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Microbial diagnosis of infection and colonization of cardiac implantable electronic devices by use of sonication

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SUMMARY

Objectives: The clinical utility of sonication as an adjunctive diagnostic tool for the microbial diagnosis of cardiac implantable device-associated infections (CIDAIs) was investigated.
Methods: The implants of 83 subjects were investigated, 15 with a CIDAI and 68 without a clinical infection. Clinical data were analyzed prospectively and sonication fluid cultures (83 patients, 100%) and traditional cultures (31 patients, 37.4%) were performed
Results: Generator pocket infection and device-related endocarditis were found in 13 (86.7%) and four (26.7%) subjects, respectively. The mean numbers of previous technical complications and infections were higher in the infected patients compared to the non-infected patients (8 vs. 1, $p < 0.001$; 2 vs. 0, $p < 0.031$, respectively). The sensitivity and specificity for detecting CIDAI was 73.3% (11/15) and 48.5% (33/68) for sonication fluid culture, and 26.7% (4/15) and 100% (16/16) for traditional culture ($p < 0.001$), respectively. A higher number of organisms were identified by sonication fluid than by tissue culture (58 vs. 4 specimens; $p < 0.001$). The most frequent organisms cultured were Gram-positive cocci (66.1%), mainly coagulase-negative staphylococci (35.5%). Thirty-five (51.5%) non-infected subjects were considered colonized due to the positive identification of organisms exclusively through sonication fluid culture.
Conclusions: Sonication fluid culture from the removed cardiac implants has the potential to improve the microbiological diagnosis of CIDAIs.

1. Introduction

The surgical implantation of cardiovascular electronic devices, including permanent pacemakers (PPMs), implantable cardioverter defibrillators (ICDs), and cardiac resynchronization devices (CRTDs), has been indicated increasingly worldwide in the last 30 years for the treatment of many different medical conditions, such as bradycardia, ventricular arrhythmia, and heart failure, and for the prevention of sudden cardiac death.[1–3] Indeed, data collected in 2009 from 61 different countries have shown a continuous rise in the number of cardiac implantable electronic devices (CIEDs) being inserted worldwide, with the highest number of implanted PPMs in the USA (225 567) and Germany accounting for 927 new implants per million population.[4]

Although infection following CIED use remains relatively uncommon, it may affect exclusively the generator pocket, intravascular electrode components, or endocardial structures, or may even present in different combinations, making the clinical suspicion often delayed and not considered.[5] Moreover, accurate and evidence-based diagnostic tools for CIED-associated infections (CIDAIs) are lacking, as the clinical presentation is highly variable, echocardiography may show low accuracy, and blood and conventional cultures of peri-implant fluid (swab) or tissue samples may show low sensitivity.[6–10] False-negative microbiological results

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varying from 12% to 49% have been associated with previous antibiotic use and to the nature of a biofilm-associated infection in which organisms are enclosed in a polymeric matrix substance exhibiting altered phenotype and gene expression.[5,11-12] Furthermore, local positive cultures in subjects showing no signs or symptoms of active infection have been associated with microbial colonization of the generator pocket.[13–15]

A few studies have recently suggested that by using techniques that dislodge bacterial cells from the biofilm, such as vortexing and sonication, microbial diagnosis is increased among CIDAI subjects.[6,13,16] Compared to conventional cultures, sonication has shown higher sensitivity and specificity for microbial diagnosis for a variety of implant-associated infections.[17–19] In the present study, the clinical utility of sonication as an adjunctive diagnostic tool for CIDAIs was investigated, by comparing it with traditional cultures (blood cultures and intraoperative peri-implant tissue cultures). Furthermore, the utility of sonication in identifying microbial colonization of the generator pocket was also assessed among subjects with no signs of infection.

2. Materials and methods

2.1. Study population

Eighty-three subjects who underwent complete or partial surgical removal of a CIED (including PPMs and ICDs) for any reason, between September 2010 and October 2013, at the Cardiac Surgery Unit of Santa Casa de Sao Paulo School of Medical Sciences, São Paulo (Brazil), were included prospectively in this study. Subjects were excluded if clinical data were unavailable for analysis, when the retrieved CIED was not submitted to the sonication technique, or when contamination occurred during implant removal, transportation, or processing in the microbiology laboratory. Subject demographics, the type of CIED, comorbidities, previous CIDAIs and surgeries, the length of time between implantation and implant retrieval, clinical signs and symptoms of infection, and the presence of endocarditis were recorded. The study protocol was reviewed and approved by the institutional review board.

2.2. Diagnosis of CIED-associated infection

Clinical features of CIDAI were considered to be present when the generator pocket showed localized cellulitis, swelling, discharge, wound dehiscence, or local pain; intraoperative tissue showed visible purulence as determined by the surgeon; a draining fistula communicating with the internal implant was evident; and when signs and symptoms of systemic infection (fever, chills, night sweats, malaise) were present. The Duke criteria were also used for the diagnosis of CIED infective endocarditis.[20–21]

2.3. Specimen collection and microbiological methods

In the surgical ward, blood cultures, sterile cotton swabs of the prosthetic (generator and leads), peri-prosthetic fluids, and tissue samples were collected and processed for microbiology and histopathology. Tissue was homogenized in 3 ml of brain–heart infusion (BHI) broth for 1 min. Homogenized tissue and wood shaft cotton swabs were inoculated onto aerobic sheep blood agar, chocolate agar, and anaerobic blood agar and into thioglycolate broth (BD Diagnostic Systems, Sparks, MD, USA). The time limit for processing samples was 6 h. Aerobic and anaerobic plates were incubated aerobically at 35–37 °C in 5–7% CO_2 for 7 days and anaerobically at 37 °C for 14 days, respectively. Additionally, 0.5 ml of tissue homogenate was inoculated in thioglycolate broth, incubated for 14 days, and the turbid thioglycolate broth was sub-cultured on blood agar plates when cloudy. Colonies of microorganisms growing on plates were identified, and their susceptibility to antibiotics was tested by standard microbiological techniques. Bacterial identification was performed with routines established in the laboratory, assessing the morphology and tinctorial properties displayed on Gram staining. Catalase tests were applied on Gram-positive colonies to identify *Staphylococcus spp* and *Streptococcus spp*. DNase tests differentiated *Staphylococcus aureus* from coagulase-negative staphylococci (CoNS), while groups of *Streptococcus spp* and *Enterococcus spp* were identified by their hemolysis (alpha, beta, or gamma), ability to hydrolyze esculin in the presence of bile, and growth on 6.5% NaCl associated with susceptibility testing to optochin and bacitracin or the CAMP test, when necessary. Gram-negative colonies were identified by biochemical methods to determine their genus and species, including glucose non-fermenting bacteria. Low-virulence microorganisms (CoNS, *Corynebacterium sp*, *Chryseobacterium sp*, *Bacillus sp*) were considered pathogens when the same organism was identified in at least two different tissue samples, or when at least one additional (culture-independent) criterion for CIDAI was also fulfilled.

2.4. CIED sonication

In the operating room, the explanted CIEDs were removed aseptically and placed in sterilized solid polyethylene containers, to which 250 ml of Ringer solution was added; these were sealed with an air-tight cover. In the microbiology laboratory, containers with the retrieved implants were vortexed for 30 s using a Vortex-Genie 2 (Scientific Industries Inc., Bohemia, NY, USA) and then sonicated (ultrasound bath BactoSonic; Bandelin GmbH, Berlin, Germany) for 5 min at a frequency of 40 ± 2 kHz and power density 0.22 ± 0.04 W/cm^2, followed by an additional 30 s of vortexing, in accordance with the technique of Trampuz et al.[17] To concentrate the resulting sonication fluid, centrifugation was performed in 50-ml aliquots at 2500 rpm for 5 min. The supernatant was aspirated, leaving 0.5 ml (100-fold concentration), and aliquots of 0.1 ml of concentrated sonication fluid were then plated onto aerobic sheep blood, chocolate, and anaerobic sheep blood agar; these were incubated aerobically at 37 °C for 7 days and anaerobically at 37 °C for 14 days, respectively, and inspected daily for bacterial growth. Additionally, 4 ml of the remaining concentrated sonication fluid was inoculated in 10 ml thioglycolate broth, plated as described above, and incubated aerobically at 35–37 °C in 5% CO_2 for 2 days and anaerobically at 37 °C for 14 days. A 4-ml aliquot of sonication fluid and all the detected organisms were frozen at −70 °C. Colonies of isolated microorganisms growing on plates were quantitated (number of colony-forming units per milliliter sonication fluid; CFU/ml), identified, and their antimicrobial susceptibility was tested using standard microbiological techniques. Due to the addition of a concentration step to the sonication fluid culture, a cut-off of 50 CFU/plate was considered positive and used for ideal sensitivity and specificity analysis.[19] Furthermore, for those subjects on antimicrobial therapy or those who had previously received antibiotics for at least 24 h in the 14 days prior to surgery, any growth of organism in the sonication fluid culture was considered positive. CIEDs explanted due to aseptic reasons were used as negative controls and processed in the same manner as described for the infected CIED implants retrieved.

2.5. Statistical analysis

Characteristics of subjects with infected and non-infected cardiovascular electronic devices were summarized as the frequency and percentage, or as the mean (range) and standard deviation (SD). Descriptive comparisons between categorical