment; mean if single value, mean ± SD or median with interquartile range (IQR); \(\text{b–d}\) approximated means from \(\text{b}^{\text{b}}\) Giamarellos-Bourboulis (2008)\(^{119}\), \(\text{c}\) Pelekanou (2009)\(^{153}\), \(\text{d}\) Sotiriou (2009)\(^{154}\); \(\text{e}\) points added for erythema, edema, pain, purulent discharge; NA, not applicable; RCT, randomized controlled trial

6.5.3 Further development of the score

There are some limitations in the use of HSS. Firstly, we have noticed that for the most severe cases, with widespread disease that affects large skin areas, the difficulty to determine HSS increases, since it is hard to define single lesions if they are coalescing. Secondly, the degree of inflammation of individual lesions, or regions, is not included in the score, which would be valuable in particular for descriptive studies or studies of non-surgical therapy. Over time, in the same patient, the grade of inflammation can vary a lot, from silent periods with relatively dry sinus openings, no erythema and non
inflamed nodules to flare-up periods with pronounced erythema, oedema, suppuration and painful inflamed nodules. Thirdly, for some cases there are difficulties to distinguish between a small fistula and a large nodulus. This is important for the total score since a fistula renders six points and a nodulus only one. Finally, subjective variables are not included in the total score. Since pain is a major feature of HS it can be added as grading the worst lesion chosen by the patient using a visual analogue scale. DLQI or another subjective scoring system of life quality is suggested to be used in complement to HSS in future prospective trials. Work is in progress in a European network of dermatologists with special interest and expertise in HS to further improve the assessment of HS severity, especially treatment effects.

### 6.5.4 Hidradenitis suppurativa registry

A Nordic HS registry is under development, with the purpose to collect clinical and basic epidemiological facts about HS patients, to enable evaluation of medical and surgical treatment effects in individual patients, and also to collect a cohort which can be followed over a long period of time. When appropriate, it would become a basis for epidemiological HS research. In addition a common Nordic registry enables the use of uniform reporting of outcome variables and side effects in multicentre clinical trials.

### 6.6 FINAL REMARKS

Improved knowledge of the mechanisms that drive the pathogenesis in HS will lead to a better understanding of this disease and shed light on what may be the critical role of the immune system together with the function of keratinocytes. Future developments aimed at the etiological mechanisms could provide new targeted opportunities to amend the therapeutic arsenal available to deal with HS. Early treatment and careful management could then further improve the outcome and quality of life for patients with HS.

I denna avhandling har olika kliniska aspekter av HS studerats: utveckling av ett kliniskt utvärderingsinstrument för bedömning av svårighetsgrad (hidradenitis suppurativa score, HSS), utvärdering av kirurgisk behandling med koldioxidlaser, förekomsten av bakterier i blodet hos HS-patienter och slutligen förekomst och utbredning av den neuroendokrina markören PGP (protein gene product) 9.5 i hudbiopsier.

I delarbete I var syftet att undersöka den nu förenklade kliniska mätmetoden HSS, där HS-förändringar räknas och poängsätts, samt studera om BMI (body mass index) respektive rökning påverkar sjukdomsgraden. Ett frågeformulär angående livskvalitet vid hudsjukdom, Dermatology life quality index (DLQI) fylldes i av 246 patienter, och 115 bedömdes enligt HSS. Undersökningen visade att det fanns ett visst positivt samband mellan HSS och DLQI och att patienter med högre sjukdomsgrad hade signifikant högre HSS. Likaså hade rökare högre HSS än icke-rökare och kraftigt överviktiga kvinnor högre HSS än normalviktiga, vilket inte kunde visas hos männen (som i studien var färre). Resultaten talar för att HSS avspeglar svårighetsgrad och samvarierar med riskfaktorer samt att det förefaller lämpat för kliniska prövningar.

I delarbete III utfördes blododlingar på 21 HS-patienter med medelsvår sjukdomsgrad, före, under samt efter operation med koldioxidlaser. I första provet fann man bakterieväxt hos nio av dessa, i andra provet hos tio och i tredje provet hos sex patienter. En patient hade bakterieväxt i alla tre proverna. Flera olika sorters bakterier odlades fram, varav koagulasnegativa stafylokocker var vanligast. Odlingarna hos sex av patienterna var genomgående negativa, vilket kan tyda på att själva operationsmetoden inte orsakar bakteriespridning till blodet.

Delarbete IV beskriver förekomst och utbredning av nervfibermarkören PGP 9.5 i hudbiopsier från 16 HS-patienter. Biopsierna delades upp i två grupper beroende på provlokal; ljumske respektive armhåla, och undersöktes med immunhistokemisk teknik i mikroskop, varvid man fann att medianantal nervprofiler (de nervdelar som räknades) var lägre i överhud hos sjuka, jämfört med kontroller. I läderhuden var det motsägendefulla resultat; en ökning av medianen i ljumskar men en minskning i armhålegruppen. Även PGP 9.5-positiva celler räknades, och man såg en ökning i sjuk läderhud, där skillnaden ljumskgruppen var signifikant. Cellerna har ännu inte undersöks närmare med avseende på typ.

HS-patienter upplever en hög sjukdomsbörda och rejält sänkt livskvalitet enligt mätningar med DLQI. För att även få ett objektivt mått att följa, t ex för att utvärdera behandlingsresultat, har ett kliniskt poängsystem (HSS) utvecklats och visat sig korrelera både till andra utvärderingsmetoder och till riskfaktorena rökning och övervikt. Koldioxidlaserkirurgisk behandling verkar ge tillfredsställande resultat och inte sprida bakterier under behandlingen. Kunskapen om sjukdomsorsakerna är fortfarande begränsad, och betydelsen av bakterier liksom även nervfiberprofiler och celler från det så kallade neuroendokrin-immuna systemet, i HS-förändringar är oklar. Ökad kunskap om sjukdomsmekanismer skulle kunna leda till utveckling av förbättrade behandlingsmetoder som riktar in sig mer direkt på orsakerna till HS. Det är dock oavsett metod viktigt med en tidig och korrekt diagnos som kan ligga till grund för behandling, med syfte att uppnå bästa möjliga resultat och därmed öka livskvaliteten för de människor som drabbas av HS.
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