Next-Generation Sequencing for the Diagnosis of Challenging Culture-Negative Endocarditis

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Diagnosis of culture-negative infective endocarditis usually implies indirect pathogen identification by serologic or molecular techniques. Clinical metagenomics, relying on next-generation sequencing (NGS) is an emerging approach that allows pathogen identification in challenging situations, as evidenced by a clinical case. We sequenced the DNA extracted from the surgically-removed frozen valve tissue from a patient with suspected infective endocarditis with negative blood and valve cultures. Mapping of the sequence reads against reference genomic sequences, a 16S rRNA gene database and clade-specific marker genes suggested an infection caused by Cardiobacterium hominis.

Keywords: next-generation sequencing, culture-negative endocarditis, clinical metagenomics, Cardiobacterium hominis, diagnosis

Pathogen identification is a cornerstone for the diagnosis of infective endocarditis (IE) and guides treatment duration and antibiotic choice. Yet, in up to 31% of patients with IE, cultures performed on blood and valve remain negative (1). Previous antibiotic treatment and fastidious growth requirements are main reasons for failure of microorganism identification (2). Bacteria of the HACEK group, intracellular germs or nutritionally variant streptococci require particular conditions for the growth in the laboratory, such as specific complex media or lysis-centrifugation blood culture system (2).

Broad-range PCR amplification which screens for bacterial pathogens using universal primers provides an adjunct in diagnosis of IE. Multimodal diagnostic assays, including serologies and specific real-time PCR amplification, further increase the diagnostic yield in blood-culture negative IE (3, 4).

Recently, metagenomic approach using high-throughput culture-independent techniques based on next-generation sequencing (NGS) has emerged and opened new perspectives for microbiological diagnosis in the clinical setting. Here, we report the identification of Cardiobacterium hominis by NGS in the vegetation of a culture negative IE, in which broad-range PCR amplification did not detect any microorganism.

In May 2018, a 58-year-old man, independent painter, consulted the emergency room complaining about breathlessness, chest tightness and chills for a couple of weeks. He had a good dental status with no history of recent dental procedure. Physical examination revealed signs of heart failure with no splenomegaly, peripheral embolic lesions, large-vessel emboli, cutaneous lesions, or retinal/conjunctival lesions. Transthoracic and then transesophageal echocardiographies showed a moderate to severe aortic regurgitation and a 15 × 8 cm mobile vegetation of the aortic valve.
vegetation on the right leaflet. Laboratory tests showed leukocyte count 12.4 × 10^9 cells/L (reference range 4–11 × 10^9 cells/L), hemoglobin 130 g/L (reference range 140–180 g/L) and C-reactive protein at 87.3 mg/L (reference range 0–10 mg/L). Suspecting an infective endocarditis, empiric treatment was started with amoxicillin, amoxicillin/clavulanic acid and gentamicin (day 0). On day 3, an aortic valve replacement was performed, and a biological valve was placed without complications. Before pathogen identification, antibiotic treatment was already adjusted by probabilistic means to ceftriaxone, a third-generation cephalosporin. After detection of C. hominis, this regimen was pursued for a total duration of 6 weeks. The patient recovered well and went back to his former performance status.

Five sets of blood cultures, taken at 30 min intervals on day 0, i.e., before antibiotic treatment, remained negative on day 7. Culture of the excised valve and vegetation on standard enriched media came back negative. Results of the all-round standard multimodal diagnostic work-up, aimed at identifying causative agents, came back negative. The final diagnosis was achieved by NGS of the aortic native valve DNA extract, which identified C. hominis, a member of the HACEK group that accounts for 1.2–3% of IE cases (14). Only 61 cases of C. hominis IE have been reported in the English-language literature between 1962 and 2005 (15).

C. hominis is a facultatively anaerobic, slow-growing, fastidious, non-motile, gram-negative rod of low virulence, commonly present in normal oral and nasal human microbiota. It is the third most common agent responsible for HACEK endocarditis (14). Failure to identify C. hominis in blood cultures, which were taken before antibiotic treatment of our patient, may be ascribed to the fastidious nature of the organism.

In previous studies, testing heart valves for bacterial pathogens in patients with suspected IE revealed higher specificity of broad-range PCR as compared to standard cultures, even though some patients with positive cultures tested negative by PCR (16, 17). Various reasons have been attributed to false negative broad-range PCR results: low bacterial load due to the time lapsing between antibiotic therapy and surgical intervention, use of a non-representative valve fragment (with low local bacterial density), and PCR inhibition (17) which may depend on DNA extraction procedure and PCR conditions. In our case, antibiotic treatment could be a possible cause of the culture-negative results.

| Sample | DNA concentration (pg/μL) | NGS reads |
|--------|--------------------------|-----------|
|        | Human    | Bacterial | % Bacterial | Raw       | Filtered non-human | Assigned to prokaryotes | % Prokaryotic |
| S1     | 16,355   | 140       | 0.84        | 1,491,700 | 2,603              | 2,437                  | 0.81        |
| S2     | 219      | 718       | 76.63       | 1,296,937 | 192,031             | 184,618                | 81.14       |
| NEC1   | 0        | 0.19      | 100         | 24,813    | 4,438               | 3,677                  | 94.4        |
| NEC2   | 0        | 1.31      | 100         | 83,731    | 7,494               | 5,141                  | 54.4        |

aAortic samples S1 and S2 and negative extraction controls (NEC1 and NEC2).
bDetermined by qPCR tests.
cRelative to the sum of bacterial and human DNA.
dAfter removal of low-quality, human and replicate reads.
eThe percentage was determined relative to the sum of (de-replicated) reads assigned with CLARK to Homo sapiens and prokaryotic phyla.
of the valve tissue but unlikely to have caused failure in pathogen detection by PCR, because bacterial DNA load (which may originate from either live or dead cells) in the extracts of valve fragments used for NGS was relatively high (>2 × 10^6 and >10^7 rRNA gene copies for S1 and S2, respectively; Table 1). It is more likely that the broad-range PCR detection was compromised by the presence of PCR inhibitors. Yet, a sampling bias cannot be ruled out, since broad-range PCR and NGS assessed different specimen fragments. In addition, it has been recently shown that specific steps in sample preparation of the valve specimen may affect the diagnostic yield (18).

In a previous study, Imai et al. found good correlation between NGS and culture data obtained on the native valve from two patients with blood culture-positive endocarditis (19). Two other cases of blood culture-negative endocarditis were reported where the pathogen could be identified by NGS (19, 20). In our case, the identification of the causative agent of IE by NGS was reinforced by the use of three different bioinformatics tools relying on different reference databases. We believe this point is of crucial importance and guarantees for a curated and redundant signal and therefore a more reliable diagnosis.

Currently, the application of NGS approach in routine diagnostic testing is relatively costly unless larger batches of samples are processed. The application of NGS to various samples is of increasing clinical interest because it allows faster pathogen identification than standard culture. A complete metagenomics analysis of a clinical sample is now possible in <30 h using Illumina sequencing platforms. This turn-around time is longer than that of PCR and qPCR assays; however, it circumvents the need for multiple assays, e.g. target-specific PCRs aimed at confirming broad range PCR results (21), and has the potential to (i) detect multiple organisms (including uncommon pathogens), (ii) perform bacterial typing and (iii) predict antibiotic resistance profile (6, 22), all in the same test. Mechanical pre-treatment of valve tissue followed by the removal of DNA from selectively lysed human cells seem to increase the likelihood of pathogen identification. NGS of cell-free circulating plasma or serum DNA is another promising diagnostic tool for the detection of bacteremia in patients with IE (23). Our case clearly demonstrates that there is a lack in conventional techniques and that there is a clinical benefit to perform NGS in selected situations such as culture- and PCR-negative results. In particular the implementation of multiple analysis approaches and the use of orthogonal assessment to validate the obtained results should be promoted further.

DATA AVAILABILITY

The datasets generated for this study can be found in European Nucleotide Archive (ENA), PRJEB25228.

ETHICS STATEMENT

Written informed consent has been obtained from the patient for the publication of this case report.

AUTHOR CONTRIBUTIONS

MK, VL, SE, ACa, ACh, CH, and JS analyzed and interpreted patient data. MG, NG, PK, and ACh performed the experiments. NG and VL analyzed the metagenomics data. MK, VL, YC, and JS wrote the manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2019.00203/full#supplementary-material
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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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