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Case report

Diagnosis of *Streptococcus canis* periprosthetic joint infection: the utility of next-generation sequencing

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ABSTRACT

A 62-year-old man who had undergone a primary knee arthroplasty 3 years earlier, presented to the emergency department with an infected prosthesis. He underwent prosthesis resection. All cultures failed to identify the infecting organism. Analysis of the intraoperative samples by next-generation sequencing revealed *Streptococcus canis* (an organism that resides in the oral cavity of dogs). It was later discovered that the patient had sustained a dog scratch injury several days earlier. The patient reports that his dog had licked the scratch. Treatment was delivered based on the sensitivity of *S. canis*, and the patient has since undergone reimplantation arthroplasty.

Introduction

The diagnosis of periprosthetic joint infection (PJI) continues to challenge the medical community. The lack of a "gold standard" test compels clinicians to rely on several tests, none of which have absolute accuracy. Despite efforts to improve the diagnosis of PJI, such as those proposed by the International Consensus Meeting [1], some challenges have proven insurmountable. Culture-negative PJI (CN-PJI) in particular, is one such issue, as the inability to isolate the infecting organism using conventional culture may cast doubt over the diagnosis, and cause uncertainty regarding optimal treatment [2]. The inability to isolate an organism leaves patients at the mercy of empiric antimicrobial therapy, and the potential failure to cover the infecting organism, thereby jeopardizing the outcome of treatment. There is currently no clear protocol for the management of patients with CN-PJI. Current recommendations state that patients with CN-PJI should receive antimicrobials covering the most common pathogens for PJI without committing to a specific protocol [3]. The fact that PJI can be caused by fungi and atypical organisms leaves infectious disease specialists to "best guess" the antimicrobial regimen for CN-PJI patients. This can lead to the true infecting organisms not being covered or indeed the administration of unnecessary antimicrobials, thus compromising the outcome of treatment and imparting adverse effects on patients. Considering the fact that the incidence of CN-PJI can reach 50% in some studies [4-8], this clinical situation is encountered commonly. Without knowing the infective organism, clinicians are unable to effectively monitor patients' response to treatment and determine if the infection has been controlled. In addition, some patients could suffer the psychological trauma of whether a joint infection existed in the first place that necessitated multiple surgical procedures and long-term antimicrobial treatment. This situation is not acceptable in modern-day medicine and further innovations are needed to address this issue in orthopaedics and other medical fields involving the use of implants and biofilm formation.

This case highlights the challenge of CN-PJI and provides an encouraging endorsement for the potential of molecular diagnostics, such as next-generation sequencing (NGS), in identifying infecting organisms in PJI. It further corroborates the belief that organisms from sources such as pets can result in PJI.

Informed consent: The patient provided written consent that data concerning the case would be submitted for publication.

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Case history

A 62-year-old male was transferred to our institution after presenting to an outside hospital with an infected left total knee arthroplasty and systemic sepsis. The patient had undergone an uncomplicated total knee arthroplasty 3 years earlier. On arrival at the outside institution, aspiration of the knee was performed. The analysis of the synovial fluid revealed a nucleated cell count of 63,700 cells/μL with a 90% neutrophil differential. The serological markers of infection were elevated with an erythrocyte sedimentation rate of 82 mm/h and a C-reactive protein of 6.2 mg/dL. The result of laboratory investigations and clinical examination led the physicians at the outside institution to reach a diagnosis of PJI. At the family's request, he was transferred to our institution for definitive care of the infected knee after stabilization. On his arrival at our institution, the left knee was found to be swollen, warm, with erythema of the overlying skin, and painful range of motion. Radiographs showed soft tissue swelling, with arthroplasty components in good position, and no evidence of fracture or loosening (Fig. 1). In light of the clinical evaluation and laboratory investigations, which met the International Consensus Meeting criteria for the diagnosis of PJI [1], the patient was scheduled to undergo resection of the infected knee. Subsequently, the patient developed hypotension, hypoxia, and atrial fibrillation, thus necessitating intubation. In view of the systemic illness, the patient was started on empiric therapy with intravenous piperacillin/tazobactam and vancomycin before surgery. Synovial fluid obtained intraoperatively was sent for analysis, including culture, in addition to performing the leukocyte esterase test in the operating room, which produced a trace result [9]. At the time of debridement, tissue samples were also obtained from the knee, and sent for routine culture and NGS at PathoGenius Laboratory (Lubbock, TX). Samples obtained for culture and NGS included synovial fluid, synovium, and tissue from the femur and tibia. All 4 samples were sent for aerobic and anaerobic bacterial, fungal, and acid-fast cultures. Bacterial cultures were held for 14 days, whereas fungal and acid-fast cultures were held for 29 and 44 days, respectively.

Culture results of the synovial fluid at the outside institution, the synovial fluid obtained at our institution, and the periarticular tissues of the infected knee retrieved intraoperatively were all culture negative. Although the patient received systemic antibiotics on arrival at our institution, which is known to result in the inability to isolate the infective agent in PJI [10], the synovial fluid aspiration at the outside institution was obtained before administration of any antibiotics. Blood cultures obtained both at our institution and the referring institution, which were taken before antibiotic administration, were also negative.

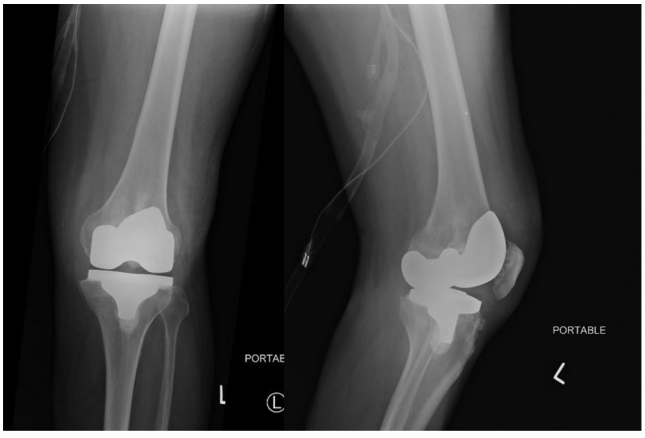

Figure 1. Preoperative anteroposterior and lateral radiographs.

NGS analysis of the fluid and periarticular tissues all revealed *Streptococcus canis*, an oral pathogen in dogs, cats, and cattle (Fig. 2). It was later discovered that the patient had sustained a scratch from his pet dog, and was continuously licked by the dog, several days before developing the knee infection.

NGS is a well-established molecular technique that involves amplification of microbial DNA using polymerase chain reaction (PCR) and subsequent sequencing of all the amplicons. The test is performed in 2 steps, with the first being a PCR to amplify the sequence of interest and subsequently sequencing the amplicons from that PCR. In this specific assay, the rRNA gene is amplified and sequenced. The 2 regions of interest for detection of bacterial and fungal species are the 16S and internal transcribed spacer sequences, respectively [11,12]. These 2 sequences are both highly conserved and variable regions of the rRNA gene, allowing for specific microbial identification.

Based on NGS results, *S. canis* was found to be sensitive to vancomycin, and hence piperacillin/tazobactam was discontinued. It should be noted that the sensitivities provided by the results of NGS assay are projections based on the presence of antibacterial resistance genes and not a “true” sensitivity determined based on the growth of the organism. Because of his systemic sepsis, the patient had a difficult postoperative recovery, which was complicated by pneumonia, placement of a tracheostomy after failing to wean off ventilation, and was finally discharged to a rehabilitation facility. The patient completed a 6-week course of intravenous vancomycin followed by a 2-week antibiotic holiday, with monitoring of erythrocyte sedimentation rate and C-reactive protein. We do not routinely aspirate patients before reimplantation at our institution. At the time of reimplantation, synovial fluid and tissue samples were obtained and sent for culture and NGS in a similar fashion to that performed at the first stage. Both NGS and culture from the reimplantation procedure were negative. At his 6-month follow-up visit, the patient was walking with a cane and did not show any clinical signs of infection.

Discussion

Culture-negative infections in general, and PJI in particular, continue to challenge the medical community. Infections associated with implants, such as prosthetic joints, are known to exist as biofilms, which cannot easily be identified using conventional culture. Although numerous strategies for improving the yield of culture have been proposed, including withholding of antibiotics before taking culture samples [2], culturing synovial fluid in blood culture bottles [13] and holding cultures for longer periods, inability to isolate the infecting organism associated with implants is common. The incidence of CN-PJI at our institution is currently 28%. This is in line with the reported rate of CN-PJI, which can range between 27% and 55% [4-8].

There are numerous issues with the use of conventional cultures in modern medicine. Based on the recommendations of the Infectious Disease Society of America and the American Society for Microbiology [14], samples obtained for culture need to be transported in a specific fashion, and processed within 2 hours, which is difficult to implement in clinical practice. The conventional culture methods that were developed in the late 19th century also rely on a medium to grow the infecting organism. Although the latter may be possible with acute infections, most chronic infections, particularly those associated with biofilms, are difficult to grow using conventional culture [15]. In addition, the use of selective media may allow for preferential growth of one organism, that may not be the true pathogen, whereas suppressing the growth of other organisms. The