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Clinical Outcomes

Usefulness of a direct 16S rRNA gene PCR assay of percutaneous biopsies or aspirates for etiological diagnosis of vertebral osteomyelitis

Sang-Ho Choi [a,1], Heungsup Sung [b,1], Sung-Han Kim [a], Sang-Oh Lee [a], Sang Hoon Lee [c], Yang Soo Kim [a], Jun Hee Woo [a], Mi-Na Kim [b,*]

[a] Department of Infectious Diseases, Asan Medical Center and University of Ulsan College of Medicine, Seoul, Republic of Korea
[b] Department of Laboratory Medicine, Asan Medical Center and University of Ulsan College of Medicine, Seoul, Republic of Korea
[c] Department of Radiology, Asan Medical Center and University of Ulsan College of Medicine, Seoul, Republic of Korea

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ABSTRACT

We performed a prospective study to evaluate the clinical usefulness of a direct 16S rRNA gene (16S rDNA) PCR assay of percutaneous biopsies or aspirates for the etiological diagnosis of vertebral osteomyelitis. During May 2009 to December 2010 and November 2011 to August 2012, consecutive patients with suspected vertebral osteomyelitis who underwent a percutaneous biopsy or aspiration were enrolled. Of 45 patients with vertebral osteomyelitis, 16S rDNA PCR was positive in 24 (53.3%), whereas culture was positive in 13 (28.9%) ($P = 0.027$). Three of PCR-positive cases (12.5%, 3/24) and 1 of culture-positive case (7.7%, 1/13) were considered to be false-positives. Of 16 patients without prior antimicrobial exposure, 75% of cases (12/16) were positive by either culture (7/16, 43.8%) or PCR (9/16, 56.3%). A 16S rDNA PCR assay with sequencing was more sensitive than routine culture for the etiological diagnosis of vertebral osteomyelitis.

1. Introduction

Vertebral osteomyelitis is a serious infectious disease that can be complicated by paravertebral, epidural, or psoas abscesses (Zimmerli, 2010). Although *Staphylococcus aureus* is the most common etiology, a variety of microorganisms have been reported to be associated with vertebral osteomyelitis, including gram-negative aerobic bacilli, streptococci, anaerobes, *Brucella* species, and *Mycobacterium tuberculosis* (Sapico and Montgomerie, 1990). Therefore, timely definite identification of the pathogens involved is fundamental for appropriate patient management. In the absence of documented bacteremia, percutaneous biopsy or aspiration are generally warranted for pathogen identification. However, it is often difficult to obtain adequate specimens for culture, and conventional culture methods frequently fail to identify etiological agents, especially when specimens are obtained after initiation of antimicrobial therapy.

Broad-range PCR assays, followed by sequencing of the amplicon, particularly those that target the 16S rRNA gene (16S rDNA), have been used in endocarditis (Bosshard et al., 2003), bacterial meningitis (Welinder-Olsson et al., 2007), and pyogenic arthritis (Yang et al., 2008). The technique allows detection of a wide range of eubacterial species by targeting both the highly conserved and hypervariable regions of the gene and improves pathogen detection. However, only a few investigators have addressed the usefulness of 16S rDNA PCR for vertebral osteomyelitis in adults (Fenollar et al., 2006; Fihman et al., 2007; Fuursted et al., 2008; Lecouvet et al., 2004), and of those previous studies, some were only small subsets of PCR study of osteoarticular infection (Fihman et al., 2007; Lecouvet et al., 2004). To our knowledge, none of the previous studies included more than 20 patients with vertebral osteomyelitis. Therefore, we performed a prospective study to evaluate the clinical usefulness of 16S rDNA PCR for the etiological diagnosis of vertebral osteomyelitis.

2. Materials and methods

2.1. Patients and specimen collection

During May 2009 to December 2010 and November 2011 to August 2012, consecutive patients with suspected vertebral osteomyelitis admitted to Asan Medical Center (a 2700-bed tertiary referral hospital in Seoul, Republic of Korea) who underwent a percutaneous biopsy or aspiration were enrolled. The decision to perform a biopsy or aspiration was at the treating physician's discretion. When the pathogen was identified from a blood culture, no biopsy or aspiration was performed. Computed tomography– or ultrasonography-guided tissue biopsy or aspiration was performed by a musculoskeletal radiologist in an aseptic manner. The specimens obtained were divided into two parts; one was used for culture, and the other was either directly processed and used for 16S rDNA PCR or frozen at −80

* Corresponding author. Tel.: +82-2-3010-4511; fax: +82-2-478-0884.
E-mail address: mnkim@amc.seoul.kr (M.-N. Kim).
[1] These authors contributed equally to this work.

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°C for subsequent use in 16S rDNA PCR. All patients were followed up for at least 6 months.

At the time of enrollment, the patient was considered to have vertebral osteomyelitis if there was evidence of 1) clinical symptoms suggestive of vertebral osteomyelitis: back pain unrelieved by rest, and/or tenderness of the back on physical examination, and/or body temperature >38 °C, in addition to 2) elevated inflammatory markers: C-reactive protein or erythrocyte sedimentation rate, and 3) MRI findings consistent with vertebral osteomyelitis: vertebral end plate/body infiltration, soft tissue inflammation, or abscess formation in the disc space, paraspinal, epidural, or psoas muscle area. The final diagnosis and the relevance of the conventional culture and 16S rDNA PCR results were evaluated based on all available laboratory and clinical data, including response to antimicrobial therapy. When skin flora or glucose non-fermentative gram-negative bacilli were identified from patients with no history of surgery or injection, these were regarded as false-positives.

2.2. Bacterial culture and identification

A Gram-stained smear of the specimen was examined for polymorphonuclear leukocytes and bacteria. For conventional cultures, specimens were disrupted by mincing using a pestle and mortar. All specimens were cultured on blood agar and MacConkey agar at 35 °C in a 5% CO_2 incubator, on *Brucella* agar anaerobically, and in thioglycollate broth at 35 °C in an ambient air incubator. Blood agar and thioglycollate broth cultures were incubated for 7 days. Culture isolates were identified using commercially available biochemical assays. The routine identification and antimicrobial susceptibility tests were performed using MicroScan Combo Panels (Siemens Healthcare Systems, Malvern, PA, USA). Vitek2 NH and ANC cards (bioMerieux SA, Marcy l'Etoile. France) and API Coryne (bioMerieux) were used for the identification of fastidious organisms, anaerobes, and Gram-positive bacteria, respectively. *Salmonella* species other than *Salmonella enterica* serovar Typhi were identified using Salmonella Grouping Antrisera (Becton Dickinson, Sparks, MD, USA). Mycobacterial culture was performed using the BACTEC MGIT 960 System (Becton Dickinson) and Ogawa medium, which isolates was identified using Seegene MTB ACE Detection (Seegene, Seoul, Korea).

2.3. DNA extraction, 16S rDNA PCR amplification, and sequencing

Specimens were disrupted and homogenized by bead beating or mincing with pestle and mortar. DNA was then extracted using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer's protocol. The final elution volume was 50 μL. For each PCR analysis, we used 5 μL DNA extract. Broad-range PCR amplification of the 16S rDNA was performed using 2 primer sets: 8FPL 5′-AGT TTG ATC CTG GCT CAG-3′ and 806R 5′-GGA CTA CCA GGG TAT CTA AT-3′; 515FPL 5′-TGC CAG CAG CCG CGG TAA-3′ and 13B 5′-AGG CCC GGG AAC GTA TTC AC-3′ (Relman, 1993). Positive and negative controls were included in each run. Amplicons were purified using a Power Gel Extraction kit (TaKaRa Bio Inc., Shiga, Japan) and directly sequenced on an ABI Prism 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator v 3.1 cycle sequencing kit (Applied Biosystems). The quality of the generated sequence was assessed according to the guideline MM18-A of Clinical and Laboratory Standards Institute (CLSI, 2008).

2.4. Interpretation of sequencing results

GenBank combined with EzTaxon-e (Kim et al., 2012) was used to compare the sequences obtained with those of reference organisms in these databases (Park et al., 2012). The organism was identified by the approved guideline for 16S rDNA results (CLSI, 2008).

2.5. Additional nucleic acid amplification tests for mycobacteria

In cases of suspected mycobacterial infection, specimens were evaluated by real-time PCR assay using Cobas TaqMan MTB PCR or with nested PCR using Absolute™ NTM&MTB PCR (BioSewoom, Seoul, Korea). All assays were conducted in accordance with the manufacturer's instructions. Testing of *M. tuberculosis*–specific PCR was at the physician's discretion.

2.6. Ethical review and statistics

This study was approved by Asan Medical Center Institutional Review Board. The sensitivity of 16S rDNA PCR and conventional culture was compared using McNemar's test.

3. Results

Of a total of 47 patients enrolled on to the study, 2 patients were eventually found not to have infections (one had metastatic cancer and the other had degenerative disease); thus, 45 patients with vertebral osteomyelitis were finally included for analysis (Table 1). Twenty-three patients were male (51.1%), and mean age was 59.0 years (range, 13–83). Nine patients had malignancy (20.0%), 8 had diabetes mellitus (17.8%), and 4 had liver cirrhosis (8.9%). As a lesion involved, the lumbar spine (66.7%) was most common, followed by the lumbo-sacral spine (13.3%), thoracic spine (11.1%), and thoracic-lumbar spine (4.4%). Fourteen patients (31.1%) had vertebral osteomyelitis in relation to prior spine surgery or injection therapy, 6 of whom had a foreign body. Five patients (11.1%) had a prior documented infection related to vertebral osteomyelitis: 3 patients with prior urinary tract infections (2 by *Escherichia coli* and 1 by *Enterococcus faecium*), 1 with central venous catheter–associated bacteremia (by methicillin-resistant *S. aureus*), and 1 with unknown source bacteremia (by *Streptococcus dysgalactiae*). Twenty-nine patients (64.4%) received antimicrobial therapy prior to diagnostic intervention, 25 of these cases (86.2%) received this treatment within 7 days of biopsy or aspiration. The median duration of prior antimicrobial therapy was 11.0 days (range, 1–115 days).

Of 45 specimens tested, 13 (28.9%) and 24 (53.3%) were positive by culture and 16S rDNA PCR, respectively ($P = 0.027$); 8 (17.8%) were positive using both techniques, 16 (35.6%) were positive by 16S rDNA PCR only, 5 (11.1%) were positive by culture only, and 16 (35.6%) were negative by both techniques. When we analyzed 16 patients without prior antimicrobial exposure, 75% of cases (12/16) were positive by either culture (7/16, 43.8%) or PCR (9/16, 56.3%). Presence of significant amount of inflammatory cells in direct smear was observed in 29 (64.4%) specimens, and bacteria were seen in only 1 specimen, which was positive for *S. aureus* in 16S rDNA PCR but negative for culture. Twenty-two (75.9%) among 29 specimens, which had inflammatory cells, were positive for bacteria by either of PCR or culture, but only 6 (37.5%) of 16 specimens, which did not have inflammatory cells, were positive for bacteria by either of PCR or culture ($P = 0.026$).

The most common etiologic organism was *S. aureus*, followed by *Staphylococcus epidermidis*, *E. coli*, and *Streptococcus agalactiae* (Table 2). From all 5 patients who had a documented infection prior to the development of vertebral osteomyelitis, the same organisms were identified from culture and/or PCR (1 from both culture and PCR [*E. coli*], 2 from culture only (*E. faecium* and methicillin-resistant *S. aureus*, respectively), and 2 from PCR only (*S. dysgalactiae* and *E. coli*, respectively). Three 16S rDNA PCR-positive cases (1 *Staphylococcus capitis*, 1 *S. epidermidis*, and 1 *Achromobacter xylosoxidans*) and 1 of culture-positive case (*Propionibacterium acnes*) were considered false-positives. Of 8 specimens positive by both 16S rDNA PCR and culture, the results of 16S rDNA PCR were concordant with the culture results in 6 (75.0%) and discordant in 2 cases; *P. acnes* by culture