Bacteraemia in Patients with Hidradenitis Suppurativa Undergoing Carbon Dioxide Laser Surgery: Detection and Quantification of Bacteria by Lysis-Filtration

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Key Words
Hidradenitis suppurativa  Bacteraemia  Staphylococcus warneri  Carbon dioxide laser surgery

Abstract
Background: Hidradenitis suppurativa (HS) is a cicatrising and persistent disease of apocrine gland-bearing areas in adults. The severity of this condition varies from a few suppurating lesions to widespread, disabling disease. The aetiology is obscure, but suggested contributory factors include a genetic predisposition, comedones occluding the pilosebaceous apparatus, bacterial infections, and hormonal factors. Treatment consists mainly of surgery, while medical therapies serve principally as adjunct therapy. Objectives: The aim of the study was to determine the number and type of bacteria circulating in the bloodstream in patients with HS undergoing surgical treatment with a carbon dioxide laser stripping-secondary intention technique. Methods: Twenty-one patients (20 females and 1 male, mean age 36, range 20–55 years) were included in the study. One blood sample (8.3 ml) was taken before surgery, one during the operation and the last one 10 min after surgery. Five healthy persons (all females, mean age 36, range 23–48 years) not undergoing any operation were used as the controls. The blood was cultured by a lysis-filtration technique which had been shown to be very sensitive. Since the filter catches the microorganisms and colonies are formed during culturing, the number of bacteria in the samples is easily determined. Results: In 6 patients, all samples were negative, which indicates that the method of surgery itself caused no spread of bacteria from the lesions. Bacterial growth in the first blood sample was found in 9 patients, from the second sample in 10 and from the third one in 6. In 1 patient, bacteria were detected in three samples. At least 12 bacterial species were identified. The dominating bacteria were coagulase-negative staphylococci of which most were subtyped as Staphylococcus warneri. Among the anaerobic microorganisms, Propionibacterium acnes and P. granulosum were the most frequently isolated bacteria. The bacterial findings in the blood samples accord well with the results from a previous study in which cultures were taken from the deep parts of HS lesions. In the 5 controls, no microbial growth was detected. Conclusion: The carbon dioxide laser stripping technique caused no additional spread of bacteria into the bloodstream. The evaluation of cultures containing microorganisms from normal skin flora is controversial. Since the bacteria encountered in this study are in close agreement with the findings in cultures from the deeper parts of HS lesions they seem to be relevant. The growth of bacteria in the first blood sample taken before surgery may indicate that some of these patients have bacteria continuously circulating in their blood.
Introduction

Hidradenitis suppurativa (HS) is a cicatrising and frequently persistent inflammation of the terminal hair follicles of apocrine gland-bearing areas in the adult [1, 2]. One or both axillae and the inguinal region may be affected, commonly with spread to the scrotum, labiae, mons pubis, mammary or perineal regions and buttocks. This condition may remain relatively mild, but distressing [3], ranging from a few but recalcitrant suppurating lesions to an advanced, widespread and disabling disease lasting for years or decades. The aetiology of HS is not clear, but genetic predisposition, bacterial infections and hormonal factors have been discussed [4, 5]. The earliest inflammatory event in HS is rupture of the follicular epithelium, followed by spilling of foreign body-like material, such as keratin, bacteria, sebum products and hair into the dermis. The ‘dumping’ of foreign products initiates an inflammatory response, resulting in a foreign-body granuloma. Epithelial strands form sinuses in this inflammatory tissue. Bacterial colonisation, usually by coagulase-negative staphylococci (CoNS), can aggravate the chronic inflammation in this milieu [6]. Treatment consists mainly of surgery, while medical therapies principally serve as adjunct therapy. We have recently described a surgical technique for HS, consisting of ablation of diseased tissue combined with preservation of healthy tissue, using a carbon dioxide laser and a miniature microprocessor-controlled flash scanner [7]. The aim of the present study was to determine the number and type of bacteria circulating in the bloodstream of patients with HS undergoing surgical treatment with this scanner-assisted carbon dioxide laser technique by using lysis-filtration [8].

Material and Methods

Subjects

A total of 21 patients (20 females and 1 male) with a mean age of 36 (range 20–55) years were included in the study. The diagnosis of HS was based on the history and clinical presentation on the initial examination. All patients had one or more active suppurating lesions and had had three to twelve recurrent abscesses during the last 12 months before examination. Some patients had continuous suppurating lesions involving the axillary, inguinal and perineal (perianal, genital, pubic) areas, buttocks, upper thighs or female breasts. One or more lesions ≤10 cm in length were selected for treatment. The patients were regarded as typical of the clinical cases referred to our centre in the Department of Dermatology, Karolinska University Hospital, Huddinge, Stockholm, Sweden, which is especially interested in this disease. They were all clinically classified as Hurley stage II [9], i.e. recurrent abscesses with tract formation and cicatrisation and single or multiple, widely-separated lesions. No patient was included with concurrent diseases having fistulising tendencies, such as regional enterocolitis, ulcerative colitis or rheumatoid arthritis. A group of 5 healthy persons, all females, with a mean age of 36 (range 23–48) years and not undergoing any kind of operation, was used as the controls. The study subjects gave informed consent and the protocol was approved by the local ethics committee.

Scanner-Assisted Carbon Dioxide Laser Surgery

The surgical technique used in this study has recently been described in detail [7]. In brief, a focusing hand-piece from a special carbon dioxide laser 1030® (Lumenis Inc., New York, N.Y., USA) is attached to the miniature optomechanical flash scanner delivery system (SilkTouch®, Lumenis Inc.) that generates a focal spot which rapidly and homogeneously spiral scans and covers a round area of tissue in the focal plane. The area selected was ablated with the laser beam by passing it over the tissues, and this was 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 was controlled by the selection of power, focal length, scanner-controlled spot size and the velocity of the movements of the hand-held scanner. We used 20- to 30-watt, 3- to 6-mm spot size and 12.5- or 18-cm focal length setting. The vaporisation procedure was repeated downwards and outwards until fresh yellow adipose tissue was exposed in the deep, 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. A typical patient from our clinic is shown in figure 1.

Blood Sampling Procedure

Before sampling, the skin of the cubital fossa was carefully washed with 10% chlorhexidine in 70% ethyl alcohol followed by 8.5% povidone iodine solution. An indwelling catheter was placed in the cubital vein of the arm under strict aseptic conditions. Using Vacutainer® tubes containing 1.7 ml of a 0.35% solution sodium polyanetholsulfonate (Becton Dickinson, Rutherford, N.J., USA), we took one blood sample (8.3 ml) before local anaesthesia and surgery, one during the operation and the last one 10 min after surgery. A total of 15 samples from the 5 controls were taken 15 min apart. All samples were transported as quickly as possible to the microbiological laboratory, situated in the same building as the skin clinic, and immediately processed.

Blood Culture Technique

The blood samples processed using the sensitive lysis-filtration technique described elsewhere [8] were injected into bottles with 193 ml of a lysis solution (pH 10) containing 0.08% Na2CO3 and 0.005% Triton X-100 (Rohm and Haas, Philadelphia, Pa., USA) plus 3.0 ml of a commercial streptokinase-streptodornase compound (Varidase®, Wyeth-Lederle, Madrid, Spain) to avoid clogging of the filters. Vacuum filtration was done using a 0.45-μm pore-size filter system (Millipore Corp, Billerica, La., USA). This system has been evaluated for aerobic [10] and anaerobic bacteria [11]. After filtration, the filters were placed onto brain-heart infusion agar (Difco Laboratories, Detroit, Mich., USA)
plates supplemented with 5% horse blood for anaerobic incubation at 37°C for up to 10 days, before the samples were judged negative. As no strictly aerobic bacteria were expected to grow from the samples, there was no need to cut the filters and incubate them in both aerobic and anaerobic atmospheres, as described in the previous study [8]. The procedure of cutting the filters into two parts was also considered to constitute a risk of contamination.

**Identification of Microorganisms**

Quantitative counts were estimated from the number of colonies visible on the filters. Different types of colonies were subcultured on blood agar plates and incubated in aerobic and anaerobic atmospheres at 37°C for 24–72 h. Staphylococci were identified by API-Staph (bioMérieux, Lyon, France), using the methods described by De Paulis et al. [12] and molecular typing. Twelve isolates of CoNS had an identical, but not recognizable biochemical
profile, when typed by the API-Staph. These strains and six other CoNS with unrecognizable biochemical profiles were evaluated with molecular typing. Other facultative anaerobic bacteria were identified with the methods described in the Manual of Clinical Microbiology [13]. Anaerobic bacteria were identified with Gramstain, gas-liquid chromatography and biochemical tests, as recommended in the Wadsworth-KTL Anaerobic Bacteriology Manual [14].

**Molecular Typing of Staphylococcus warneri**

DNA Isolation. Small rice grain-sized bacterial colonies from blood agar plates were suspended in sterile Milli Q water. The genomic DNA from the bacteria was then prepared using the Qia-gen DNA mini kit (Qiagen, Valencia, Calif., USA).

PCR, DNA Sequencing and Analysis. A region of 16S rRNA gene was amplified by PCR using universal primers and ampliTaq Gold DNA polymerase. Primers and free nucleotides from the PCR products were then removed by using the QIAquick-spin PCR purification kit (Qiagen). The purified PCR products were processed for DNA sequencing by BigDye Terminator Cycle Sequencing using capillary electrophoresis technology in the ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, Calif., USA). Both strands of the PCR-amplified DNA fragment were sequenced to avoid an error of sequencing [15]. The DNA sequence was then analysed by DNA software and used to search matching in the Blast DNA database for bacterial identification and typing [15, 16].

**Results**

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 1). The dominating group of bacteria was CoNS (29 isolates). Of these, the species level of 18 strains was difficult to determine by conventional methods; therefore, they were subjected to molecular typing. DNA sequence analysis showed that 15 of the unidentified isolates 15 were S. warneri and three were S. epidermidis/caprae. The other facultative anaerobic bacteria were α-streptococci and Enterococcus faecalis. Among the anaerobic isolates, Propionibacterium acnes and P. granulosum were the commonest bacteria. A few other anaerobes, like peptostreptococci, Eubacterium spp. and Veillonella, were also seen. Facultative anaerobic strains were more frequently isolated than anaerobic ones (46 vs. 29 isolates, respectively).

No microorganisms were found in 6 of 21 patients, which suggests that the surgical approach itself caused no general spread of bacteria from the lesions. The total incidence of bacteraemia was 71% (table 2), i.e. bacteria in at least 1 sample. In table 3, the incidence and severity of bacteraemia in relation to the numbers of CoNS and propionobacteria are given. Bacterial growth in the first blood sample was found in 9 patients, from the second in 10 and from the third in 6. Bacteria were detected in three samples from 1 patient.

**Discussion**

HS is a common skin disease which causes severe symptoms and is difficult to treat. At present, the treatment is usually based on whether it is regarded as an infection or a type of acne, but its pathogenesis is not entirely understood. Treatments with immunosuppressive agents, monoclonal antibodies, anti-inflammatory drugs, antibiotics and oestrogens are of value in some patients [17]. We have found that in patients with chronic HS, macroscopically-controlled, tissue-selective and skin-preserving scanner-assisted carbon dioxide laser treatment is a safe and rapid surgical method with satisfactory cosmetic and functional results. The view underlying this surgical method is that radical removal and vaporisation of macroscopically-active HS tissue will prevent recurrences. It is thought that epithelial keratin-producing sinuses harbour debris and bacteria, which may serve as a locus of recurrence.

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<table>
<thead>
<tr>
<th>Table 1. Microorganisms obtained from blood samples collected before, during and 10 min after carbon dioxide laser surgery for HS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria isolated</strong></td>
</tr>
<tr>
<td><strong>Facultative anaerobic bacteria</strong></td>
</tr>
<tr>
<td>S. warneri</td>
</tr>
<tr>
<td>S. epidermidis</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp.</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp.</td>
</tr>
<tr>
<td>E. faecalis</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
</tr>
<tr>
<td><strong>Anaerobic bacteria</strong></td>
</tr>
<tr>
<td>P. acnes</td>
</tr>
<tr>
<td>P. granulosum</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> spp.</td>
</tr>
<tr>
<td>Veillonella spp.</td>
</tr>
<tr>
<td>Eubacterium spp.</td>
</tr>
</tbody>
</table>

Figures represent numbers of patients from whom species were isolated in relation to the time of surgery (total colony count of species).

1 Total colony count, all species.

Sartorius/Lapins/Jalal/Emtestam/Hedberg
The normal microflora of the skin varies significantly in moist and dry areas as well as rich and poor in sebaceous glands. It is well known that the degree of hydration and availability of substrate are the most important factors governing the composition of the skin’s microflora. 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 [18]. This may affect the onset of HS. Although Propionibacterium species are rarely regarded as pathogens, these organisms cause serious infections in some cases. All propionibacteria produce propionic acid as the main metabolic end product. Apart from lipases, propionibacteria produce other enzymes, such as chondroitin sulphatase, hyaluronidase, gelatinase and lecithinase, compounds that may contribute to tissue damage [19]. P. acnes has been associated with late postoperative infections and the implantation of foreign bodies, such as prosthetic heart valves, intraocular lenses and ventriculoperitoneal shunts [20].

As a group, CoNS are among the most frequently isolated bacteria and are becoming increasingly important, especially as a cause of hospital-acquired infections. S. epidermidis is commonly isolated from patients with predisposing factors such as indwelling or implanted polymer bodies. 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 S. epidermidis include the formation of extracellular enzymes and toxins, such as proteases, lipases and ß-toxins [21]. S. warneri also produces proteases and lipases [22, 23].

The results of studies of 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 of bacteraemia after carbon dioxide laser treatment of HS patients, the lysis-filtration technique was used with subsequent anaerobic incubation. The incidence and severity of bacteraemia were higher than those reported for other surgical procedures, which accords with our previous study of bacteraemia in various dental surgical procedures [8, 11]. This finding supports the view that the combination of lysis-filtration and brain-heart infusion agar has high sensitivity [11]. In 6 patients, all samples were negative, which suggests that...

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Table 2. Incidence and severity of bacteraemia before, during and 10 min after carbon dioxide laser surgery for HS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total number of bacteraemic patients (%)</th>
<th>Mean CFU (range) of bacteria/ml</th>
<th>Mean CFU (range) of facultative anaerobic bacteria/ml</th>
<th>Mean CFU (range) of anaerobic bacteria/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>9 (43)</td>
<td>0.51 (0.12–1.2)</td>
<td>0.32 (0.12–1.2)</td>
<td>0.19 (0.12–0.72)</td>
</tr>
<tr>
<td>During</td>
<td>10 (48)</td>
<td>0.23 (0.12–0.6)</td>
<td>0.13 (0.12–0.36)</td>
<td>0.096 (0.12–0.24)</td>
</tr>
<tr>
<td>10 min after</td>
<td>6 (29)</td>
<td>0.36 (0.12–0.72)</td>
<td>0.22 (0.12–0.72)</td>
<td>0.14 (0.12–0.36)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (71)*</td>
<td>0.36 (0.12–0.72)</td>
<td>0.22 (0.12–0.72)</td>
<td>0.14 (0.12–0.72)</td>
</tr>
</tbody>
</table>

* Bacteria in at least 1 sample.

Table 3. Incidence and severity of bacteraemia before, during and 10 min after carbon dioxide laser surgery for HS in relation to the numbers of CoNS and propionibacteria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total number of samples</th>
<th>Total number of bacteraemic patients</th>
<th>Number of bacteraemic patients with S. warneri</th>
<th>Number of bacteraemic patients with CoNS other than S. warneri</th>
<th>Number of bacteraemic patients with P. acnes and P. granulosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>21 (0.22)</td>
<td>9 (0.51)</td>
<td>4 (0.48)</td>
<td>2 (0.30)</td>
<td>5 (0.29)</td>
</tr>
<tr>
<td>During</td>
<td>21 (0.11)</td>
<td>10 (0.23)</td>
<td>3 (0.16)</td>
<td>3 (0.16)</td>
<td>5 (0.14)</td>
</tr>
<tr>
<td>10 min after</td>
<td>21 (0.10)</td>
<td>6 (0.36)</td>
<td>3 (0.12)</td>
<td>3 (0.32)</td>
<td>3 (0.28)</td>
</tr>
</tbody>
</table>

Figures in parentheses represent mean CFU/ml.
the surgical method itself did not cause the spread of bacteria from the lesions, because if it had we would have found an increase in the number of isolated microorganisms in the second sample followed by a marked decrease in bacteria in the third one. Halpern et al. [24] studied the incidence of transient bacteraemia by in 45 patients undergoing skin surgery on the sebaceous-rich areas of the head and neck and all their baseline blood culture results were negative, but three of them developed transient bacteraemia within the first 15 min after the start of the procedures.

Certain microorganisms requiring special conditions or nutrients may not grow or survive in many common blood culture systems. Because of the rapid growth of some bacteria, they may inhibit or outnumber other organisms. Phagocytic cells and antimicrobial substances in blood can prevent the growth of microorganisms in blood cultures. The presence of the latter in blood from patients on antimicrobial treatment may inhibit the growth of susceptible bacteria. Various methods have been used to overcome these problems. An increase in the number of bacteria isolated from patients with transient postoperative bacteraemia was reported after the introduction of prereduced anaerobic media [25], an increase in the volume of blood samples [26], the addition of a pepsin digest of ox liver to a brain-heart infusion cysteine broth [27], or use of a blood culture system based on lysis-filtration [11]. Asymptomatic bacteraemia and a high frequency of contamination have been found with the use of blood culture systems based on the lysis of blood [11, 28], and therefore, preoperative blood samples must also be taken to estimate the true postoperative bacteraemia.

The interpretation of blood cultures that are positive for CoNS or propionibacteria is often difficult. In the present study, we found a surprisingly high number of positive preoperative samples, which may indicate a high frequency of contamination unlike in other studies using this method after various oral surgical procedures [8, 11]. 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. A contamination rate as high as 10% would still not explain the incidence of bacteraemia in the preoperative samples. Another possibility is that bacteria from the chronic lesions continue to leak into the bloodstream of a subgroup of HS patients, and that they are detected with this very sensitive method of culture. This was certainly an unexpected finding, and more studies are needed to determine its clinical relevance. However, our findings must be interpreted with caution. First, due to the small number of patients, the results of bacteriological findings can be due to chance effects. Second, our patients were referred to us by other specialists, who had treated them unsuccessfully with other methods. Therefore, we may have selected HS patients with a relatively therapy-resistant type of HS. Third, our patients had Hurley stage II disease [9], which we regarded as operable with this method. Most patients seen in Departments of Dermatology and many of those with HS have a milder course, usually Hurley stage II [9], i.e. recurrent abscesses with tract formation, cicatrization, single or multiple, widely separated lesions that have not yet become more severe. In our Department, patients classified as Hurley III are referred to plastic surgeons for wide excision and reconstructive surgery. However, Hurley stage II is the commonest type of HS [5]. We have no data on bacteraemia in milder (Hurley stage I) or more severe cases (Hurley stage III). Fourth, and the most surprising result of this study, the incidence of bacteria in the blood samples taken before surgery was high. This needs careful consideration, since 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. In this study, we evaluated blood cultures with the very sensitive lysis-filtration technique and the findings accorded with those of a previous study of cultures from deeper HS lesions. In that study we used the carbon dioxide laser method to evaporate the diseased tissue, which enabled us to take bacteriological cultures at the same time and thereby minimize contamination [6]. The carbon dioxide laser permits heat sterilization [29–31]. We found sixteen species or sub-species. S. aureus and CoNS were most frequent, while Peptostreptococcus species and P. acnes were also common [6]. These findings indicate a need to re-evaluate the significance of bacteria in the development of HS and that CoNS are probably 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, will increase the pathogenic properties of CoNS in HS. In the present study, it is noteworthy that molecular typing of CoNS showed that 15 of 29 staphylococci isolates were S. warneri, which has been regarded as a contaminant, but more recently considered to be a pathogen that can be associated with significant morbidity and even mortality in hospitalised patients. S. warneri has been isolated from
subdural empyemas, urinary tract infections, vertebral osteomyelitis and bacteraemias in infants in intensive care nurseries [32, 33]. Moreover, and perhaps of more relevance to HS, S. warneri has been found in chronic conditions associated with a foreign body. These conditions resemble certain aetiopathological features of HS, as discussed above, including central venous catheters, prosthetic heart valves and neurosurgical ventricular shunts [32, 33]. Unfortunately, no data are available on the CoNS subtypes from our first study of bacteria in HS lesions [6], because the CoNS strains were not saved. In our experience of almost 300 treated HS patients and from the literature, this infection 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. The ability of CoNS isolates to express resistance to several antibiotics is well known and a reduction in susceptibility to vancomycin by S. warneri isolates from a neonatal intensive care unit has been reported [34]. In the present study, we evaluated antimicrobial susceptibility to methicillin, fusidic acid, clindamycin and vancomycin in various strains. S. warneri was susceptible to all antimicrobial agents tested (11 strains) and the three S. epidermidis/ caprae isolates were resistant to methicillin, but susceptible to the other agents tested (data not shown).

In conclusion, the carbon dioxide laser stripping technique followed by healing by secondary intention for treatment of HS caused no 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 by us previously in cultures from deeper parts of HS lesions, they seem to be relevant. The growth of bacteria in the first blood sample taken before surgery suggests that some of these patients have bacteria continuously circulating in the blood. Therefore more and larger studies should be done in different types of patients. Perhaps studies of the relation between S. warneri and the innate immune responses [35] would add further insights.

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