

A case of acquired rifampin resistance in *Mycobacterium bovis* bacillus Calmette-Guérin-induced cystitis: Necessity for treatment guidelines

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A case of presumed bacillus Calmette-Guérin (BCG) cystitis in an elderly female patient following direct intravesical BCG instillation treatment for papillary transitional cell carcinoma is reported. The organism cultured from urine samples was eventually identified as a rifampin-resistant *Mycobacterium bovis* BCG isolate. Because the patient had received rifampin monotherapy during the course of treatment for presumed BCG disease, the clinical picture favoured acquired rifampin resistance. Sequencing of the target gene for rifampin (*rpoB*) confirmed a known mutation responsible for conferring high levels of resistance to both rifampin and rifabutin (Ser531Tyr). To the authors' knowledge, this is the first reported case of *M bovis* BCG disease in a non-HIV patient where the organism had acquired drug resistance to rifampin, and the second reported case of *M bovis* BCG that had acquired drug resistance. The present case demonstrates the necessity to re-evaluate appropriate guidelines for the effective treatment of BCG disease.

Key Words: BCG; Rifampin resistance; *rpoB*

CASE PRESENTATION

In November 2001, an elderly Caucasian female presented to her urologist with painless gross hematuria. Her medical history included chronic renal insufficiency, hypertension and gout. Her medications at presentation were allopurinol, etidronate and diltiazem. She had no HIV risk factors. Urine cytology was positive for malignant cells. A cystoscopy revealed multifocal papillary bladder wall tumours. A renal ultrasound confirmed small, echogenic kidneys, while a computed tomography scan of the abdomen demonstrated bilateral simple renal cysts with calcification. Transurethral resection of all visible tumours confirmed papillary transitional cell carcinoma (grade III/IV with superficial invasion). She received local treatment with intravesical instillations of bacillus Calmette-Guérin (BCG) (TICE strain BCG, Organon Pharmaceuticals USA). Repeat cystoscopy and transurethral resection after a six-week series of BCG instillations confirmed recurrent papillary transitional cell carcinoma (grade III/III with no superficial invasion), for

Un cas de cystite induite par le *Mycobacterium bovis* variété bacille de Calmette-Guérin devenue résistante à la rifampine : Le besoin de lignes directrices de traitement

Un cas de cystite au bacille de Calmette-Guérin (BCG) présumé chez une femme âgée après un traitement d'instillation intravésicale du BCG en raison d'un carcinome papillaire transitionnel est présenté. L'organisme cultivé à partir des échantillons d'urine a fini par être identifié comme un isolat du *Mycobacterium bovis* variété BCG. Puisque la patiente avait reçu une monothérapie de rifampine pendant le traitement de la maladie au BCG présumée, le tableau clinique favorisait l'acquisition d'une résistance à la rifampine. Le séquençage du gène cible de la rifampine (*rpoB*) a confirmé la présence d'une mutation connue responsable de conférer des taux élevés de résistance à la fois à la rifampine et à la rifabutine (Ser531Tyr). En autant que le sache l'auteur, c'est le premier cas déclaré de maladie à *M bovis* variété BCG chez un patient non atteint du VIH où l'organisme acquiert une résistance à la rifampine, et le deuxième cas déclaré de *M bovis* variété BCG où le patient acquiert une pharmacorésistance. Le présent cas démontre la nécessité de réévaluer les lignes directrices pertinentes pour le traitement efficace de la maladie au BCG.

which she received a further six-week course of BCG instillations. Follow-up biopsies showed moderate chronic inflammation, focal granulomatous inflammation and no evidence of malignancy. Maintenance BCG treatment was then instituted but after the second dose, the patient developed marked frequency and dysuria. Follow-up urine cytology and cystoscopy was negative for malignancy, although bladder volume was noted to be significantly contracted. Nonspecific inflammation on direct visualization was thought to be related to BCG cystitis, although tissue samples and urine specimens were not sent for acid-fast bacilli (AFB) smear and culture at that time.

In November 2002, the patient was placed on isoniazid (INH) 300 mg and vitamin B₆ daily to treat presumed BCG cystitis. She developed leg weakness and numbness within a week of initiating treatment, and these symptoms recurred upon reintroduction of INH. A trial of rifampin 600 mg daily for two months in July 2003 improved her frequency and dysuria. These symptoms recurred six months later (January

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TABLE 1
First- and second-line susceptibility results obtained from a rifampin-resistant *Mycobacterium bovis* bacillus Calmette-Guérin isolate*

Antimicrobial	Critical concentration(s) (µg/mL)	Result
Isoniazid	0.1	S
	0.4	S
Rifampin	2.0	R (MIC ≥32 µg/mL)
Ethambutol	2.5	S
	7.5	S
Pyrazinamide	100	R
Streptomycin	2.0	S
Capreomycin	1.25	S
Kanamycin	5.0	S
Ofloxacin	2.0	S
p-aminosalicylic acid	2.0	S
Ethionamide	1.25	R
Rifabutin	0.5	R

*Results obtained using the BACTEC 460TB system, Becton Dickinson, Canada. MIC Minimum inhibitory concentration; R Resistant; S Susceptible

2004) and were nonresponsive to the reintroduction of rifampin monotherapy. In total, the patient received approximately three months of rifampin monotherapy. Because of three months of persistent symptoms, daily multidrug treatment (INH 300 mg, rifampin 600 mg, ethambutol 800 mg and levofloxacin 250 mg) for presumed BCG cystitis was prescribed in March 2004, after urine was sent for AFB smear and culture. She again developed fatigue, diarrhea, slurred speech and leg weakness, which resolved when INH was discontinued from the regimen.

The patient's urine sample on March 2004 was positive for the presence of AFB (+1 to +3 Gaffky count) and the culture was identified as *Mycobacterium bovis* (BCG). First-line antimicrobial susceptibility testing results at the local laboratory indicated that the isolate was sensitive to INH (0.1 µg/mL and 0.4 µg/mL), streptomycin (2.0 µg/mL) and ethambutol (2.5 µg/mL and 7.5 µg/mL). As expected, the organism was resistant to pyrazinamide. Resistance to rifampin was also detected (2.0 µg/mL), and the isolate was then forwarded to the National Reference Centre for Mycobacteriology in Winnipeg, Manitoba, for confirmation of identification and first-line drug susceptibility results. Extended antimicrobial susceptibility testing determined using the BACTEC 460TB system (Becton Dickinson, Canada) demonstrated susceptibility to streptomycin (2.0 µg/mL), capreomycin (1.25 µg/mL), kanamycin (5.0 µg/mL), ofloxacin (2.0 µg/mL) and p-aminosalicylic acid (2.0 µg/mL), and resistance was noted to ethionamide (1.25 µg/mL) and rifabutin (0.5 µg/mL) (Table 1).

Identification of the isolate as *M bovis* BCG was confirmed by spoligotyping and major polymorphic tandem repeat region sequencing. Genotypic resistance markers to the first-line antimicrobials (rifampin/rifabutin and INH) were examined with polymerase chain reaction and sequencing of the *rpoB* (1), *inhA*, *katG* and *aphC* genes (2). The organism was determined to have a mutation within the *rpoB* gene at codon 531 (TCG to TTG). Fragments of the *inhA*, *katG* and *aphC* genes demonstrated no mutations.

The patient was maintained on rifampin 300 mg (a lower prescribed dose after two weeks due to diarrhea), ethambutol

800 mg and levofloxacin 500 mg daily, with improvement in symptoms. Cultures were documented as negative after seven months of therapy (October 2004). One year of treatment was completed (discontinued) in February 2005. A follow-up cystoscopy with biopsies was performed in July 2005 and showed a marked reduction in inflammation.

DISCUSSION

Since 1976, *M bovis* BCG as formulated for vaccine has been used in the treatment of bladder cancer due to its immunotherapeutic properties (3). Today, direct intravesical instillation of BCG is a well-established means of treating carcinoma in situ and reduces the risk of recurrent disease post-transurethral resection (4,5). The vast majority of patients tolerate this treatment with no serious side effects (6). However, local and disseminated BCG disease can complicate intravesical treatment of bladder carcinoma. The prevalence of the following complications was determined in a study of 2602 patients by Lamm et al (4,6): fever (2.9%); hematuria (1.0%); granulomatous prostatitis (0.9%); pneumonitis/hepatitis (0.7%); arthralgia (0.5%); sepsis or epididymitis (0.4%); rash or ureteral obstruction (3%); contracted bladder (0.2%); and renal abscess or cytopenia (0.1%). The overall complication rate was reported at less than 5%. More recent estimates indicate that systemic sepsis, the most concerning of complications, occurs in approximately one of 15,000 patients treated with intravesical BCG (4). Symptoms of cystitis or bladder irritation are expected and develop in the majority of patients treated with intravesical BCG (7). These symptoms usually present within hours of instillation and resolve without specific therapy within a few days. Whether these symptoms reflect a local hypersensitivity reaction versus a true local infection remains an area of debate.

To better understand the spectrum and pathogenesis of BCG complications after intravesical immunotherapy, the clinical presentations have been usefully divided into early and late presentations of disease by Gonzalez et al (8). Early presentation of disease was often within three months of BCG initiation and related to a generalized granulomatous or hypersensitivity response, with self-limited symptoms and negative cultures. The hypothesis in this situation is that the systemic infection occurs from exposure to a relatively low-grade pathogen in an immunocompetent host. The host's immune system responds to this infection through granulomatous formation leading to successful immunological control. Late presentation of disease has been associated with focal symptoms and frequent positive cultures. In this case, it is postulated that BCG-related disease develops from the 'reactivation' of infection after early immunological control. The pathogenesis of early and late presentations of disease, as described by Gonzalez et al (8), mirror the pathogenesis of primary and reactivated tuberculosis from *Mycobacterium tuberculosis*. The patient described in our clinical case most closely reflects the 'late' presentation model, given that her symptoms persisted after discontinuation of BCG instillations and that cultures were eventually documented as positive, well after the clinical diagnosis of BCG cystitis. The fact that *M bovis* BCG could be cultured from the urine longer than 15 months after the last BCG instillation is highly suggestive of invasive local disease.

All variants of BCG are documented to be susceptible to the first-line antituberculous drugs except pyrazinamide, and with the exception of all Danish strains, which inherently

have INH resistance (9). Therefore, the present case and others reported previously have raised concerns about acquired drug resistance and the appropriate use of antimicrobial therapy for BCG disease. Hesselting et al (9) recently described acquired rifampin resistance, while Sicevic (10) has described acquired resistance to ethambutol and isoniazid. Without initial tissue specimens for pathology and culture being taken at the time of diagnosis, confirmation of BCG cystitis with acquired rifampin resistance in this case remains debatable. Despite this, the sequence of events including the lack of initial specimens argues for a focused treatment strategy based on the best available evidence.

At this time, there are no established guidelines for treatment of *M bovis* BCG disease and no randomized, controlled trials to assess the most appropriate regimen. If this organism is the suspected or proven causative agent of active disease, then convention dictates that drug therapy be initiated using a combination of first-line antituberculosis drugs, such as rifampin, isoniazid and often ethambutol. Pyrazinamide is not administered in the case of an *M bovis* or *M bovis* BCG infection because a *pncA* gene mutation inherent to the species confers primary drug resistance (11). The specific regimen and duration of treatment often varies depending on the severity of disease, and steroid therapy has also been used in some instances (12). For example, the treatment recommendations for BCG cystitis include INH alone for one to two weeks, followed by the addition of rifampin for up to three months. Severe infections, usually outside the bladder, often require

treatment with at least INH and rifampin, as well as adjunctive steroids.

Of note, the *rpoB* mutation present in this isolate (Ser531Tyr) is the same mutation that was found by Hesselting et al (9). This mutation has been demonstrated in our isolate and several others in the literature to confer a high level of resistance to rifampin, with a minimal inhibitory concentration of 32 µg/mL or higher. While 95% of mutations of the *rpoB* gene that confer phenotypic resistance are localized to an 81 base pair stretch, this codon is the one most frequently affected (65%) (13,14). Interestingly, in the report from Hesselting et al, adherence to antimicrobial therapy was good throughout, leading them to suggest that the Danish strains' inherent resistance to INH was the key to the subsequent acquisition of rifampin resistance. In contrast, we have presented a case of presumed acquired rifampin resistance where antituberculous therapy was discontinuous and included prolonged monotherapy.

CONCLUSION

In summary, cystitis is a complication of BCG bladder instillations, and the diagnosis can be confirmed with tissue culture and histopathology. Treatment of this condition should include multiple antituberculous drugs due to concerns for the development of resistance.

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