**Mycobacterium bovis** BCG Vertebral Osteomyelitis after Intravesical BCG Therapy, Diagnosed by PCR-Based Genomic Deletion Analysis

Takuya Nikaido, Kei Ishibashi, Koji Otani, Shoji Yabuki, Shinichi Konno, Shuichi Mori, Kazutaka Ohashi, Takashi Ishida, Michiko Nakano, Osamu Yamaguchi, Tatsuo Suzutani, and Shinichi Kikuchi

Department of Orthopedics, Department of Microbiology, Department of Urology, and Department of Pulmonary Medicine, Fukushima Medical University, Fukushima 960-1295, Japan

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We report a case of Mycobacterium bovis BCG vertebral osteomyelitis 1.8 years after intravesical BCG therapy for bladder cancer. We differentiated BCG from other Mycobacterium tuberculosis complex members by PCR analysis of deletion regions and started an appropriate chemotherapy regimen resulting in the remission of symptoms within 1 month.

**CASE REPORT**

An 86-year-old man was referred to our institution for unrelenting back pain. He first noticed the pain without any particular cause in December 2004. He had no fever, chills, cough, or motor or sensory deficits, but his ability to perform daily activities progressively decreased due to his back pain. The patient had a history of hypertension but denied any history of active tuberculous infection or ever having received Mycobacterium bovis bacillus Calmette-Guérin (BCG) vaccine. He also had a history of left ureteral transitional cell carcinoma and bladder cancer diagnosed in 2002. As he elected bladder preservation, these tumors were surgically resected by total left nephroureterectomy and transurethral resection followed by cisplatin-based chemotherapy in September 2002. As the follow-up cystoscopy and biopsy in December 2002 demonstrated a carcinoma in situ, treatment with intravesical BCG (Tokyo strain) immunotherapy was started in January 2003. Eight weekly instillations were administered with no significant side effects, and the patient was immediately placed on a three-drug regimen of isoniazid, rifampin, and ethambutol. Drug susceptibility analysis using bacterial culture (MGIT; Becton Dickinson, NJ) yielded a positive culture for mycobacteria. At the Department of Clinical Laboratory of our hospital, the initial examinations for the differentiation of the acid-fast bacilli used PCR (Amplificor PCR; Roche Diagnostic, Basel, Switzerland) and immunochromatography testing (CapiliaTB; Becton Dickinson, NJ). On the basis of those examinations, the acid-fast bacilli from the biopsy samples were reported to belong to the Mycobacterium tuberculosis complex (MTC). However, using those methods, the examinations could not differentiate between Mycobacterium tuberculosis and other acid-fast bacilli including M. bovis, M. bovis BCG, Mycobacterium africanum, and Mycobacterium microti. To identify the causative bacillus among those of the MTC, a cultured sample was sent to the Department of Microbiology, and we performed genomic deletion analysis using multiplex PCR targeting deletion region 1 (region of difference 1; RD1), RD9, and RD10 (12). Briefly, the sample from the bacterial culture was centrifuged at 3,500 × g for 15 minutes and the pellet was washed three times with acetone and then suspended in phosphate-buffered saline. DNA was extracted using a QIAamp DNA minikit (Qiagen, CA) according to the manufacturer’s protocol. Primer sets and PCR method were described in the literature (12). The PCR deletion analyses revealed 200-bp, 206-bp, and 202-bp bands, indicating the absence of RD1, RD9, and RD10, respectively (Fig. 2). These results indicated that the isolate was M. bovis BCG and not any other mycobacterium. Consequently, the patient was diagnosed with osteomyelitis as a complication of the intravesical M. bovis BCG administered 1.8 years earlier. The patient was immediately placed on a three-drug regimen of isoniazid, rifampin, and ethambutol. Drug susceptibility analysis using bacterial culture (MGIT; Becton Dickinson, NJ) confirmed that the isolate was resistant to pyrazinamide as is characteristic of BCG. To confirm our...
results, a commercially available system based on DNA hybridization technology and using nitrocellulose strips (GenoType MTBC; Hain Diagnostika, Nehren, Germany) was used as previously reported (7, 13, 14). The band pattern 4, 7, 9, 10, 13 obtained from the results of the GenoType MTBC based on the gyrase B gene polymorphisms also indicated that the isolate was \textit{M. bovis} BCG. Surgical intervention was not required, as the patient’s symptoms were improved 1 month after starting the three-drug chemotherapy regimen. There was no recurrence of symptoms of osteomyelitis or the bladder cancer for 1 year, but the patient later died of heart disease.

BCG is a live-attenuated strain of \textit{M. bovis} that was first used for immunization against tuberculosis in 1921. After half a century, Morales, Eidinger, and Bruce introduced BCG as an intravesical treatment for superficial bladder cancer (10). Since then, BCG has been applied to the treatment of and prophylaxis against Ta and T1 tumors and carcinoma in situ, and recent meta-analysis by the European Organization for Research and Treatment of Cancer has shown that BCG can lower the risk of cancer progression (15). Although it has been considered that complications of BCG instillations for bladder cancer therapy are relatively rare, they are more frequent than in tuberculosis vaccination programs. It is crucial for clinicians to distinguish the causative bacillus from acquired infections from other sources. However, it has been difficult to distinguish \textit{M. bovis} BCG because of the high degree of sequence conservation and similar biochemical characterizations among the members of the MTC.

Intravesical BCG therapy has been regarded as an effective treatment for Ta and T1 tumors and carcinoma in situ. Though
the therapy is considered to be safe, many kinds of adverse reactions were noted by Lamm et al., including fever, granulomatous prostatitis, pneumonia, hepatitis, arthralgia, hematuria, rash, ureteral obstruction, epididymitis, contracted bladder, renal abscess, sepsis, and cytopenia (8). While adverse effects such as hematuria, cystitis, and fever are considered to be relatively common, extravesical complications are rare.

BCG osteomyelitis is a rare complication of BCG vaccination for the prophylaxis of tuberculosis. The incidence of osteitis following BCG vaccination was calculated to be 0 to 36.9 cases per 100,000 (16). Several factors, such as age at vaccination and BCG strain, may contribute to the frequency of osteitis after BCG vaccination (9). However, BCG osteomyelitis following intravesical BCG therapy is an extremely rare complication, with only seven cases reported in the English-language literature (1). Among those reports, four cases were diagnosed with *M. bovis* BCG infection using high-performance liquid chromatography (2, 4, 5, 11).

The MTC includes *Mycobacterium tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*. In our case, though the isolate was revealed to be BMT by conventional bacterial examinations, identification of the causative bacillus was not possible due to the fact that commercial DNA probes and amplification assays based on 16S rRNA gene sequences, which show a high degree of sequence conservation, identify all MTC members. Although it is remotely possible that, in the patient we describe, the *bovis* or *africanum* isolate was acquired from another source, differentiation of the members of the MTC is necessary both for treatment and for epidemiological purposes. Although high-performance liquid chromatography has been reported to be a reliable method for the identification of mycobacteria (6), it is cumbersome and expensive and is used in only a very few laboratories. Recently, on the basis of data from comparative genomic studies of mycobacteria that found that some regions are deleted among the members of the MTC (3), rapid and simple assays for precise identification of the MTC have been reported (12). Using molecular amplification methods that showed that RD1, RD9, and RD10 were all region absent for the isolate, the patient was diagnosed with osteomyelitis as a complication of the intravesical *M. bovis* BCG administered 1.8 years earlier.

In addition to the PCR deletion analysis, the GenoType MTBC identified *M. bovis* BCG based on gyrase B gene polymorphisms. Our case indicates that these methods of genotypic identification would be clinically useful.

Although all strains of *M. bovis* are intrinsically resistant to pyrazinamide, *M. bovis* BCG is generally susceptible to antituberculosis drugs, such as isoniazid, rifampin, para-aminosalicylic acid, ethambutol, and streptomycin. In our case, successful treatment was obtained using a three-drug regimen of isoniazid, rifampin, and ethambutol following the rapid diagnosis.

In conclusion, intravesical BCG therapy may disseminate the bacilli to the bone. The PCR amplification methods targeting RD1, RD9, and RD10 are rapid, simple, and precise compared with conventional examinations and allow the immediate introduction of appropriate treatment.

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REFERENCES


