CASE REPORT

Case 1. In May 2010, a 60-year-old man was diagnosed with pTaG3 urothelial cell carcinoma of the bladder. He was treated with a transurethral resection of the bladder tumor. Afterward, he was started on intravesical *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) instillation maintenance therapy. After 10 BCG instillations, he presented at the emergency department with a fever of 40°C, and vomiting. The patient had no other localizing symptoms. He had a C-reactive protein (CRP) level of 104 mg/liter and a leukocyte level of 8.6 × 10^9/liter. Blood cultures and urine were sampled. Afterward, the patient was started on intravenous ciprofloxacin therapy. Blood and urine cultures stayed negative. After a switch to oral ciprofloxacin therapy, the patient developed fever again. Computed tomography (CT) of the thorax showed fine nodular lesions with an infiltrate in the lower left lobe, which could fit miliary tuberculosis (TB), sarcoidosis, or diffuse metastases. There were no enlarged lymph nodes visible. A bronchoalveolar lavage (BAL) was performed. BAL fluid showed negative auramine staining, no pathogenic microbes (including mycobacterial cultures), and a negative PCR result for *Mycobacterium tuberculosis* complex. An enzyme-linked immunosorbent spot (ELISPOT) assay of peripheral blood mononuclear cells (PBMCs) was negative, while an ELISPOT assay of purified protein derivative (PPD) was marginally reactive with 8 spots. An ELISPOT PPD assay on the BAL fluid was strongly reactive (>100 spots), while an ELISPOT TB assay was negative (see Fig. 1). The patient was suspected of systemic BCG infection and treated with ethambutol, isoniazid, and rifampin for 6 months, after which he recovered. A chest CT 6 months after cessation of the medication showed a decreased number of nodules and disappearance of the infiltrate in the lower left lobe compared with the first chest CT.

Case 2. An 82-year-old male was treated for high-grade non-muscle-invasive bladder carcinoma with intravesical BCG instillations. The patient had a transurethral resection of the bladder tumor in March 2008. In April 2008, he started intravesical BCG instillations. After 4 intravesical BCG instillations, he presented with fever of 40°C, chills, fatigue, and diminished appetite. The patient had a CRP level of 173 mg/liter, a leukocyte level of 8.9 × 10^9/liter, and an erythrocyte sedimentation rate of 56 mm/h. CT of the thorax showed some aspecific nodular lesions that were centrilobular and others in the upper left lobe. Urine culture showed an *Escherichia coli* infection, for which the patient was treated with ceftriaxone. Auramine staining was negative, and mycobacterial culture of the urine remained negative. Blood cultures stayed negative as well. ELISPOT TB and ELISPOT PPD assays of PBMCs were also negative. On 19 May 2008, CT of the abdomen showed an abscess ventrally of the bladder and prostate, which was drained. *Mycobacterium tuberculosis* complex PCR of the abscess was positive, and *Mycobacterium bovis* BCG type Pasteur was cultured after 12 days. The antibiotic susceptibility pattern showed sensitivity to ethambutol, streptomycin, isoniazid, and rifampin and resistance to pyrazinamide. On 3 June 2008, an ELISPOT TB assay of PBMCs remained negative, while the ELISPOT PPD assay was reactive with 7 spots. In July 2008, an ELISPOT TB assay on blood still remained negative, while the ELISPOT PPD assay rose further to 80 spots. This finding in combination with the systemic symptoms made us conclude that the patient suffered from a systemic BCG infection; he was treated with ethambutol, isoniazid, and rifampin for 9 months and recovered.

Case 3. A 74-year-old male was treated for intermediate-grade non-muscle-invasive bladder carcinoma with intravesical BCG instillations. In November 2005, the patient was treated with a transurethral resection of the bladder tumor. In December 2006, he was treated with a second transurethral resection of the bladder tumor and intravesical mitomycin instillations for recurrence of disease. In September 2007, he was treated with a transurethral resection of the bladder tumor once more for recurrence, after which he started intravesical BCG instillations. In November 2007, 1 day after the second BCG instillation, the patient developed fever, nausea, vomiting, and erythrocyturia. The general practitioner, suspecting cystitis, treated him with trimethoprim-sulfamethoxazole. Because the patient did not recover, antibiotics were switched to ciprofloxacin. Twelve days after onset of the symptoms, the patient started coughing and became dyspneic. At the same time, he developed night sweats. The patient was referred to the pulmonary outpatient clinic on suspicion of a systemic BCG infection. On physical examination, normal breath sounds with
diffuse crackles were heard. The patient had a CRP level of 84 mg/liter, a leukocyte level of 4.6 × 10^3/liter, and a erythrocyte sedimentation rate of 40 mm/h. Chest X-ray showed fine disseminated nodules in all lung fields with hilar and mediastinal lymphadenopathy. Urine, BAL fluid, bone marrow aspirate, a lung biopsy specimen, and blood cultures were sampled. Auramine staining results of urine, BAL fluid, and a lung biopsy specimen were negative. Mycobacterium tuberculosis complex PCR and culture of the urine, BAL fluid, lung biopsy specimen, and bone marrow were also negative. No bacteria were cultured from the lung biopsy specimen or urine. Cultivation of the BAL fluid showed hemolytic streptococcus group G and Candida albicans. Blood cultures remained negative. In blood cultures, an ELISPOT TB assay was negative and an ELISPOT PPD assay was marginally reactive with 4 spots. In this patient, no ELISPOT analysis of BAL fluid was performed. Because the patient was suspected of disseminated BCG sepsis, he was treated with ethambutol, isoniazid, and rifampin, and he responded well on this. In March 2008, an ELISPOT TB assay of PBMCs was still negative, while an ELISPOT PPD assay of PBMCs rose to more than 100 spots. The patient had no clinical sign at that moment, and medication was stopped. In December 2008, an ELISPOT PPD assay of PBMCs decreased to 25 spots, while the ELISPOT TB assay was still negative. A CT of the chest showed a decreased number and size of the nodules with no pathological lymphadenopathy.

**Case 4.** A 74-year-old male was given adjuvant therapy with intravesical BCG instillations after transurethral resection of a high-grade non-muscle-invasive bladder cancer. In September 2007, the patient had a transurethral resection of the bladder tumor, after which he started intravesical BCG instillations. After the 10th BCG instillation, the patient developed dyspnea, night sweats, chills, and fatigue. Physical examination was unremarkable. A chest CT showed small nodules in all lung fields compatible with miliary tuberculosis. The patient had a CRP level of 11 mg/liter and a leukocyte level of 5.8 × 10^3/liter. Auramine staining results of urine, BAL fluid, and bone marrow were negative. Mycobacterium tuberculosis complex PCR results for the bone marrow and BAL fluid were negative. Cultures of urine, bone marrow, and BAL fluid were negative. An ELISPOT TB assay of BAL fluid was negative, while an ELISPOT PPD assay on the same BAL fluid showed more than 100 spots. An ELISPOT TB assay on blood was negative, while an ELISPOT PPD assay was marginally reactive with 6 spots. The patient was treated for a suspected BCG infection with isoniazid, rifampin, and ethambutol. After 3 months of treatment, the symptoms were resolved and the chest CT was almost normalized. An ELISPOT PPD assay of PBMCs showed 1 spot, whereas an ELISPOT TB assay was negative. After 6 months, the medication was stopped and the ELISPOT PPD assay of PBMCs was now showing 12 spots, while the ELISPOT TB assay remained negative. A chest CT 3 months after cessation of the medication showed a decreased number of nodules compared with the first chest CT. An ELISPOT PPD assay on PBMCs revealed 10 spots, whereas an ELISPOT TB assay remained negative.

To date, all four cases do not have any signs of recurrence of infection.

Worldwide, hundreds of thousands of people are treated with intravesical bacillus Calmette-Guérin (BCG) instillations for bladder carcinoma each year (1). Fewer than 5% of these patients develop severe complications of this therapy, including BCG pneumonitis and systemic infection (16). One in 15,000 patients develops a septic reaction and needs to be treated with antimycobacterial antibiotics (15). Unfortunately, it is cumbersome to confirm the diagnosis of BCG pneumonitis or sepsis because cultures often remain negative and, if they are positive, it may take up to 12 weeks until mycobacteria are grown.

This hampers distinguishing between (systemic) BCG infections and other causes of infection or other complications of BCG instillations. Hence, in the case of a systemic BCG infection rapid and accurate diagnosis is important because BCG instillations should be stopped and treatment with antimycobacterial medication should be initiated, generally for a long period (3 to 6 months) (14).

We hypothesized that in the case of a (systemic) BCG infection as a complication after BCG instillations, a specific immune response will be initiated, which could be detected using purified protein derivative (PPD) as an antigen. The tuberculin skin test (TST) detects a cell-mediated immune response in the form of a delayed-hypersensitivity reaction to PPD (18, 22). This test can be used for diagnosing Mycobacterium tuberculosis infections and has cross-reactivity with other mycobacterial strains, including the BCG strain and nontuberculous strains. However, TST has some limitations, such as interobserver variations, the need to recall people for test reading, low specificity in people who are vaccinated with BCG, and false-negative results in patients with immunosuppression (5, 9, 13, 20).

A newer immunological method, the gamma interferon release assay (IGRA), overcomes these limitations. Using this method, T cells of patients sensitized to mycobacterial antigens will produce gamma interferon when stimulated by mycobacterial antigens (18). Two commercial variants of this test exist: QuantiFERON is an enzyme-linked immunosorbent assay (ELISA) which measures gamma interferon production in patients’ plasma and T-Spot.TB is an enzyme-linked immunospot (ELISPOT) assay which enumerates the gamma interferon-producing T cells. Initially, both methods used PPD as a stimulating antigen. To avoid the high degree of antigenic cross-reactivity of PPDs from different mycobacterial species, including the BCG strain and nontuberculous mycobacterial strains (12), IGRA nowadays use Mycobacterium tuberculosis-specific antigens, such as recombinant early secretory antigenic target 6 (ESAT-6) and recombinant culture filtrate protein 10 (CFP-10). IGRA for Mycobacterium tuberculosis have a good sensitivity in immunocompromised patients, such as HIV patients (6, 17). Jafari et al. have shown that IGRA could also be used on specimens from the site of infection, such as cells obtained from bronchoalveolar lavage (BAL) fluid (10).

We developed an in-house IGRA using PPD as a stimulating antigen and hypothesized that this assay could be useful for rapid detection of BCG infections resulting from intravesical BCG therapy. In this case series, we describe 4 cases in which the use of an IGRA with PPD was able to detect PPD-specific immune activation, which could be of value for adequately detecting infections due to BCG.

In this report, we describe four cases with systemic BCG infections after intravesical BCG instillations for bladder carcinoma. All patients were treated for systemic BCG infections and survived; only in 1 case the culture turned positive for BCG. This case series shows that IGRA using PPD as the stimulating antigen are able to detect immune activation in both blood and BAL fluid, which could be valuable in diagnosing infections with BCG strains in patients treated with intravesical BCG instillations.
Yearly, 357,000 patients are diagnosed with bladder carcinoma worldwide (19), of which 70 to 75% are non-muscle invasive (25). Intravesical BCG instillations are recommended as adjuvant therapy for patients with intermediate-risk and high-risk non-muscle-invasive bladder carcinoma (1). After instillation, BCG induces a complex immune response in the bladder with an increase in cytokines and chemokines. Repeated instillations lead to a local type 1 cellular immune response, which lasts for 6 months. The exact antitumor mechanism is not known (25). The success of BCG immunotherapy relies on the intravesical administration of live BCG strains and the generation of a localized immune response in the bladder. Since BCG contains viable bacteria, it has the potential to produce (systemic) adverse events. Major adverse reactions, such as pneumonitis, prostatitis, or sepsis, occur in fewer than 5% of patients with intravesical BCG instillations (16). Therefore, all patients with fever after intravesical BCG instillations are suspected of BCG sepsis. BCG sepsis might be the result of systemic absorption (26), which may induce a hypersensitivity reaction in which elevated levels of cytokines are released in the bloodstream (16). Historically, poor technique in intravesical instillations and nonrecognition of BCG-related adverse events have led to serious morbidity and, in some cases, to mortality. Risk factors for BCG sepsis include traumatic catheterization and absorption through the inflamed bladder wall in patients to whom BCG was given soon after the transurethral resection of the bladder tumor (14,25). Cultures generally stay negative, and treatment is started based on clinical suspicion (14). Patients with a BCG infection are treated for 3 to 6 months with antimycobacterial medication, and intravesical BCG instillations are stopped until the patient is recovered. To distinguish between BCG infection and other causes of infection or other complications of intravesical BCG instillation, IGRAs using PPD as a stimulating antigen gives rise to a signal comparable to that of the positive control, indicating the presence of large amounts of PPD-specific T cells. In the bottom row, it seems that the positive control of the blood is not strongly positive. However, the wells are measured by an automatic reader, which is a more objective way to enumerate the activated T cells and which gives a clear positive result. Cells stimulated with M. tuberculosis-specific antigens ESAT-6 and CFP-10 give no signal, whereas stimulation with PPD antigen gives rise to clearly visible spots, indicating the presence of PPD-specific T cells in peripheral blood, although at a much lower level than that in the BAL fluid.

In cases 1 and 4, the ELISPOT PPD assay in BAL fluid is strongly reactive while the ELISPOT PPD assay in blood is weakly reactive. During active infection, T cells are clonally increased and recruited to the site of infection (2, 8). In patients with active Mycobacterium tuberculosis infection, some studies have been performed on this issue. Wilkinson et al. showed a much higher concentration of ESAT-6-specific T cells in pleural effusions than in peripheral blood in patients with pulmonary tuberculosis (24). Jafari et al. showed that, in sputum acid-fast bacillus smear-negative tuberculosis, IGRAs on BAL fluid mononuclear cells are superior to IGRAs on peripheral blood mononuclear cells in diagnosing pulmonary tuberculosis (11). Thus, performing IGRAs on cells obtained at the actual site of infection could be more sensitive than performing them on peripheral blood (10), which could explain the discrepancy in ELISPOT PPD assay results between the BAL fluid and the peripheral blood, where the reactive T cells have been recruited to the site of infection and are therefore not available in circulating blood.

Figure 1 shows the results of the ELISPOT assay of case 1. The negative control of the BAL fluid shows more background signals, probably due to the impurity of the material and the presence of macrophages in the BAL fluid. These background signals are on the same level as that of the samples stimulated with the M. tuberculosis-specific antigens ESAT-6 and CFP-10. PPDb-stimulated cells give rise to a signal comparable to that of the positive control, indicating the presence of large amounts of PPD-specific T cells. In the bottom row, it seems that the positive control of the blood is not strongly positive. However, the wells are measured by an automatic reader, which is a more objective way to enumerate the activated T cells and which gives a clear positive result. Cells stimulated with M. tuberculosis-specific antigens ESAT-6 and CFP-10 give no signal, whereas stimulation with PPD antigen gives rise to clearly visible spots, indicating the presence of PPD-specific T cells in peripheral blood, although at a much lower level than that in the BAL fluid.

As direct specimens are sometimes difficult to obtain and therefore not always available, it is useful to repeat ELISPOT PPD assays on PBMCs, as they can become more reactive over time. In case 3, the initial ELISPOT PPD assay on PBMCs was negative, while the ELISPOT PPD assay on blood became reactive a few months after the start of the BCG sepsis. In case 2, the ELISPOT PPD assay was more reactive 1 month after the start of the infection. This could be explained by the fact that during active infection T cells are clonally increased and recruited to the site of infection. Consequently, the ELISPOT assay of a direct specimen is...
positive early in the infection, while the ELISPOT assay of PBMCs becomes positive later during the infection.

Patients who are BCG vaccinated or are exposed to nontuberculous mycobacteria (NTM) are at risk for false-positive results in the ELISPOT PPD assay (4, 5, 23). Brock et al. showed that 47% of patients who are BCG vaccinated respond to PPD, while 10% respond to the Mycobacterium tuberculosis-specific antigen ESAT-6 and CFP-10 (5). However, in The Netherlands BCG vaccination is rare and not commonly used. In addition, the prevalence of NTM in The Netherlands is low. Not much is known about the specificity of the ELISPOT PPD assay in this group of patients. In an unpublished study by our department, 70% and 86.4% of 103 patients with interstitial lung diseases had fewer than 4 spots or fewer than 14 spots on an ELISPOT PPD assay of PBMCs, respectively (S. F. T. Thijssen, M. van der Wel, J. J. M. Bouwman, and A. W. J. Bossink, unpublished data).

For intradermal PPD, reactivity is expected 12 weeks after exposure to BCG or mycobacteria. However, this time frame is arbitrary and conservative; in other words, after 12 weeks most exposed patients are responsive to intradermal PPD. Nevertheless, a local immune response to PPD can be expected earlier. Patients who are treated with intravesical BCG instillations receive much higher doses of antigen (i.e., BCG) than do those given intradermal BCG vaccination. In BCG vaccination, 0.8 × 10^6 to 1.2 × 10^6 CFU of Mycobacterium bovis is given percutaneously, while in intravesical BCG instillations 2 × 10^6 to 8 × 10^6 CFU of Mycobacterium bovis is given. Bilen et al. showed that 68% of patients became positive for intradermal PPD reactivity 1 week after they were treated for the first time with intravesical BCG instillations (3). Thus, intravesical BCG instillations may provoke a T-cell-mediated immune response to PPD which can be expected earlier than the 3-month period for TST which is used in TB contact tracing.

Our cases were treated for 4 to 9 months with antimycobacterial medication. Pyrazinamide was not given because Mycobacterium bovis is usually resistant to pyrazinamide. This is confirmed in case 2, in which Mycobacterium bovis was cultured. In our department, treatment for 9 months is given to patients with a positive culture for Mycobacterium bovis. The patients for whom there was no positive culture were treated with antimycobacterial medication until clinical recovery.

In conclusion, this case series shows that IGRAs using PPD as a stimulating antigen are potentially valuable in diagnosing infection with BCG, especially when fluids of affected organs such as BAL fluids can be tested. BCG instillations are widely used in the treatment of non-muscle-invasive bladder carcinoma. IGRAs using PPD as a stimulating antigen are a promising new tool in diagnosing and/or monitoring complications of this treatment and should be evaluated in greater series.

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