

Clinical significance of the isolation of *Staphylococcus epidermidis* from bone biopsy in diabetic foot osteomyelitis

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Introduction: Coagulase-negative staphylococci are considered as microorganisms with little virulence and usually as contaminants. In order to establish the role of *Staphylococcus epidermidis* as a pathogen in diabetic foot osteomyelitis, in addition to the isolation of the sole bacterium from the bone it will be necessary to demonstrate the histopathological changes caused by the infection.

Methods: A consecutive series of 222 diabetic patients with foot osteomyelitis treated surgically in the Diabetic Foot Unit at La Paloma Hospital (Las Palmas de Gran Canaria, Canary Islands, Spain) between 1 October 2002 and 31 October 2008. From the entire series including 213 bone cultures with 241 isolated organisms, we have analyzed only the 139 cases where Staphylococci were found. We analyzed several variables between the two groups: *Staphylococcus aureus* versus *Staphylococcus epidermidis*.

Results: Of the 134 patients included in this study, *Staphylococcus epidermidis* was found as the sole bacterium isolated in 11 cases and accompanied by other bacteria in 12 cases. *Staphylococcus aureus* was found as the sole bacterium isolated in 72 cases and accompanied by other bacteria in 39 cases. Histopathological changes were found in the cases of osteomyelitis where *Staphylococcus epidermidis* was the sole bacterium isolated. Acute osteomyelitis was found to a lesser extent when *Staphylococcus epidermidis* was the sole bacterium isolated but without significant differences with the cases where *Staphylococcus aureus* was the sole bacterium isolated.

Conclusion: *Staphylococcus epidermidis* should be considered as a real pathogen, not only a contaminant, in diabetic patients with foot osteomyelitis when the bacterium is isolated from the bone. No differences in the outcomes of surgical treatment have been found with cases which *Staphylococcus aureus* was isolated.

Keywords: *diabetic foot; osteomyelitis; bone infection; diabetic foot infections; foot ulcer*

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Osteomyelitis is a challenging complication of diabetic foot ulcers that may threaten the limb of the patient. In nearly every series reported in the literature, *Staphylococcus aureus* was the most common pathogen cultured from bone samples in the feet of diabetic patients, followed by *Staphylococcus epidermidis* (1). Despite coagulase-negative staphylococci being considered as microorganisms with little virulence (2) and usually considered as contaminants in such conditions (3), studies with microbiological examination of bone samples have shown isolation rates of *S. epidermidis* between 10 and 50% (4–7). Surprisingly, Senneville et al. (4) found a higher rate of coagulase-negative staphylococci in bone biopsy samples than in swab samples

(25.6 vs. 4.6%; $p < 0.001$). This finding may support the idea that coagulase-negative staphylococci were true pathogens in such cases, but the importance of this finding is unclear because of the absence of histological confirmation of osteomyelitis (4). Recently, a study has been published that emphasizes the importance of *S. epidermidis* as a pathogen in foot ulcers with protruding bone, which suggests underlying osteomyelitis, and the authors propose that *S. epidermidis* should be recognized as a nosocomial pathogen (8). In order to establish the role of *S. epidermidis* as a pathogen in diabetic foot osteomyelitis, in addition to isolating the sole bacterium from the bone, it will be necessary to demonstrate the histopathological changes caused by the infection.

Furthermore, since *S. epidermidis* is usually associated with lower pathogenicity, it would be interesting to compare the outcomes of the treatment with the cases in which *S. aureus* is isolated. We are not aware of any paper correlating the isolation of *S. epidermidis* from bone biopsy with histopathological changes in diabetic patients with foot osteomyelitis. The purpose of this study is to analyze the bone histopathology when *S. epidermidis* is the sole bacterium isolated from an operative biopsy. In addition, we analyze the clinical presentation and outcomes of the treatment, comparing the cases of *S. epidermidis* with *S. aureus*.

Patients and methods

A consecutive series of 222 diabetic patients with foot osteomyelitis were treated surgically in the Diabetic Foot Unit at La Paloma Hospital (Las Palmas de Gran Canaria, Canary Islands, Spain) between 1 October 2002 and 31 October 2008. From the entire series, including 213 bone cultures with 241 isolated organisms, we analyzed only 139 cases where staphylococci were found. We excluded four cases without histopathological confirmation of osteomyelitis, despite the isolation of the bacteria from the bone sample. A further case where *S. aureus* and *S. epidermidis* were found together was also excluded. In total, the series studied included 134 patients. The diagnosis of infection was established on the basis of the presence of purulent secretion or ≥ 2 signs or symptoms of inflammation (9). The diagnosis of osteomyelitis was made on the basis of a combination of a probe-to-bone test (10) and a radiological study of the foot. Limb ischemia was diagnosed if any of the following criteria were met: absence of both distal pulses, an ankle/arm index below 0.9 or transcutaneous oxygen pressure of < 30 mmHg. Data about the patients were prospectively collected using a computerized database, which contains data on patient demographics, clinical evaluation, presence of co-morbidities, prior amputations or reulceration, previous antibiotic treatment and results of laboratory tests. Blood cultures were not obtained. All patients were operated on within the first 12 h after admission to our hospital. All patients were given an initial empirical antibiotic treatment with ampicillin/sulbactam in the operating theatre during anesthetic induction, except in cases of associated allergy. From 31 October 2007, we began to use amoxicillin/clavulanic acid as the empirical preoperative antibiotic treatment. In patients with a penicillin allergy, intravenous clindamycin and ciprofloxacin were chosen as treatment. During surgical intervention, samples were extracted from the affected bone for analysis in microbiological and pathological laboratories. No anaerobic cultures were performed, as this is not the normal practice in our centre. Surgical treatment was defined as conservative surgery if only the infected bone and non-viable soft tissue, of

whatever size, were removed without amputating any part of the foot; as minor amputations if they involved a partial amputation of the foot that did not involve the ankle joint, and as major amputations if located above the ankle joint. Surgical treatment protocols were the same irrespective of the bacteria isolated. This means that re-operations were performed when local or general sepsis was found, which could not be controlled using antibiotics or minor in-bed debridements. Wounds were left open to heal by second intention except in cases of major amputations. Moist wound healing management was always used, but it was varied according to the needs of the patient. Once cultures and antibiograms were available, the antibiotic administered was modified according to the results. The length of postoperative antibiotic treatment varied considerably, but, generally, when the wound was totally occupied by granulation tissue, antibiotic treatment was discontinued. Histopathological findings in bone specimens were defined as acute osteomyelitis when necrosis, destroyed bone, and infiltration by polymorphonuclear granulocytes at cortical sites and inside the bone marrow were present. Congestion or thrombosis of the medullary or periosteal small vessels were also frequently found. Osteomyelitis was defined as chronic when there was destroyed bone and infiltration by lymphocytes, histiocytes, and/or plasmatic cells at cortical sites and inside the bone marrow. The histopathological changes were defined as acute exacerbation of chronic osteomyelitis when there was a background of chronic osteomyelitis with infiltration of polymorphonuclear granulocytes. All three forms of osteomyelitis exhibited areas of fibrosis in variable forms and medullar edema (7). Healing was defined as complete epithelization of the ulcer and/or the surgical wound resulting from the removal of bone. Patients were followed until healing. Postoperative death is defined as that occurring within 30 days of surgery.

The study was conducted in accordance with the Declaration of Helsinki as revised in 2000. This retrospective study did not require approval by an ethics committee since the procedures did not alter or exceed the scope of our standard medical care. All patients gave written informed consent for surgery, for photographs taken of their wounds, to be included in our computer database and for their (anonymized) cases to be published.

Statistical analysis

We analyzed several variables between the two groups: *S. aureus* vs. *S. epidermidis*. Qualitative variables were analyzed by Fisher's exact test and non-normally distributed quantitative variables by using the non-parametric Mann-Whitney *U* test. Differences were considered statistically significant when *p*-values were less than 0.05. Statistical analysis was performed using Statplus statis-

tical analysis software (AnalystSoft, Vancouver, BC, Canada).

Results

From the 134 patients included in this study, *S. epidermidis* was found as the sole bacterium isolated in 11 cases and accompanied by other bacteria in 12 cases. *S. aureus* was found as the sole bacterium isolated in 72 cases and accompanied by other bacteria in 39 cases. Methicillin-resistant *Staphylococcus aureus* [MRSA] was isolated in 39 out of these 111 cases (35.1%). *S. epidermidis* was vancomycin-resistant on two occasions (8.6%). The bacteria that were found together with staphylococci are shown in Table 1. Histopathological changes were found in the cases of osteomyelitis where *S. epidermidis* was the sole bacterium isolated. No statistically significant differences in the duration of the ulcer or previous antibiotic treatment were found. Acute osteomyelitis was found to a lesser extent when *S. epidermidis* was the sole bacterium isolated, but without significant differences with the cases where *S. aureus* was the sole bacterium isolated. Clinical presentation and outcomes between the two groups are summarized in Table 2.

Discussion

S. epidermidis, a non-pathogenic member of common cutaneous microbial flora, expresses few virulence factors under normal conditions (11). Coagulase-negative *Staphylococcus* spp., such as *S. Epidermidis*, are responsible for the majority of chronic osteomyelitis associated with orthopedic implants (12). *S. epidermidis* has been regarded as a contaminant in a series of osteomyelitis (3), (13), but the isolation of this bacterium is very frequent in a series using bone biopsies. Authors show 9% (14), 10.7% (7), 11% (5), and 50% (6) of *S. epidermidis* in their series. Other authors found 25.6% (4) and 40% (15) of coagulase-negative staphylococci in bone biopsy samples.

Table 1. Bacteria isolated from bone biopsy together with staphylococci

	<i>Staphylococcus aureus</i> (n = 39)	<i>Staphylococcus epidermidis</i> (n = 12)
<i>Escherichia coli</i>	10 (25.6%)	0
<i>Enterobacter</i> species	8 (20.5%)	3 (25%)
<i>Pseudomonas</i> species	8 (20.5%)	2 (16.6%)
<i>Proteus</i> species	4 (10.2%)	5 (41.6%)
<i>Acinetobacter</i> species	3 (7.7%)	0
<i>Citrobacter</i> species	2 (5.1%)	1 (8.3%)
<i>Klebsiella</i> species	1 (2.5%)	1 (8.3%)
<i>Serratia odorifera</i>	1 (2.5%)	0
<i>Shigella</i> species	1 (2.5%)	0
<i>Streptococcus viridians</i>	1 (2.5%)	0

Lavery et al. (5) state that a possible explanation for the difference between their series and Newman's series (6) may be the fact that intraoperative bone cultures used by the Lavery team may be less prone to contamination than the percutaneous technique. The technique chosen for sampling (needle puncture or transcutaneous bone biopsy) may also have some influence on the isolation of coagulase-negative staphylococci: 7.1% in bone biopsy specimens and 19.5% in needle puncture specimens (16). Although histopathology was performed in some of these studies, authors did not show the histopathological findings in the cases where coagulase-negative staphylococci were isolated. All the patients in our series were operated on because the clinical signs and exploration suggested the presence of osteomyelitis. Samples from subsequent bone biopsies where *S. epidermidis* was isolated, showed histopathological changes that confirmed the diagnosis. In this way, we are able to propose that *S. epidermidis* behaved as a true pathogen agent. This proposition cannot be definitively proved since no anaerobic cultures were performed. Anaerobic bacteria could also have been responsible for the histopathological changes in the cases where *S. epidermidis* was the sole bacteria isolated. Nonetheless, the role of anaerobic bacteria in diabetic foot osteomyelitis is not well understood because bone biopsy-based osteomyelitis studies only show 3% (14), 4% (6), 4.8% (4), 11.9% (16), and 14% (5) of anaerobes. We think, as do others, that the mixed aerobic-anaerobic infections commonly identified in soft tissue infections in diabetics are not characteristic of bone infections of the foot in diabetic patients (5). For these reasons, we believe that anaerobic bacteria played little part, if any, in the histopathological changes when *S. epidermidis* was the sole bacterium isolated and these changes were caused by *S. epidermidis* itself. Our postoperative management is to leave the surgical wounds open for healing by secondary intention and this is a hostile environment for the growth of anaerobic bacteria. Furthermore, we think, like others (17), that anaerobes were easily eliminated by aggressive debridement and making anaerobic cultures could not therefore contribute to optimizing the treatment of these patients (18).

Recently, it has been reported that diabetic patients with ischemic foot ulcers differ from neuropathic foot ulcer patients, having a higher frequency of *S. epidermidis* skin colonization and ulcer infection (8). The authors of this prospective study found significant differences in the rate of isolation of *S. epidermidis* between neuropathic and ischemic ulcers when the toe web surface was swabbed and when they performed curettage of the ulcers. They suggest a predilection of *S. epidermidis* for ischemic tissue. However, there was no statistically significant difference when the sample was obtained by biopsy: 12.5% in neuropathic ulcers vs. 10% in ischemic ulcers. This may mean that *S. epidermidis* colonized the

Table 2. Clinical presentation and outcomes between *Staphylococcus epidermidis* and *Staphylococcus aureus*

	<i>Staphylococcus epidermidis</i> as sole isolated (n = 11)	<i>Staphylococcus aureus</i> as sole isolated (n = 72)	P
Age (years) ^a	68 (8)	65.5 (16)	0.34
Years since diagnosis of diabetes ^a	16 (18)	19 (10)	0.87
Glycated hemoglobin, proportion of total hemoglobin ^a	0.086 (0.029)	0.082 (0.026)	0.29
Previous ulcer, n (%)	7 (63.6)	45 (62.5)	1
Previous amputation, n (%)	4 (36.4)	37 (51.4)	0.52
Ulcer as point of entry of infection, n (%)	11 (100)	65 (90.3)	0.58
Ulcer duration in days ^a	60 (120)	30 (106)	0.06
Previous antibiotherapy, n (%)	7 (63.6)	60 (83.3)	0.21
Fetid smelling, n (%)	4 (36.4)	22 (30.6)	0.73
Necrosis, n (%)	3 (27.3)	22 (30.6)	0.56
Exposed bone, n (%)	3 (27.3)	17 (23.6)	0.72
Probing to bone test positive, n (%)	9 (81.8)	66 (91.7)	0.28
Positive signs in X-ray, n (%)	8 (72.7)	61 (84.7)	0.38
Ischemia, n (%)	8 (72.7)	38 (52.8)	0.33
White blood cell count, × 10 ⁹ /l ^a	7,380 (6,500)	9,400 (3,838)	0.3
Leukocytosis, n (%)	4 (36.4)	18 (25)	0.47
Erythrocyte sedimentation rate (mm/h) ^a	72.5 (81)	78 (50)	0.55
Alkaline phosphatase (U/l) ^a	219 (308)	146 (186)	0.15
Conservative surgery, n (%)	7 (63.6)	42 (58.3)	1
Minor amputations, n (%)	4 (36.4)	29 (40.3)	1
Major amputations, n (%)	0 (0)	1 (1.4)	1
Number of surgical procedures, n (%)	1 (0)	1 (1)	0.43
Acute osteomyelitis, n (%)	3 (27.3)	39 (54.2)	0.11
Chronic osteomyelitis, n (%)	4 (36.4)	15 (20.8)	0.26
Acute exacerbation of chronic osteomyelitis, n (%)	4 (36.4)	18 (25)	0.47
Perioperative death, n (%)	1 (9.1)	1 (1.4)	0.24
Hospital stay (days) ^a	15 (16)	16 (39)	0.48
Time to healing (days) ^a	75 (69)	102 (103)	0.06

^aValues are expressed as median (interquartile range).

ischemic tissue more frequently, but it is probable that factors other than ischemia led to infection. The difference between infection and colonization in diabetic foot ulcers defined in terms of microbiological factors is still an unresolved issue. We have not found any significant differences in the presence of ischemia between the groups. No differences in clinical presentation, metabolic control or ulcer duration have been found in our study. Authors investigated the hypothesis that like *S. aureus*, *S. epidermidis* can also invade bone cells evading antimicrobial therapy and may therefore explain the difficulties in treating such infections (19) They found that *S. epidermidis* was capable of invading bone cells, but that there were significant strain-dependent differences in this ability (19). The virulence and pathogenicity of strains of *S. epidermidis* may be related more to genetic factors in the bacteria than to local conditions in the hosts. We speculate that the identification of such strains in bone biopsies may help in the choice between surgical

treatment and antibiotic treatment. However, this may be as difficult here as it has been shown in other clinical settings (20).

We have not found significant differences in the outcomes of treatment in the cases where *S. epidermidis* was found compared with *S. aureus*. This finding may be surprising, but we believe that surgical treatment determines the outcomes of the treatment irrespective of the bacteria present. We have previously reported that MRSA bone infections treated by early and aggressive surgical treatment are not associated with worse prognosis (21). Another limitation of this study is the small number of cases where *S. epidermidis* was isolated. This is a determining factor that limits the statistical conclusions. However, the total number of cases where staphylococci were isolated is large enough to contribute to the knowledge of the role of *S. epidermidis* in diabetic foot osteomyelitis.

In conclusion, we have presented a series of cases in which *S. epidermidis* was isolated from a bone biopsy

and histopathological studies confirmed the diagnosis. *S. epidermidis* should be considered as a real pathogen, not only as a contaminant, in diabetic patients with foot osteomyelitis when the bacterium has been isolated from the bone. No differences in the outcomes of surgical treatment have been found with cases in which *S. aureus* was isolated.

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