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# Microbiological diagnosis in cardiac implantable electronic device infections detected by sonication and next-generation sequencing

Thomas Olsen, MD, PhD,[*†] Ulrik Stenz Justesen, MD, DMSc,[†‡] Jens Cosedis Nielsen, MD, PhD, DMSc,[§¶] Ole Dan Jørgensen, MD, PhD,[‖**] Niels Christian Foldager Sandgaard, MD, PhD,[*] Christen Ravn, MD, PhD,[††] Christian Gerdes, MD, PhD,[§] Anna Margrethe Thøgersen, MD, DMSc,[‡‡] Sabine Gill, MD, PhD,[*] Kurt Fuursted, MD, DMSc,[§§] Jens Brock Johansen, MD, PhD[*†**]

*From the *Department of Cardiology, Odense University Hospital, Odense, Denmark, †Department of Clinical Research, University of Southern Denmark, Odense, Denmark, ‡Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark, §Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark, ¶Institute of Clinical Medicine, Aarhus University, Aarhus, Denmark, ‖Department of Heart, Lung and Vascular Surgery, Odense University Hospital, Odense, Denmark, **Danish Pacemaker and ICD Register, Odense, Denmark, ††Department of Orthopedic Surgery, Odense University Hospital, Odense, Denmark, ‡‡Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark, and §§Statens Serum Institut, Copenhagen, Denmark.*

**BACKGROUND** Device-related infection (DRI) is a severe complication of treatment with cardiac implantable electronic devices. Identification of the causative pathogen is essential for optimal treatment, but conventional methods often are inadequate.

**OBJECTIVE** The purpose of this study was to improve microbiological diagnosis in DRI using sonication and next-generation sequencing analysis. The primary objective was identification of causative pathogens. The secondary objective was estimation of the sensitivity of different microbiological methods in detecting the causative pathogen.

**METHODS** Consecutive patients with clinical signs of DRI between October 2016 and January 2019 from 3 tertiary centers in Denmark were included in the study. Patients underwent a diagnostic approach, including blood cultures and perioperative collection of microbiological samples (pocket swab, pocket tissue biopsies, generator, and leads). Conventional culturing was performed, and device components were sonicated and examined with an amplicon-based metagenomic analysis using next-generation sequencing. The results were compared with a reference standard–identified causative pathogen.

**RESULTS** In 110 patients with clinical signs of pocket (n = 50) or systemic DRI (n = 60), we collected 109 pocket swabs, 220 pocket tissue biopsies, 106 generators, 235 leads, and a minimum 1 set of blood cultures from 102 patients. Combining all findings, we identified the causative pathogen in 95% of cases, irrespective of DRI type. The usability of each microbiological method differed between DRI types. In pocket DRI, next-generation sequencing analysis of generators achieved sensitivity of 90%. For systemic DRI, blood cultures reached sensitivity of 93%.

**CONCLUSION** Using a strategy including sonication and next-generation sequencing, we identified the causative pathogen in 95% of DRI. Sensitivity of microbiological methods differed according to the type of DRI.

**KEYWORDS** Cardiac implantable electronic device; Cardiac implantable electronic device infection; Device-related infection; Infection; Molecular microbiology; Next-generation sequencing; Sonication

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## Introduction

Implantation of a cardiac implantable electronic device (CIED) is the treatment of choice for several cardiac arrhythmias. Device-related infections (DRIs) are an infrequent[1] but severe complication that increases both morbidity and mortality.[2,3] DRI traditionally is divided into localized pocket DRI (limited to the device pocket) or cardiac device-related infective endocarditis (systemic bloodstream infection

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involving the leads, cardiac valves, or endocardial surface).[4–6] DRI presents with a wide array of symptoms, and diagnosis can be challenging in nonobvious cases. Treatment of DRI necessitates complete CIED system removal[4,7] in combination with a prolonged period of antibiotics.[5,6,8] Therefore, exact microbiological diagnosis is needed but often is not possible[9,10] using conventional culturing. Reasons are thought to be previous antibiotic treatment, the fastidious nature of some bacteria, and biofilm formation on device components.

Sonication is a novel technique that disrupts the biofilm and has shown promising results in smaller series of DRIs[9,11] and orthopedic prosthetic joint infections.[12,13] Recently, various amplicon-based metagenomic approaches involving next-generation sequencing (NGS) have emerged as a diagnostic tool, enhancing pathogen detection in infected patients.[14,15]

The purpose of this study was to evaluate the usefulness of a diagnostic approach including sonication and NGS in clinically suspected DRI. The primary objective was identification of the causative pathogen, defined by a multicriteria reference standard. The secondary objective was estimation of the sensitivity of different microbiological methods.

## Methods

### Study design, population, and diagnostic approach

The project was designed as a descriptive, prospective, multicenter study and performed according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[16] We included consecutive patients with clinical suspicion of DRI who were referred for device removal at 1 of the 3 participating tertiary hospitals (Odense, Aarhus, and Aalborg University Hospitals) between October 2016 and January 2019. Patients younger than 18 years, who were pregnant, or had contraindications for transesophageal echocardiography (TEE) were excluded. Patients were assessed,[6] DRI diagnosed according to the proposed Mayo classification criteria (Figures 1 and 2),[8] and patients categorized as having either pocket or systemic DRI. Further examination included (1) preoperative TEE, blood sampling, and 2 sets of blood cultures; (2) perioperative collection of microbiological samples; and (3) postoperative conventional and advanced microbiological analysis (Figure 3).

Baseline characteristics and clinical data were acquired from patient record files. Device history was obtained from the Danish Pacemaker and Implantable Cardioverter Defibrillator Register (DPIR), which contains prospectively registered data on all device operations in Denmark. Written informed consent was obtained from all patients. The study was approved by The Regional Committees on Health Research Ethics for Southern Denmark (Jrn. S-20160080) and performed according to the principles of the Declaration of Helsinki.

### CIED removal and intraoperative sampling

CIED systems were removed in the cardiac electrophysiological laboratory or in a hybrid room by experienced interventional cardiac electrophysiologists using a transvenous procedure involving general anesthesia, temporary pacemakers, and femoral sheaths, when needed (Supplemental Appendix A). Pocket swabs (eSwab®, COPAN, Brescia, Italy) were obtained just after the device pocket was opened. The generator was explanted and placed directly into a sterile,

| | Device-Pocket Findings | Systemic findings |
|---|---|---|
| Major criteria | Erosion of generator or leads through the skin | Two or more positive blood culture with organisms typical* for CIED infection and no alternative source |
| | Wound dehiscence | TEE findings with mobile vegetation susceptible of infection on the device-leads, endocardium or hearth valves |
| | Purulent drainage from the device-pocket | 18-FDG-PET/CT findings suggestive of DRI |
| | Sinus tract | |
| Minor Criteria | | |
| | Tenderness, pain, or discomfort at the device-pocket | Two or more positive blood cultures with microorganisms not typical for CIED infection |
| | Swelling or fluctuance of the device-pocket | Fever (38.0 °C or higher) |
| | Erythema over or in proximation of the device-pocket | Embolic phenomena (typical septic pulmonary emboli from lead vegetations or right-side endocarditis) |
| | Adherence of the device to the skin | Infective endocarditis |
| | | Persisting/recurrent signs of systemic infections without any other obvious cause of infection |

**Figure 1** Clinical signs of cardiac implantable electronic device (CIED)-related infection. Based on the proposed Mayo CIED infection classification criteria[8] and current guidelines.[4–6] **Staphylococcus aureus,* coagulase-negative staphylococci, or enterococci. 18-FDG-PET/CT = 18-fluorodeoxyglucose positron emission tomography/computed tomography; DRI = device-related infection; TEE = transesophageal echocardiography.

| | Device-Pocket Related Infection | Systemic Device Related Infection |
|---|---|---|
| Definite CIED infection | One major device-pocket finding | Two or more major systemic findings |
| Likely CIED infection | Two or more minor device-pocket findings | One major systemic finding and 1 or more minor systemic findings |
| Possible CIED infection | Suspected CIED infection that does not meet the criteria for definite or likely infection | Suspected CIED infection that does not meet the criteria for definite or likely infection |
| Not likely CIED infection | Not fulfilling any of the above criteria | Not fulfilling any of the above criteria |

**Figure 2** Classification of cardiac implantable electronic device (CIED)-related infections. Based on the proposed Mayo CIED infection classification criteria[8] and current guidelines.[4–6]

airtight polypropylene container (HPL806®, Lock&Lock). Two biopsy samples approximately 1 × 1 × 1 cm were obtained from the device pocket and placed in separate eSwab tubes. The leads were extracted using passive or active manual sheaths. The distal 5–8 cm of the leads were cut and placed directly in separate sterile containers. All samples were transported to the Department of Clinical Microbiology at Odense University Hospital and processed without delay or kept at 5°C until processing.

## Microbiological methods

The generator and leads were processed individually by the sonication culture method.[17] Approximately 10 mL of saline (0.9% NaCl) was added to each box to cover the device parts. The container was vortexed for 30 ,seconds followed by 60 seconds of sonication at maximum power (40 kHz) using an ultrasound bath (BactoSonic®, Bandelin GmbH, Berlin, Germany) and vortexed again for 30 seconds. Aliquots of 0.2 mL were sampled and cultured aerobic and anaerobic on agar plates along with pocket swabs and pocket tissue biopsies (Supplemental Appendix B). In addition, aliquots of sonication fluids were added to thioglycolate enrichment broth and incubated for 14 days

## NGS and analysis

Two aliquots of 1 mL were sampled from the sonication fluid and stored in a freezer at –80°C until processing. After collection of all specimens, the samples were transferred to the Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark for further processing.[18] In brief, the DNA was extracted from the specimens with a DNA Mini Kit and amplified with a 2-step polymerase chain reaction with different primers (Supplemental Appendix C). Amplicons were sequenced on a desktop sequencer using a v2 reagent kit (Supplemental Appendix D).

NGS analysis provided numerous DNA sequences (reads), which were interpreted to determining significant pathogens. We followed a predefined algorithm (Supplemental Appendix E) evaluating both the amount of reads and the virulence of the microorganisms.

## Microbiological presentation and reference standard

An errorless algorithm for identification of the causative pathogen in DRI does not exist. Therefore, we created a multicriteria reference standard based on all the test results and clinical findings.[19] A multidisciplinary team interpreted the microbiological findings based on a predefined algorithm to establish the likely causative pathogen (Supplemental Appendix F). All pathogens were evaluated based on to their virulence and their likelihood of causing DRI. Environmental microorganisms and commensals were evaluated as possible contaminants. Any pathogens found on leads were evaluated as potential device pocket contamination occurring during extraction.

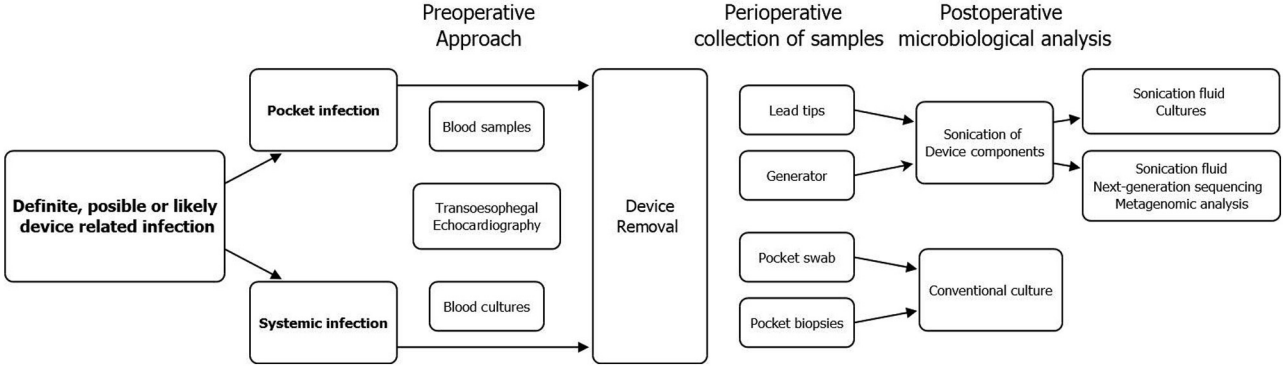


**Figure 3** Consort diagram of the diagnostic approach. Preoperative collection of blood samples, 2 sets of blood cultures and a transesophageal echocardiography; perioperative collection of microbiological samples; and postoperative microbiological analysis with conventional cultures, sonication, and targeted next-generation sequencing analysis.

**Table 1** Baseline characteristics of patients with DRI

| | Pocket DRI | Systemic DRI |
|---|---|---|
| No. of infections | 50 | 60 |
| Males | 37 (74) | 47 (78) |
| Age (y) | 73.5 (68–78) | 74.5 (67–75) |
| Total no. of device operations | | |
| 1 | 10 (21) | 37 (79) |
| 2 | 19 (53) | 17 (47) |
| 3 | 12 (71) | 5 (29) |
| ≥4 | 9 (90) | 1 (10) |
| Device type | | |
| PM | 27 (40) | 41 (60) |
| ICD | 7 (33) | 14 (67) |
| CRT-P | 4 (57) | 3 (43) |
| CRT-D | 12 (86) | 2 (14) |
| BMI (kg/m$^2$) | 28.7 (27.0 ± 30.4) | 26.3 (24.9 ± 27.8) |
| CRP (mg/L) | 35 (21 ± 50) | 159 (134 ± 184) |
| Temperature (°C) | 37.5 (37.3 ± 37.7) | 38.7 (38.4 ± 39.0) |
| Days from last device operation | 338 (68–824) | 1194 (432–2202) |
| TEE vegetation [n/N total (%)] | 18/48 (38) | 40/59 (68) |
| Bloodstream infection [n/N total (%)] | 7/42 (17) | 54/60 (90) |
| Clinical signs | | |
| Redness | 38 (76) | 3 (5) |
| Warmth | 27 (54) | 3 (5) |
| Tenderness | 38 (76) | 2 (3) |
| Thinning of skin | 29 (58) | 1 (2) |
| CIED adherence to skin | 23 (46) | 0 (0) |
| Swelling | 20 (40) | 3 (5) |
| Minor skin defect | 30 (60) | 0 (0) |
| Secretory skin defect | 18 (36) | 0 (0) |
| Fever | 10 (20) | 47 (78) |
| Sepsis | 4 (8) | 35 (58) |

Values are given as n, n (%), median (interquartile range), or mean ± SD unless otherwise indicated.

BMI = body mass Index; CIED = cardiac implantable electronic device; CRP = C-reactive protein; CRT-D = cardiac resynchronization therapy–defibrillator; CRT-P = cardiac resynchronization therapy–pacemaker; DRI = device-related infection; ICD = implantable cardioverter-defibrillator; PM = pacemaker; TEE = transesophageal echocardiography.

### Statistical analysis

Baseline characteristics and device history are summarized as categorical variables and presented as number and/or frequency. Continuous variables are presented as either mean (95% confidence interval) or median (interquartile range). Positive findings obtained by the different microbiological methods are given as number and frequency. Positive findings subsequently were compared to the causative pathogen as the efficiency to detect this specific pathogen (true positive) or false positive (detecting another pathogen). Negative findings were compared to the causative pathogen (false negative) or true negative in the cases where we could not identify a causative pathogen. Based on these factors, we calculated the sensitivity and positive predictive value (PPV) for each method with confidence intervals. All statistical analyses were performed using Stata Statistical Software Release 15 (StataCorp., College Station, TX).

## Results

### Study population

One hundred sixty-four DRI patients underwent removal of their CIED. Informed consent and sample collections were achieved in 110 patients. Of the 54 nonparticipants, 19 were not included due to logistic issues, 1 was younger than 18 years, and informed consent could not be obtained before extraction in the remaining 34 patients. Systemic DRI (n = 60) was associated with higher C-reactive protein and temperature than pocket DRI (n = 50). TEE revealed mobile vegetations in both groups but more frequently in systemic DRI than pocket DRI (68% vs 38%). Nearly half the DRI (43%) occurred after *de novo* implantation and were mainly systemic infections (79%). The remaining 57% followed a CIED reintervention and consisted primarily of pocket DRI (63%). Pocket DRI had a median time to infection of 338 (68–824) days, whereas systemic DRI occurred significantly later at a median of 1194 (432–2202) days since the preceding CIED operation (Table 1).

### Microbiological sampling

In 110 patients, we collected 109 pocket swabs, 220 pocket tissue biopsies, 106 generators, 235 leads, and at least one set of blood cultures from 102 patients. More than 75% (511/670) of the samples were analyzed within 48 hours of CIED extraction; the remaining were analyzed up to 3 days later due to weekends and holidays. Only 3 patients did not receive any preoperative antibiotics, whereas 13 patients had their first dose on the day of CIED removal. The period of preoperative antibiotic treatment was considerably shorter for pocket DRI [median 2 (0–7) days] compared to systemic DRI [median 11 (8–21) days]. In general, NGS analysis provided a considerable number of DNA sequences. However, 21 samples (6.2%) had to be omitted due to very low number of reads (<1000) probably due to polymerase chain reaction inhibitors. The remaining 319 samples had an average of 43666 reads (range 5973–173,032) per sample.

### Conventional culture, sonication, and NGS

Of the 109 pocket swabs, only 26% (n = 28) showed growth of a microorganism using conventional culturing: 43% (n = 21) of the pocket DRI and 12% (n = 7) of systemic DRI. In a subgroup analysis of pocket DRI, 64% of pocket tissue biopsy samples were culture positive, which increased to 75% when sonication fluid from generators or leads was analyzed. In systemic DRI, we found dissimilar results with low rates of positive cultures from all methods, except blood cultures (90%) (Table 2).

Separate aliquots of sonication fluid from device components were subjected to NGS analysis. Microbiome examination provided a wide array of microorganisms (Supplemental Appendix G), which were examined to determine significant

**Table 2** Microbiological characteristics of the methods

| | Pocket DRI | | Systemic DRI | | Total | |
|---|---|---|---|---|---|---|
| | n/N (%) | Poly (n) | n/N (%) | Poly (n) | n/N (%) | Poly (n) |
| Pocket swabs | 21/49 (43) | 4 | 7/60 (12) | 1 | 28/109 (26) | 5 |
| Pocket tissue biopsies | 32/50 (64) | 7 | 15/60 (25) | 2 | 47/110 (43) | 9 |
| Generator sonication fluid, cultures | 36/47 (77) | 10 | 18/59 (31) | 2 | 54/106 (51) | 12 |
| Lead sonication fluid, cultures | 38/49 (78) | 5 | 19/60 (32) | 2 | 57/109 (52) | 7 |
| Generator sonication fluid, NGS | 40/45 (89) | 3 | 19/57 (33) | 2 | 59/102 (58) | 5 |
| Lead sonication fluid, NGS | 39/49 (80) | 5 | 30/58 (52) | 2 | 69/107 (64) | 7 |
| Blood cultures | 7/42 (17) | 0 | 54/60 (90) | 1 | 61/102 (60) | 1 |

Positive findings by the different methods stratified by infection type. Pocket swabs and pocket tissue biopsies were cultured on agar plates and in thioglycolate. Generators and leads were sonicated. Aliquots of the sonication fluid were cultured. Different aliquots of the sonication fluid underwent molecular analysis.

DRI = device-related infection; n = test with ≥1 positive cultures; N = total number; NGS = next-generation sequencing analysis; Poly = number of tests with >1 positive microbiological finding.

pathogens (Supplemental Appendix E). NGS analysis of generators and leads identified a significant pathogen in 89% (40/45) and 75% (35/47), respectively, of pocket DRI in contrast to 18% (10/57) and 48% (27/56), respectively, of systemic DRI.

### Reference standard–determined causative pathogen

After completion of microbiological analyses, all cases were evaluated according to the reference standard algorithm (Supplemental Appendix F). This identified the likely causative pathogen in 105 of 110 cases (95%); 5 cases did not reveal any plausible pathogen. The most common pathogens were *Staphylococcus aureus* (n = 31) and *Staphylococcus epidermidis* (n = 25), followed by *Cutibacterium acnes* (formerly *Propionibacterium acnes*) (n = 10). *Staphylococcus aureus, Enterococcus faecalis,* and *Streptococcus* species were the key pathogens in systemic DRI, whereas *S. epidermidis, C. acnes, S. aureus,* and *Corynebacterium* species dominated pocket DRI (Figure 4). In 35% (38/110) of DRI, additional pathogens were identified (Table 2 and Supplemental Appendix G), but these were not recognized as causative because they were mainly commensal microorganisms or were found in only a few samples.

For pocket DRI, PPVs were high (>85%) for all modalities except for blood cultures, but sensitivities were low for conventional microbiological methods (Figure 5). Opposing results were found for systemic DRI, with low sensitivity for all methods except blood cultures (93%). Likewise, PPVs were quite low except for blood cultures (98%) and the analysis of leads (Figure 5).

## Discussion

Using advanced microbiology methods with sonication and NGS analysis, we identified the causative pathogen in 95% of DRI cases. For pocket DRI, pathogens were identified on both the generator and leads in >80%, whereas for systemic, DRI pathogens were identified from <25% of generators.

### Infections

In our cohort, the majority of patients endured systemic DRI in contrast to most reports from the existing literature. This may partly be explained by recent guidelines,[4,6] which have increased awareness of systemic DRI, and by the tertiary setting of this study. Age and sex distribution were in line with previous studies.[10,20–22] No differences in gender distributions or mean age were observed between DRI types.

Gram-positive cocci were responsible for 75% of all pathogens. *Staphylococcus aureus* and *S. epidermidis* were the main pathogens, causing >50% of the infections.[5,10,20] Of note, the main pathogen differed according to the type of infection. *Staphylococcus aureus* was the causative pathogen in 42% of systemic DRI, whereas *S. epidermidis* caused 38% of pocket DRI. This is not surprising due to the different virulence factors of the 2 types of staphylococci. Bacteremia with *S. aureus* often caused very severe infections,[23] whereas *S. epidermidis* rarely causes systemic infections but is one of the dominating pathogens in prosthetic infections.[24] In approximately one-third of cases we found >1 pathogen. Some of these findings probably were due to contamination, but we cannot exclude that some of the cases were polymicrobial infection.

### Microbiological methods

Traditional microbiological methods require living and metabolic active microorganisms, hence the importance of

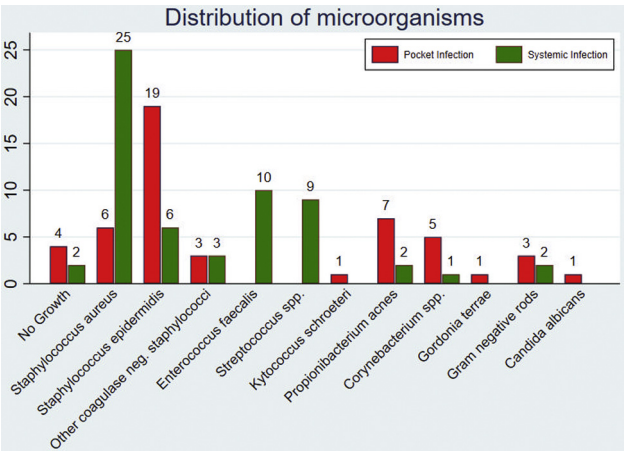


**Figure 4** Distribution of microorganisms in 110 cases with clinical device-related infection (DRI), stratified by type of infection as pocket DRI (n = 50) or systemic DRI (n = 60).

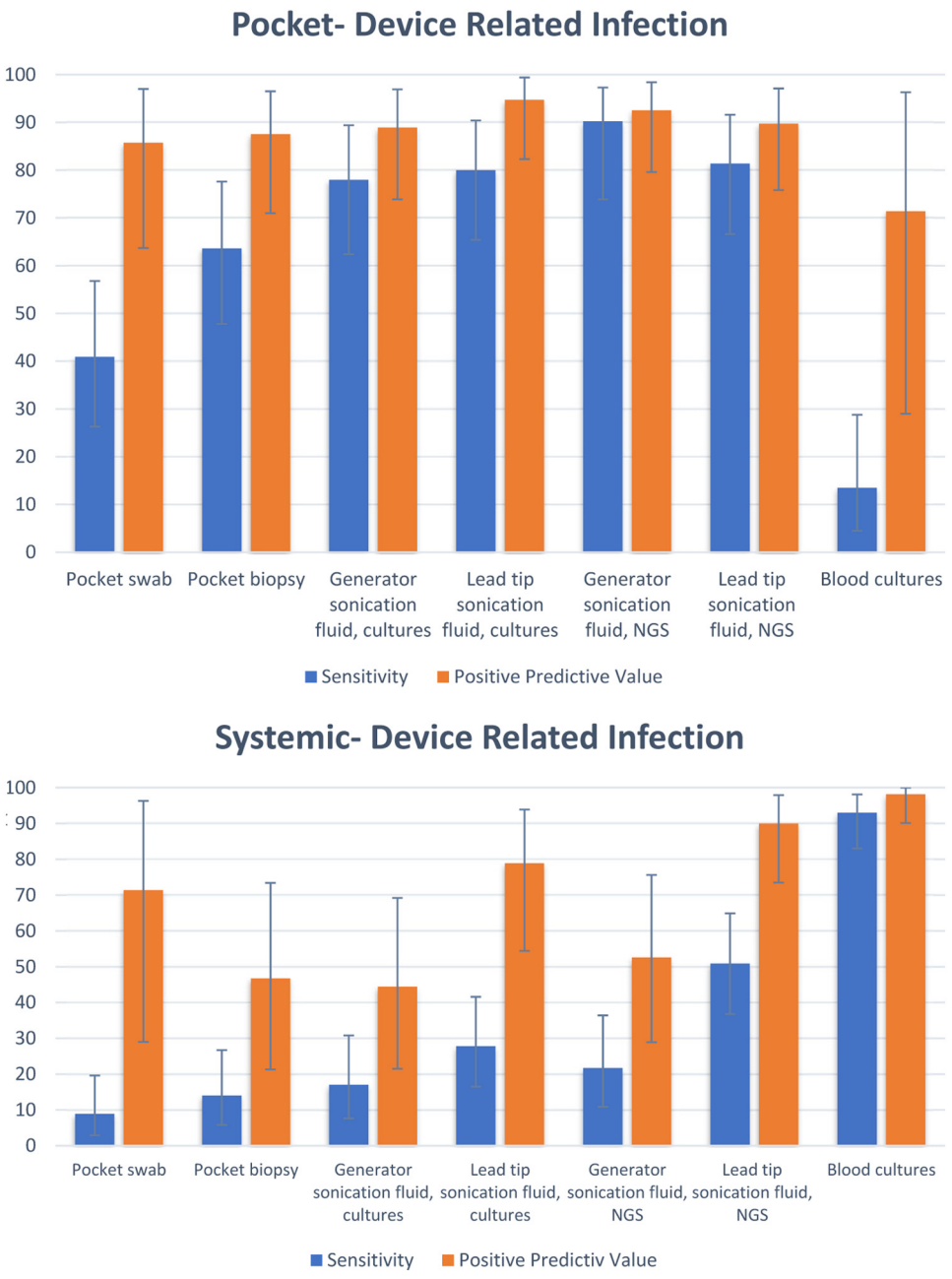


**Figure 5** Diagnostic ability of microbiological methods to detect the causative pathogen established through a reference standard, based on clinical and microbiological data. Sensitivity and positive predictive values of the various tests are shown for the different types of infection. NGS = next-generation sequencing.

acquiring samples before administering antibiotics. As expected, pocket swabs and pocket tissue biopsies had the lowest sensitivities, especially for systemic DRI. This may partially be explained by a longer period of preoperative antibiotics but also by differences in pathogenesis. Systemic DRI often originates from distant foci and may not necessarily colonize the device pocket before symptoms are displayed.

In the biofilm mode of growth that is characteristic of prosthetic infections, bacteria live in complex structured sessile microbiological communities, with both metabolic active and dormant bacteria. The metabolic active bacteria are susceptible to antibiotics, whereas the dormant bacteria are much more resistant but also more difficult to culture.[25] Culturing of the leads has been shown to be more accurate than pocket tissue biopsies,[9,10,26] but other investigators have demonstrated the superiority of sonication in comparison to traditional methods.[27] In our study, we did not culture either the generator or the leads conventionally, as all the device components were sonicated before culturing. In sonication, we aimed to disrupt the biofilm, thereby releasing dormant, metabolic passive microorganisms as free-floating nonsessile metabolic active bacteria, the so-called *planktonic state*.

For pocket DRI, cultures of the sonicated device components increased sensitivity in accordance with previous studies.[9,10,26,28] Opposing results were found in blood

cultures, with sensitivity of 93% for systemic DRI in contrast to 14% for pocket DRI. However, this was expected as blood culture is a major diagnostic criterion for systemic DRI. The low incidence of positive blood cultures in pocket DRI is not surprising, as only a few of these patients displayed signs of bloodstream infection.[29] In addition, the biofilm mode of growth expected to play a major role in pocket DRI only occasionally releases bacteria in the *planktonic state*.[25,30,31]

### NGS, reference standard, and causative pathogen

NGS is a new molecular approach in which all DNA fragments are amplified, sequenced, and subsequently categorized into species, allowing identification of nonliving microorganisms. To our knowledge, NGS analysis has not previously been used to identify causative pathogens in suspected DRI. In our cohort, NGS analysis increased pathogen detection; however, it carries an inevitable risk of misinterpreting clinical insignificant pathogens as causative. Potential pathogens of unknown significance have been detected in asymptomatic patients undergoing elective CIED operations,[11,28,32] and a few other studies have found an association with increased risk of DRI.[33,34]

We created a reference standard to minimize the risk of falsely identifying contaminants as causative pathogens. For pocket DRI, we detected the primary causative pathogen on 80% of the generators and on 75% of the leads, supporting removal of the complete CIED system when infected. However, for systemic DRI, we only found a matching pathogen on less than half of the leads and less than one-fifth of the generators. This may be explained by several factors. First, we might have sampled a wrong part of the leads. Second, patients with systemic DRI had a longer period of treatment with preoperative antibiotics. Third, pocket DRI pathogens often are less virulent and might mask the infection until they have migrated extensively along the leads, whereas the pathogens in systemic DRI are highly virulent and trigger a rapid systemic response. Finally, it is possible that some of the cases of systemic DRI with a strong suspicion of DRI did not involve the CIED system. Nevertheless, these patients had clinical signs of systemic DRI and had to be treated even though certainty of true systemic DRI cannot always be obtained before system removal.

### Contamination of samples

All CIED systems were removed under sterile conditions and immediately placed in sterile airtight containers. However, contamination of the device components during removal or the processing in the laboratory cannot be completely excluded. We retracted leads inside sheaths and only sampled the distal portion to avoid direct contact with device pocket tissue.

In 12 of 47 pocket DRI, we could not detect the causative pathogen on any leads even though they were extracted through infected pockets. This is in accordance with other studies[10] and shows that leads can be extracted without contamination.

### Study strengths and limitations

To our knowledge, this is the largest study of DRI evaluating several different microbiological methods and one of the first studies to use NGS in a clinical context. Consecutive patients underwent removal of their CIED system due to clinical suspicion of DRI, but we cannot exclude that a few patients without DRI were included. However, this reflects clinical practice, as involvement of the implanted CIED system often is uncertain in suspected systemic DRI.

Consecutive patients were included, but one-third did not complete the study protocol. Typically, their clinical state required urgent operation before informed consent could be obtained. This selection bias may have affected the distribution of pathogens and underestimated the usability of the methods, especially for systemic DRI.

Using highly sensitive microbiological methods complicates distinguishing between contamination and causative pathogens. All results were interpreted by a multidisciplinary team of experts according to a predefined algorithm. However, as different samples can be equally contaminated, there is a risk of falsely identifying contaminants as causative. There was also a risk of falsely discarding causative pathogens as contamination.

Estimating the sensitivity of microbiological methods in DRI comes with several limitations. First, we included patients with clinical DRI (true positive) but cannot guarantee that a few did not have DRI (false positive). Second, controls (true negative) were not included because recent publications have suggested that some clinical noninfected patients have bacterial colonization with unknown significance (false negative).[28,33,34] Lastly, we cannot guarantee that we identified the real causative pathogen and thereby may have produced false estimates of the sensitivities. Nevertheless, we consider our results a reliable estimate of sensitivity.

## Conclusion

Using a strategy including sonication and NGS resulted in identification of a microbiological pathogen in 95% of DRI cases. Sensitivity differed among the microbiological methods according to the type of DRI. Sonication and NGS may add value to the existing methods, but further studies are needed to establish the applicability in clinical practice.

## Appendix

### Supplementary data

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.hrthm.2022.01.039.

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