Problems in diagnosis and treatment of \textit{Staphylococcus haemolyticus} endocarditis in a haemodialysis patient


We report a case of \textit{Staphylococcus haemolyticus} endocarditis in which diagnosis could be achieved only by use of DNA-fingerprinting methods. Coagulase-negative staphylococci (CNS) were isolated over a 2-year period during most of a total of 14 periods of fever. It was difficult to assess whether these subsequent isolations could be explained by recurrent contamination with different strains and species or by persistent endocarditis caused by a single strain, since different colony morphologies, API-Staph (BioMérieux, Marcy U Etoile, France) profiles and antibiotic susceptibility patterns were observed. Moreover, no clinical signs of endocarditis could be established during the first period of fever and glycopeptide treatment, started after the first period of fever, was ineffective, although in vitro susceptibility testing indicated glycopeptide susceptibility.

\textbf{Case report}

A 38-year-old male patient had been treated with chronic haemodialysis since June 1993, because of IgA nephropathy. He also suffered from chronic liver cirrhosis due to alcohol abuse, cardiomyopathy without the presence of cardiac valvular disease, intermittent claudication and chronic obstructive pulmonary disease. In July 1993, continuous ambulatory peritoneal dialysis (CAPD) catheter was inserted in a procedure which was associated with technical problems. Prophylaxis with teicoplanin \((2 \times 400 \text{ mg/day})\) was started. Two months later, recurrent fever and positive blood cultures suggested endocarditis and the patient was treated for 6 weeks with teicoplanin (in combination with gentamicin for 2 weeks). Echocardiography was normal at this time, but 6 months later echocardiography revealed vegetations on the aortic valve with valvular insufficiency of degree III. Six weeks of therapy with vancomycin \((500 \text{ mg every two dialysis sessions})\), combined with gentamicin \((40 \text{ mg after every dialysis})\) for the first 2 weeks, was given. Three months later \((9.5 \text{ months after catheter insertion})\), the same treatment was administered since blood cultures were again positive for CNS. From then onwards a second major criterion for the clinical diagnosis of infectious endocarditis \([1]\) was fulfilled by the fact that most blood culture sets were positive. Thirteen months after catheter insertion, the patient was admitted to the intensive care unit for cardiac failure, and a combined regimen of vancomycin, gentamicin and rifampicin was given. Echocardiography revealed an old and a new vegetation. At that moment, excision of the infected valves was not considered because of depressed left ventricular function and coagulation problems from the liver cirrhosis. The patient suffered new episodes of fever, and was treated twice with vancomycin and also with other antibiotics (ceftriaxone, amoxycillin/clavulanic acid) because of pneumonia. After 23 months it was decided to replace the infected valves, because of the persistent occurrence of septic episodes. A myxoid degenerated aortic valve was found, combined with a vegetation on the ventricular side of the left coronary cusp and healed endocarditis on the non-coronary cusp with shrinkage of the valvular leaflet. Cultures of the excised valves yielded small colony variants of \textit{S. haemolyticus}.

Seven isolates of CNS from the positive blood cultures, and two from the excised valve, were studied in more detail. Because the early isolates had been considered CNS contaminants, blood culture isolates were available only from 16 months after the first febrile episode. At that moment, the isolated organisms formed pinpoint colonies (small colony variants, SCV) after 24 h of incubation, which became white non-hemolytic small colonies \((1–2 \text{ mm})\) after 2–3 days of incubation, quite different from the early isolates, which were larger and hemolytic. Also, some of the API-Staph identification results of the blood culture isolates were rather confusing, since the five \textit{S. haemolyticus} isolates were identified as \textit{S. hominis} \((4)\) or \textit{S. aureus} \((1)\). Application of tRNA inter-repeat spacer length polymorphism analysis (tRNA-PCR), which has been described as a potential tool for the identification of staphylococci \([2,3]\), identified most of the blood culture isolates as \textit{S. haemolyticus} (Figure 1). One of the blood cultures could be identified as \textit{S. capitis} subsp. \textit{urealyticus} and one as \textit{S. sciuri} (Figure 1). At this stage, it was possible to suspect that \textit{S. haemolyticus} was the causative agent and this was later confirmed by identification by tRNA-PCR of the two valve isolates as \textit{S. haemolyticus}. In addition, \textit{Smal} chromosomal DNA restriction digestion \([4]\) indicated that the \textit{S. haemolyticus} isolates were very similar and were clearly different from the \textit{S. sciuri} and \textit{S. capitis} isolates (Figure 2).

The clonal relatedness of the \textit{S. haemolyticus} isolates was studied by \textit{Smal} chromosomal DNA restriction analysis \([4]\) and with arbitrarily primed PCR (AP-PCR) \([5]\), carried out independently with primer 7.
Figure 1  tDNA-PCR profiles of clinical isolates from blood cultures and valves and of reference strains. B, blood culture isolates; V, valve isolates. Molecular sizes are indicated in base pairs.

(GTG GAT GCG A) and primer ERIC1 (AAG TAA GTG ACT GGG GTG ACG C). According to Smal chromosomal DNA restriction analysis, the overall similarity between the blood culture and valve *S. haemolyticus* isolates was obvious, although some variation was observed (Figure 2). The AP-PCR fingerprints of the *S. haemolyticus* isolates from the patient obtained with two independent primers were comparable (Figure 3, only results for primer 7 presented). The fingerprints of isolates B2, B3, B4, B6, B7, V1 and V2 showed a high resemblance, were clearly different from that of the non-*S. haemolyticus* patient isolates (Figure 3, isolates B1 and B5) and showed some distinct differences—apparent for fragments above 800 bp—from the *S. haemolyticus* reference isolates (Figure 3, lanes 10–12), suggestive of a clonal relatedness between the *S. haemolyticus* patient isolates. Using different typing techniques, it has been shown that there is substantial genotypic variability within the species *S. haemolyticus* [6].

Several blood culture isolates from the presumptively identical *S. haemolyticus* strain with normal colony morphology were found to be susceptible to vancomycin as tested by disk diffusion [7]. Teicoplanin, although administered initially, was not tested because disk diffusion with this antibiotic is carried out only on request in this laboratory. Vancomycin was given several times, but resolution of the clinical symptoms was only transient. During October 1995, rifampicin was added to the treatment. A few days later, the isolates from positive blood cultures were rifampicin resistant and subsequently the isolates were all SCV. Susceptibility
Figure 2  Dendrogram (UPGMA method) of the Dice coefficients of the Smal chromosomal DNA restriction profiles (pulsed-field gel electrophoresis) of the clinical blood culture and valve isolates. Normalization of the fragment positions was carried out by GelCompar analysis. An Smal digest of S. aureus NCTC 8325 was used as molecular size marker. Fragments from 36 to 800 kb were analyzed.

Figure 3  AP-PCR profiles (primer 7 (GTG GAT GCG A)) of clinical isolates from blood cultures and valves and of reference strains. B, blood culture isolates; V, valve isolates. Molecular sizes indicated in base pairs.
testing from this moment on was difficult and could only be done with deviation from the standard technique [7], i.e. by the use of blood agar, heavy inoculum and prolonged incubation in a CO2-enriched atmosphere. Nevertheless, these SCV were determined to be susceptible to vancomycin.

Glycopeptides have good activity against Gram-positive organisms, including when these are methicillin resistant, and therefore are indicated for the treatment of endocarditis [8]. Moreover, in anephric patients high antibiotic levels can be obtained, remaining above the MIC value for a prolonged time, conditions which are essential for good treatment with glycopeptides [8]. Since the isolate of our patient did not develop glycopeptide resistance and since vancomycin peak serum levels were kept between 10 and 20 μg/mL, it remains unclear why we were unable to eradicate the causative agent. Cell wall deficiency has been described as leading to glycopeptide resistance [9], but this explanation can probably be excluded because cell wall-deficient isolates revert to normal colony morphology after prolonged incubation, which was not the case for the glycopeptide-susceptible S. haemolyticus isolates described here. Reversal to normal colony morphology could not be achieved on tryptic soy agar and tryptic soy agar supplemented with 5% sheep blood or by addition of X-factor, V-factor or menadione, or incubation in 5% CO2, but growth was slightly enhanced on GC Agar with IsoVitalex (Becton Dickinson Microbiology Europe, Meylan, France). The uneven distribution of glycopeptides in the cardiac vegetation has been proposed for in vivo resistance [10], and the intracellular location of SCVs may cause their exposure to only subtherapeutic concentrations of many antimicrobial agents [11]. SCVs tend to be more resistant to cell wall-active antibiotics [11], and defects in electron transport—which are held responsible for the SCV phenotype—may cause a decrease in aminoglycoside uptake [11]. The occurrence of staphylococcal SCVs has been reported previously [e.g. 11,12].

In summary, several factors prevented the timely recognition of a single causative agent. Although CNS are the most frequently isolated pathogens in prosthetic valve endocarditis and cause 5% of infections involving native valves [13,14], distinguishing significant septicemia caused by CNS from contamination poses a problem, since these organisms belong to the normal skin flora. CNS blood contaminants can be expected to be more common in hemodialysis-treated patients, because of frequent intravascular manipulations; indeed, such contaminants were isolated from our patient (S. sciuri and S. capitis subsp. ureolyticus). Furthermore, limitations in phenotypic identification of Staphylococcus sp. indicated erroneously the presence of additional species (S. aureus and S. hominis). Also, the causative agent underwent phenotypic changes during infection, suggesting again the presence of still another species. From the data presented here, it appears that the SCV phenotype was induced by rifampicin treatment. The presence of a single causative organism became clear only with hindsight at a moment when review of all the data was possible and after application of genotypic identification and typing techniques.

This case confirms that in vitro susceptibility of S. haemolyticus towards glycopeptides is not necessarily predictive of in vivo eradication of the organism from endocarditis and that recovery from CNS endocarditis without surgery is unlikely [15,16]. The occurrence of SCV variants of Staphylococcus species—which may be more difficult to treat than wild-type strains—should not be overlooked. In spite of new and promising genotypic techniques, endocarditis with CNS remains difficult to diagnose and manage.

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References
Clinical spectrum of infections due to
Propionibacterium acnes

Propionibacterium acnes is a Gram-positive, non-spore-forming, anaerobic rod that is a common inhabitant of the skin, anterior nares, conjunctiva, mouth, and upper respiratory tract [1,2]. It is a common contaminant of cultures obtained percutaneously (surgical wound swabs, blood or other body fluids). However, P. acnes has been implicated alone or as part of a mixed flora as a cause of several human infections [3]. In this report, we analyze the clinical, epidemiologic and microbiological features of 44 cases of infection with P. acnes diagnosed in our hospital between 1988 and 1996.

We retrospectively reviewed the clinical charts of the Microbiology Department of the Fundación Jiménez Díaz (a 600-bed teaching hospital in Madrid, Spain) from January 1988 to February 1996. We selected for review all cases with: (1) isolates of P. acnes with moderate or heavy growth, in pure or predominant culture, and evidence of inflammatory reaction (as detected on Gram smear by the presence of polymorphonuclear leukocytes), or (2) growth of P. acnes in at least two blood culture sets (Hemoline, bioMérieux, France).

During the study period we identified 118 specimens with moderate or heavy growth of P. acnes from 112 patients. Forty-four patients (39.3%) were considered to have significant infection. Twenty-one cases were reported in previous articles [4-6]. In 29 cases, P. acnes was the only organism isolated. In 15 patients, these isolates were recovered with scanty growth of one or two other organisms: coagulase-negative Staphylococcus spp. (12 cases), Streptococcus anginosus (two), Staphylococcus aureus (one), Corynebacterium spp. (one), and Clostridium perfringens (one).

The ages of patients ranged from 17 to 80 years (median, 44 years). Thirty-two were males. Twenty-five patients (57%) had cutaneous or subcutaneous infections (16 wound surgical infections, seven abscesses, one pacemaker generator box infection, one post-traumatic fasciitis), 13 (31%) had central nervous system infections (six brain abscess, four subdural empyma, two epidural empyma, one meningitis) and six had other infections (two transient bacteremia, one endocarditis, one osteomyelitis, one mastoiditis, one botryomycosis). Predisposing conditions were observed in 37 patients (84%), 26 of whom (59.1% of the total) were admitted in the neurosurgery ward. The conditions noted in these 37 patients were previous surgery (29), presence of foreign bodies (22), malignancy (17), immunosuppression (five), trauma (five), and diabetes mellitus (two).

Forty patients (91%) received antibiotic therapy with a duration ranging from 7 to 90 days (median, 18 days); in 37 cases, antimicrobial treatment was combined with surgical drainage, debridement or removal of foreign material. Surgical treatment alone was applied in two patients.

Two patients did not receive medical or surgical treatment (transient bacteremia). Three patients died (7%), but in only one case could death be attributed to infection with P. acnes. This patient had a primary brain abscess which spontaneously discharged to the cerebral