EXTENDED REPORT

# The pustular skin lesions in Behçet's syndrome are not sterile

**G Hatemi, H Bahar, S Uysal, C Mat, F Gogus, S Masatlioglu, K Altas, H Yazici**

*Ann Rheum Dis* 2004;**63**:1450–1452. doi: 10.1136/ard.2003.017467

See end of article for authors' affiliations

Correspondence to: Professor H Yazici, Cerrahpasa Tip Fakultesi, Ic Hastaliklari ABD, 34300 Aksaray, Istanbul, Turkey; hyazici@attglobal.net

Accepted 21 December 2003

**Background:** The pustular skin lesions of Behçet's syndrome (BS) are clinically and histopathologically similar to ordinary acne, but BS patients get lesions at sites not commonly involved in acne, such as the legs and arms. The microbiology of these lesions has not been studied adequately.
**Objective:** To make a detailed study of the microbiology of BS lesions.
**Methods:** Subjects were patients with BS and acne vulgaris. Material was extracted from pustular lesions and directly plated to aerobic and anaerobic media by sterile swab. Anaerobic bacteria were identified using a commercial kit (API 20A). Aerobic bacteria were defined by standard procedures.
**Results:** 58 BS patients and 37 acne patients were studied. Pustules were cultured from the following sites: BS patients (70 pustules): face (17), back (30), chest (2), arm (4), leg (17); acne patients (37 pustules): face (27), back (6), chest (1), arm (2), leg (1). At least one type of microorganism was grown from each pustule. *Staphylococcus aureus* (41/70, 58.6%, p=0.008) and *Prevotella* spp (17/70, 24.3%, p=0.002) were significantly more common in pustules from BS patients, and coagulase negative staphylococci (17/37, 45.9%, p=0.007) in pustules from acne patients.
**Conclusions:** The pustular lesions of BS are not usually sterile. The microbiology of these lesions is different from ordinary acne. It remains to be determined whether the infection is secondary or has any pathogenic implications.

Pustular skin lesions in Behçet's syndrome (BS) are clinically similar to ordinary acne; however, they appear both on the usual acne sites, such as the face, back and chest, and at unusual sites such as the arms and legs. The histopathology of papulopustular lesions in BS does not differ from that of ordinary acne.[1]

It is generally thought that pustular lesions in BS are sterile, but to the best of our knowledge the microbiology of these lesions has not been formally examined. This study was designed to explore the microbiology of pustular lesions in BS patients in comparison with the pustules in acne vulgaris. The information obtained might prove useful in determining the obscure pathogenesis of the syndrome, especially in the light of recent observations from our unit that acne and arthritis in BS may be related.[2 3]

## METHODS

We studied 58 BS patients (37 male, 21 female, mean (SD) age 32.6 (8.8) years) attending a dedicated multidisciplinary Behçet's syndrome outpatient clinic, along with 37 patients with acne vulgaris (22 male, 15 female, mean age 25.7 (9.2) years). BS patients fulfilled the International Study Group criteria.[4] We studied only those BS patients who were not using steroids or other immunosuppressive drugs and who had active pustules during their visit. The patients with acne vulgaris were attending the dermatology outpatient clinic and had active pustules. They were not using any drugs and did not have any associated diseases. All patients were first examined by a rheumatologist and a dermatologist.

### Collection of samples

For sample collection the patients were sent to a microbiologist who was unaware of the diagnosis. Samples were collected from the usual acne sites such as the face, back, and chest, and unusual acne sites such as the arms and legs from both BS and acne patients. If the patient had pustules on more than one site (such as face and leg), samples were collected from one pustule from each site. Before the collection of samples, the skin on and around the pustule was cleaned with 70% alcohol and allowed to dry. Pustules were opened with a sterile needle and the content was taken with sterile thin cotton swabs.[5]

### Microbiological analysis

#### Aerobic bacteria

For the isolation of aerobic bacteria, blood agar, chocolate agar, and McConkey agar were used. After an incubation period of 48 hours at 37°C, the morphology of each colony with a distinct appearance was marked and Gram stains were prepared from these colonies. *Staphylococcus aureus* was identified by haemolysis on blood agar plates, DNase activity, and plasma coagulase characteristics. The API Staph kit (bioMerieux sa, Marcy-l'Etoile, France) was used to confirm the identification of staphylococci. Streptococci were identified by their α and β haemolytic characteristics and by their PYR activity. *Escherichia coli* were identified using the API E kit (bioMerieux).

#### Anaerobic bacteria

For the isolation of anaerobic bacteria, Schaedler agar enriched with 5% sheep blood and 1 mg/ml vitamin $K_1$ was used. This anaerobic blood agar was supplemented with either 0.25% phenyl ethyl alcohol, forming phenyl ethyl alcohol blood agar, or 7.5 mg/ml vancomycin and 100 mg/ml kanamycin, forming kanamycin-vancomycin blood agar. Anaerobic incubation was carried out in anaerobic jars (Oxoid, Columbia, Maryland, USA) for a minimum of seven days before initial examination. Anaerobic conditions were obtained with Anaero-Gen (Oxoid and Mitsubishi Gas Company). After a seven day incubation, primary anaerobic plates were examined on a colony microscope; colonies were described from each anaerobic medium and semiquantitated. One colony of each type described was Gram stained and subcultured onto two chocolate agar plates and an anaerobe blood agar plate to obtain pure cultures of each colony. One of the chocolate agar plates was incubated at 37°C and the other in $CO_2$ to verify the aerobic and aero-tolerant character

**Table 1** The demographic features and distribution of pustules from patients with Behçet's syndrome and acne vulgaris according to location

| | Behçet's syndrome | Acne vulgaris |
|---|---|---|
| Number of patients | 58 | 37 |
| Male/female | 37/21 | 22/15 |
| Age (years) (mean (SD)) | 32.6 (8.8) | 25.7 (9.2) |
| Number of lesions | 70 | 37 |
| Number of lesions on: | | |
| Face | 17 | 26 |
| Back | 30 | 6 |
| Chest | 2 | 1 |
| Arms | 4 | 3 |
| Legs | 17 | 1 |

of the colony. The API 20 A kit (bioMerieux) was used to identify the anaerobic bacteria.[6 7]

### Statistics

Student's *t* test and Fisher's exact test were used for statistical analyses.

## RESULTS

Pus from cultures was studied in 58 BS patients and 37 acne patients. Some of the BS patients had pustules at more than one site, and material from each of these pustules was cultured separately. Altogether 70 pustules from 58 BS patients were cultured. One pustule was cultured from each acne patient. The demographic features of the subjects and the distribution of the pustules are presented in table 1.

The microorganisms grown in pustules from BS patients and their distribution according to location are given in table 2. *Staphylococcus aureus* was the most common microorganism grown in pustules from BS patients (58.6%), especially those at unusual acne sites (18 of 21, 85.7%). Gram negative anaerobic bacteria of *Prevotella* spp were grown in 17 pustules from BS patients.

When the microorganisms grown from pustules at unusual acne sites such as the arms and legs were compared with those at the usual acne sites such as the face, back, and chest we found that *S aureus* was significantly more common in the unusual acne sites among BS patients ($p = 0.003$).

The microorganisms grown in pustules from the acne patients and their distribution according to location are given in table 3. *Propionibacterium acnes* and coagulase negative staphylococci were the most commonly grown bacteria.

The microorganisms grown from pustules from BS and acne patients are shown in table 4. *S aureus* were significantly more common ($p = 0.008$) in the pustular lesions of Behçet's syndrome, while coagulase negative staphylococci were significantly less common ($p = 0.007$). *Prevotella* spp were grown from 17 pustules from BS patients, but from none of the acne pustules. The microbiology of pustules at usual acne sites was similar in BS and acne vulgaris. Only coagulase negative staphylococci were slightly more common in acne vulgaris patients ($p = 0.048$).

The number of type of microorganisms grown in each pustule was also calculated. The mean number of types grown in each pustule was 1.9 in BS patients and 1.7 in patients with acne vulgaris ($p = 0.074$).

## DISCUSSION

In this study we showed that pustules were not sterile in our Behçet's patients. Their microbiology was somewhat similar to acne vulgaris, except that *S aureus* was more common and coagulase negative staphylococci less common in the BS patients. Previous studies had shown that *Propionibacterium acnes* and coagulase negative staphylococci were the main microorganisms growing in acne vulgaris pustules.[8] In our acne patients these were also the most common pathogens, but in addition *S aureus* was grown in 29.9% of our patients. In previous studies from other centres *S aureus* was reported to grow with such high frequency in acne vulgaris only after prolonged antibiotic treatment.[5] A Japanese group showed the growth of *S aureus* in 8.3% of their patients with acne vulgaris.[9] In two previous studies by another group from our university, *S aureus* was grown in acne vulgaris pustules at frequencies of 20% and 29.6%, respectively.[10 11] These investigators studied only the pustular lesions and not comedos or papules. Secondary infection of pustules with S *aureus* could explain this high frequency. One study showed that the number of types of microorganism and the colony counts increased as the age of the lesion increased.[12] In our study group, most patients were not able to report the age of their lesions accurately. A study designed to examine the microbiology of pustules according to their age, and including microbiological analysis of biopsies of early papular lesions, could be helpful in determining the role of microorganisms in the pathogenesis of these lesions. Another explanation for the high frequency of *S aureus* could be that these organisms are indeed more frequent in the Turkish population.

Examination of the differences between microorganisms grown in our BS and acne patients showed that the higher frequency of *S aureus* observed in BS pustules was a feature of pustules at unusual acne sites. *S aureus* was grown in 18 of 21 pustules (85.7%) on the arms and legs. The frequency of microorganisms grown in pustules at usual sites was similar in BS and acne patients except for a slight difference in coagulase negative staphylococci ($p = 0.048$), which grew more often in pustules from the acne patients. A study of the comparative microbiology of the occasional pustules observed on the arms and legs of otherwise healthy individuals to those of pustules of BS patients at similar sites would be necessary to clarify this issue.

**Table 2** The microorganisms grown in pustules from patients with Behçet's syndrome and their distribution according to location

| | Usual acne site | | | | Unusual acne site | | | |
|---|---|---|---|---|---|---|---|---|
| | Face | Back | Chest | Total | Arm | Leg | Total | Total |
| Number of lesions | 17 | 30 | 2 | 49 | 4 | 17 | 21 | 70 |
| *S aureus** | 10 | 13 | – | 23/49 | 4 | 14 | 18/21 | 41/70 |
| *Propionibacterium acnes* | 9 | 15 | – | 24/49 | – | 5 | 5/21 | 29/70 |
| Coagulase negative staphylococci | 3 | 7 | 2 | 12/49 | – | 2 | 2/21 | 14/70 |
| α-Haemolytic streptococci | 4 | 8 | 1 | 13/49 | 1 | 2 | 3/21 | 16/70 |
| *Propionibacterium granulosum* | 3 | 1 | – | 4/49 | 1 | – | 1/21 | 5/70 |
| *E coli* | 5 | 3 | – | 8/49 | – | 1 | 1/21 | 9/70 |
| *Prevotella* spp | 6 | 5 | – | 11/49 | 1 | 5 | 6/21 | 17/70 |

*S aureus was significantly more common at unusual acne sites, $p = 0.03$.

**Table 3** The microorganisms grown in pustules from patients with acne vulgaris and their distribution according to body location

| | Usual acne site | | | | Unusual acne site | | | |
|---|---|---|---|---|---|---|---|---|
| | Face | Back | Chest | Total | Arm | Leg | Total | Total |
| Number of lesions | 26 | 6 | 1 | 33 | 3 | 1 | 4 | 37 |
| *S aureus* | 5 | 6 | – | 11/33 | – | – | – | 11/37 |
| *Propionibacterium acnes* | 12 | 3 | – | 15/33 | 1 | 1 | 2/4 | 17/37 |
| Coagulase negative staphylococci | 14 | – | 1 | 15/33 | 2 | – | 2/4 | 17/37 |
| α-Haemolytic streptococci | 5 | 1 | – | 6/33 | – | – | – | 6/37 |
| *Propionibacterium granulosum* | 1 | – | – | 1/33 | – | – | – | 1/37 |
| *E coli* | 2 | – | – | 2/33 | – | – | – | 2/37 |
| *Prevotella* spp | – | – | – | – | – | – | – | – |

The other important difference was the growth of *Prevotella* spp bacteria in 17 pustules from BS patients. Prevotella are Gram negative anaerobic bacilli, which are not commonly grown in pustular lesions. They are generally found in mixed anaerobic infections such as abscesses and pulmonary and ear infections.[13] In one review of secondary bacterial infections complicating skin lesions, *Prevotella* spp were reported in 22 of 150 secondarily infected skin lesions, consisting of psoriasis, eczema, eczema herpeticum, scabies, and poison ivy.[14] It is not known whether the skin in Behçet's syndrome shares common features with these lesions. It could be suggested that the reaction of skin to infections is different in BS. Previous work by Fresko *et al* from our group, showing that surgical cleaning diminishes the pathergy reaction (skin hypersensitivity to needle prick) may support this hypothesis.[15]

The role of the microorganisms we described in the pathogenesis of pustules in BS is not clear. The same is true for ordinary acne. It is thought that microorganisms may activate inflammation in acne, but they are not responsible for the initiation of acne lesions.[16] It is not known whether the effects of antibiotics such as tetracycline and erythromycin in acne reflect their antimicrobial or their immunomodulatory actions.[16]

In an earlier study by Diri *et al* it was shown that pustular lesions were more common in BS patients with arthritis.[2] A factor analysis of the clinical manifestations of BS also supported the view that pustular lesions and arthritis are associated.[3] It was suggested that arthritis in BS could be related to acne associated reactive arthritis. The effect of penicillin on mucocutaneous lesions and arthritis in BS has been examined in two different studies and it was found that prophylactic penicillin treatment reduced both the mucocutaneous lesions and the arthritis episodes.[17 18] Our findings in the current study lend some support to our previous contention that the pathogenesis of joint involvement in BS could be similar to that in reactive arthritis.[2]

**Table 4** The microorganisms grown in patients with Behçet's syndrome and acne vulgaris

| | Behçet's syndrome | Acne vulgaris |
|---|---|---|
| Number of patients | 58 | 37 |
| Number of lesions | 70 | 37 |
| *S aureus** | 41 (58.6%) | 11 (29.7%) |
| *Propionibacterium acnes* | 29 (41.4%) | 17 (45.9%) |
| Coagulase negative staphylococcus† | 14 (20%) | 17 (45.9%) |
| α-Haemolytic streptococci | 16 (22.9%) | 5 (13.5%) |
| *Propionibacterium granulosum* | 5 (7.1%) | 1 (2.7%) |
| *E coli* | 9 (12.9%) | 2 (5.4%) |
| *Prevotella* spp‡ | 17 (24.3%) | – |

*p=0.008; †p=0.007; ‡p<0.001.

## Conclusions

Our results show that the pustular lesions in BS are infected. Whether these pustules are secondarily infected or whether the infections play a pathogenic role in the development of pustular lesions and possibly of arthritis remains to be determined.


## Authors' affiliations

**G Hatemi, F Gogus, S Masatlioglu, H Yazici,** Istanbul University, Cerrahpasa Medical School, Division of Rheumatology, Istanbul, Turkey
**H Bahar, K Altas,** Istanbul University, Cerrahpasa Medical School, Department of Microbiology
**S Uysal, C Mat,** Istanbul University, Cerrahpasa Medical School, Department of Dermatology


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