

Bloodstream Infections Caused by Small-Colony Variants of Coagulase-Negative Staphylococci Following Pacemaker Implantation

Christof von Eiff, Pierre Vaudaux, Barbara C. Kahl, Daniel Lew, Stefan Emler, Andrej Schmidt, Georg Peters, and Richard A. Proctor

From the Institute of Medical Microbiology, University of Münster, Münster, and the Department of Internal Medicine, University of Erlangen-Nürnberg, Erlangen, Germany; the Division of Infectious Diseases, University Hospital of Geneva, Geneva, Switzerland; and the Laboratory of Bacterial Pathogenesis and Immunology, Rockefeller University, New York, and the Departments of Medical Microbiology/Immunology and Medicine, University of Wisconsin Medical School, Madison, Wisconsin, USA

Small-colony variants (SCVs) of *Staphylococcus aureus* cause persistent and relapsing infections. Relatively little is known regarding infections caused by SCVs of coagulase-negative staphylococci. We report two cases of pacemaker electrode infections due to SCVs of *Staphylococcus epidermidis* and *Staphylococcus capitis*. Sequence analysis of a portion of the 16S rRNA gene (16S rDNA) confirmed the identity of the staphylococcal species as *S. capitis* and *S. epidermidis*. Isolates from cultures of blood obtained over at least a 2-week interval were compared by pulsed-field gel electrophoresis and found to be clonal even though the colony morphology was very different. Analysis for auxotrophism revealed hemin dependencies for all isolated SCVs. The two cases have several clinical and laboratory characteristics (which are also seen with *S. aureus* SCV infections) and strongly suggest that SCVs of coagulase-negative staphylococci must be actively sought, because they grow very slowly and can be easily missed.

Although *Staphylococcus aureus* small-colony variants (SCVs) have been recognized for many years, the connection of this phenotype with persistent infections has only recently been appreciated [1, 2]. They have been reported to produce relapsing infections in bone, heart valves, lung, and soft tissues [1]. The significance of these variants particularly in patients with chronic osteomyelitis and patients with cystic fibrosis has been demonstrated in prospective studies [3, 4]. Herein, we report two cases of bloodstream infections due to SCVs of coagulase-negative staphylococci (CoNS) following endocardial pacemaker electrode implantation and compare their clinical and laboratory characteristics with those of infections caused by *S. aureus* SCVs.

Diagnostic Methods

Identification. CoNS were identified on the basis of a variety of conventional phenotypic characteristics, the biochemical profile (which was determined by using the API System

[ATB32 Staph Strips, 26 tests; and API Staph Strips, 19 tests; bioMérieux, Marcy l'Etoile, France] after 48 hours of incubation at 37°C), and the absence of the *S. aureus*-specific *nuc* gene [5]. SCVs were identified as slow-growing, nonpigmented, nonhemolytic pinpoint colonies that were observed after 48–72 hours of incubation on rabbit blood agar [3, 4]. Very small colonies on Columbia agar were suspected as SCVs if they grew nearly normally on Schaedler's agar (with CO₂ or anaerobically) [4].

Auxotrophism studies. Auxotrophy for hemin (Unipath, Basingstoke, United Kingdom) was tested by using standard disks, and autotrophy for thymidine (Fluka Chemie, Buchs, Switzerland) or menadione (Sigma Aldrich Chemie, Deisenhofen, Germany) was tested by impregnating disks with 15 µL of thymidine or menadione at 100 µg/mL. Test isolates were inoculated on chemically defined medium as previously described [3, 4].

Analysis of 16S rDNA. Extraction of DNA and amplification of 1 kilobase of the bacterial 16S rDNA were carried out with use of primers 285 and 261 as previously described [6]. Briefly, DNA was prepared from single colonies of both the species *Staphylococcus epidermidis* and *Staphylococcus capitis* grown on blood agar and disrupted mechanically. A 300-bp portion of the amplified DNA was sequenced by direct sequencing with the primer 285 and the internal primer 244 [6] by using fluorescent dideoxy-dye terminators on an automated DNA sequencer (ABI 373A; Perkin-Elmer, Rotkreuz, Switzerland). Results were controlled for sequencing errors and compared with NCBI GenBank entries by using a BLAST algorithm [7].

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Reprints or correspondence: Dr. Christof von Eiff, Institute of Medical Microbiology, Westfälische Wilhelms-Universität Münster, Domagkstraße 10, 48149 Münster, Germany (eiffc@uni-muenster.de).

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Molecular typing. To examine the clonality of phenotypically normal CoNS and SCV isolates, we analyzed these strains with pulsed-field gel electrophoresis as previously described [4]. *Sma*I digests of total bacterial DNA were resolved, and strains were considered clonal if less than two bands varied on a gel.

Case Reports

Case 1. A 62-year-old man presented for evaluation of a 3-month history of relapsing episodes of fever and chills. History revealed pacemaker placement for bradycardia 4 months earlier. Physical examination was unremarkable except for a soft decrescendo aortic murmur. Laboratory examinations showed mild anemia, elevated C-reactive protein (CRP) level, and elevated erythrocyte sedimentation rate (ESR), but the WBC count and differential cell counts were normal. Transesophageal echocardiography and CT of the chest and abdomen were unremarkable. Initial blood cultures yielded CoNS, which were interpreted as contaminants and not further differentiated. However, eight additional cultures of blood obtained over the following 17 days were positive for *S. epidermidis*.

Visible growth (very small colonies) on a subculture plate started after 48 hours of incubation at 37°C. The organisms isolated after 48–72 hours were so heterogeneous in their colonial morphology that polymicrobial infection was suspected. Biochemical testing with API Staph Strips and conventional macrotube testing were not possible (although catalase positivity was found in all seven isolates) because limited growth over 48 hours was not sufficient enough to provide positive test results in the reaction vials. Therefore, two approaches were used to identify the positive blood cultures. First, by analyzing the 16S rDNA, the sequences of the amplicons over 250 nucleotides were found to be identical to previously reported sequences of *S. epidermidis* (accession no. L37605). Second, *Sma*I digests of the whole bacterial genome of all isolates were typed by pulsed-field gel electrophoresis and were found to be clonal, despite large differences in their colony phenotype. Thus, the seven isolates were the same species and clonal.

Analysis of auxotrophism revealed that the SCVs were hemin auxotrophic. The organisms were susceptible to ceftriaxone, and because of penicillin allergy, therapy with ceftriaxone was initiated for 6 weeks. The pacemaker was not removed because fever quickly resolved, and the CRP level and ESR returned to normal. A follow-up transesophageal echocardiogram, total-body CT, and bone scans were also unremarkable. Although the source of infection remained unproved, the number of positive blood cultures, the temporal relationship of the onset of fever with the placement of the pacemaker, and the absence of other foreign bodies suggested that the pacemaker was the most likely focus of infection. Seven months after stopping antibiotic therapy, no signs of infection were observed.

Case 2. A 64-year-old man presented for evaluation of a 2-week history of recurrent fever (temperature up to 40°C) and

chills following a tooth extraction 10 days before the initial onset of symptoms. The patient had a pacemaker placed 3 years ago for bradycardia due to sick sinus syndrome. Laboratory examinations showed severe anemia and an elevated WBC count, CRP level, and ESR. While a chest roentgenograph and an abdominal sonograph were unremarkable, a transesophageal echocardiogram indicated a 2.5-cm vegetation on the transvenous endocardial pacemaker electrode. There was no evidence of infection of the skin and soft tissue overlying the implanted generator.

Four cultures of blood obtained over a 2-week period, which were recognized as positive after 3 days of incubation of the subculture plates, yielded *S. capitis*. Sequence analysis of a portion of the 16S rRNA gene (16S rDNA) confirmed the identity of the isolates as *S. capitis* (accession no. L37599). The isolates, which grew mostly as pinpoint colonies, proved to be hemin-auxotrophic SCVs. Upon subculture from the solid medium, some colonies grew rapidly. All isolates were compared by pulsed-field gel electrophoresis and found to be clonal even though the colony morphology was very different.

Oxacillin and gentamicin treatment was started, and fever promptly resolved. Because the vegetations could still be detected 5 weeks after initiation of antibiotic therapy, the pacemaker was removed. The electrode of the pacemaker was cultured, and pinpoint colonies grew after 72 hours of incubation. In a following subculture, these organisms did not grow, making further differentiation impossible. After a further 2-week course of the same antibiotic regimen, the patient remained afebrile, and there was no recurrence in the subsequent 10 months.

Discussion

SCVs are a naturally occurring subpopulation that grow slowly and produce small colonies (hence, their name). *S. aureus* SCVs were first described >80 years ago, and a number of studies support a pathogenic role for SCVs in diseases [1–4]. *S. aureus* SCVs have been reported to produce persistent and often recurrent infections and have a number of characteristics that are atypical for *S. aureus*, including reduced coagulase production, failure to ferment mannitol, and increased resistance to aminoglycosides and cell wall-active antibiotics [1, 2]. Biochemical characterization of most clinical SCVs reveals defects in electron transport. Clinical and laboratory-generated SCVs are frequently auxotrophic for menadione and hemin, two compounds required in the biosynthesis of the electron transport chain components menaquinone and cytochrome, respectively [1, 3, 4, 8]. In addition, *S. aureus* SCVs have been found to persist inside of cultured endothelial cells, probably because these variants produce very little α -toxin [8, 9]. The intracellular location may shield SCVs from the host immune response and decreases exposure to antibiotics [4, 8, 9].

Compared with *S. aureus* SCVs, very little is known regard-

ing infections caused by SCVs of CoNS. There are only two well-described cases of infection due to phenotypic variants of CoNS; however, strains were not characterized as slow-growing electron transport–deficient SCVs regarding their auxotrophisms [10, 11]. Infections caused by SCVs of *S. capitis* have not yet been reported. The two cases reported here have several characteristic laboratory features, which have also been seen with *S. aureus* SCV infections. First, the organisms grew very slowly and could have been easily missed if the blood subcultures were examined only for 48 hours, particularly when large-colony forms were also present. Second, SCVs were difficult to identify because of their slow and atypical biochemical reactions. They had changed biochemical characteristics, such as reduced lactose and turanose fermentation and/or no nitrate reduction. In a previous study in which a stable genetically defined mutant with a SCV phenotype was used, the absence of sugar alcohol (e.g., mannitol) fermentation was explained by interrupted electron transport [8].

Third, the presence of heterogeneous colony forms initially suggested a mixed infection; however, all of the isolates proved to be from the same species, and the subpopulations were also from the same clone. Fourth, all SCVs isolated from these patients were hemin auxotrophs. Fifth, SCVs grow more rapidly in the presence of CO₂ and on rich medium, such as Schaedler's agar (which contains hemin). Hence, these SCVs can be construed as anaerobic organisms since these conditions are often used for anaerobic blood cultures. Because oxygen does not enhance the growth of electron transport–deficient SCVs, their growth in an anaerobic chamber on Schaedler's agar can make laboratory personnel believe that anaerobes are present [1].

Thus, SCVs of CoNS are similar to *S. aureus* SCVs in many ways. However, differences regarding antibiotic susceptibilities, which were observed when normal phenotypes and SCVs of *S. aureus* were compared, were not seen in the cases presented. The SCVs of CoNS from both patients were susceptible to the antibiotics tested, but testing was extended to 48–72 hours to allow sufficient growth of the controls. Finally, because of the large differences in colony morphology and the slow growth of these SCVs of CoNS, molecular techniques may be required for identification.

The clinical presentations of our two patients are of interest in view of the rather benign symptoms of endovascular infections. These benign symptoms are typical for infections caused by *S. aureus* SCVs because the ability of the SCV strain to lyse mammalian cells and to produce toxins is less than that of the rapidly growing parent strain [2, 8, 9]. Both patients responded well to antibiotic therapy with no recurrences of signs or symptoms, although deposits on the pacemaker wire in one case were still detectable after treatment with antibiotics. This course is somewhat different than that seen with both normal phenotypes and SCVs of *S. aureus*, where breakthrough bacteremia during therapy and recurrences after termination of

antibiotic therapy are common. This course, however, is consistent with the expected responses to antibiotic therapy when the efficacy of antibiotic treatment alone for *S. aureus* prosthetic device infections is compared with that of antibiotic treatment alone for prosthetic device infections due to CoNS. Specifically, *S. aureus* prosthetic device infections are very rarely cleared without removing the device, whereas coagulase-negative organisms may be clinically eradicated about one-half of the time [12].

Therefore, although some differences are seen, many of the clinical and laboratory characteristics of *S. aureus* SCVs are also found with SCVs of CoNS. Thus, SCVs of CoNS must be actively sought, because they can be easily missed due to their slow growth and may cause serious infections.

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