Clinical use of BCG and its complications: a case series

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SUMMARY

Bacillus Calmette-Guérin (BCG), a live, attenuated strain of *Mycobacterium bovis*, is the essential constituent of the vaccine against tuberculosis and the gold-standard adjuvant treatment for urothelial cancer of the bladder. Being a live, attenuated strain with a potential pathogenic action, bacilli can cause several complications, both locally near the inoculation site and remotely through blood dissemination. BCG-related disease can represent a side effect of anti-TB vaccination in patient with congenital or acquired immunodeficiency or a complication of the therapeutic schedule in oncologic patients. Herein we report five cases of BCG-related disease which occurred at the Infectious Diseases Department of the University Hospital of Palermo during a five-year period from January 2014 to December 2019.

Keywords: BCG, bladder cancer, tuberculosis, BCG vaccination.

INTRODUCTION

Bacillus Calmette-Guérin (BCG) is a live, attenuated strain of *Mycobacterium bovis* which is part of the Mycobacterium Tuberculosis Complex (MTC). *BCG* vaccine strain was obtained from an isolate of *M. bovis* and it was used for the first time as a human vaccine in 1921 [1-4]. Today WHO recommends as part of childhood immunization programs a single dose of BCG vaccine only in countries with a high TB burden. In countries with low TB incidence rates, like Italy, this may be limited to neonates and infants in recognized high-risk groups (all TST-negative immunocompetent newborns and breastfeeding infants aged <6 months and all TST-negative children, aged between 6 months and 5 years, moving to highly epidemic areas or whose parents come from highly endemic areas or who have been in contact with a family member with active TB without contracting the disease themselves, health care and prison workers) [5]. Possible complications following BCG vaccination are more prevalent in the immunocompromised hosts [6, 7].

In 1990 FDA approved BCG adjuvant therapy for recurrent Non-Muscle-Invasive-Bladder Cancer (NMIBC). Today BCG immunotherapy is the gold-standard adjuvant treatment for NMIBC with high progression risk (stage T1 tumors, high-grade carcinoma, carcinoma in situ, and multiple and recurring stage Ta tumors >3 cm) and is also recommended for intermediate-risk NMIBC [8-11]. The immunotherapy with BCG is generally considered safe but post-instillation disease could occur and could be both localized and disseminated [12-19]. The local disease is usually a rapid-resolving cystitis, glans ulcerations, granulomatous prostatitis, epididymitis, epididymo-orchitis; also, possible although uncommon clinical manifestations could be renal abscesses and ureteral obstructions. Less usually systemic disease can be due to lymphatic or hematic BCG dissemination. [14, 16-20].

Current knowledge of late local and systemic BCG infections predominantly comes from individual case reports [19-21].

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We describe here all the patients admitted with a suspected diagnosis of BCG-related disease in the past 5 years in the Department of "Infectious Disease" of "P. Giaccone" Hospital, University of Palermo.

Case 1

A thirty years old woman, born in Switzerland, was admitted to the Infectious Diseases Unit of A.O.U.P. "P. Giaccone" of Palermo, Italy, on April 2017 because of chronic osteomyelitis mostly involving the right wrist. Her medical history revealed that at birth she had been vaccinated with BCG over the left gluteus muscle and after 48 hours developed a left inguinal abscess with lymphadenopathy with spontaneous perforation. The lymph node biopsy showed a granulomatous lymphadenitis with acid – fast bacilli and BCG strain was isolated. Treatment with isoniazid was started but one month later the patient presented a right homers fracture with a radiological finding of osteolytic areas in the right ulna and clavicle and in the lower limbs. A diaphysectomy was necessary and ethambutol and rifampicin were added to isoniazid. The antibiotic treatment was continued for four years.

When the patient was 8 years old she went through a left pneumonectomy because of recurrent lung infection episodes with bronchoscopic evidence of granulomatous tissue occluding the main left bronchus. An impaired immune response was suspected and an innate interferon-γ receptor R1 defect was detected by molecular analysis. At the age of 12 the patient developed a right supraclavicular and left mammary masses and a lacrimal gland infiltration. Incision and drainage showed caseification and acid-fast bacilli in the specimen. Rifabutin at the dose of 300 mg once daily and pain therapy were prescribed till the admission to our Unit.

At the admission the patient was apyretic and complained widespread joint pain. A recent Magnetic Resonance Imaging (MRI) showed a bone marrow oedema, bone expansion, lytic areas and periosteal reaction of the right wrist. Our Computed Tomography CT) scan showed lytic bone lesions interesting right homer distal epiphysis and carpus bilaterally. Quantiferon TB Gold (Cellestis, Ltd., Carnegie, Australia) was negative. Three sputum samples and urine cultures revealed no growth. A 8-Fluorine fluodeoxiglucose positron emission tomography combined with CT scan (18F-FDG PET/CT) confirmed a pattern of osteolytic lesions with high inflammatory activity. A treatment with isoniazid, rifampin and ethambutol was started and continued for two months. Isoniazid and rifampin were kept up for other four months till the following visit. The patient improved, she didn't need pain therapy anymore and a 18F-FDG PET/CT one year later confirmed a lower number of lesions of high intensity 18F-FDG uptake. Because of both the intricate history and the immune defect the patient is still assuming isoniazid and rifampicin and is continuing her follow up at our outpatient clinic.

Case 2

A 72 years old Italian male was admitted to the Infectious Diseases Unit of A.O.U.P. "P. Giaccone" of Palermo, Italy, on April 2019 because of fever preceded by shivering, malaise, anorexia, irritative lower urinary tract infection symptoms and severe hypotension leading to syncope. Seven months before he had undergone excision of a superficial bladder cancer and subsequently received a six-week course of intravesical BCG (one dose per week) followed by the first monthly instillation. Last instillation was administered two weeks before admission. Due to clinical history a BCG disease was suspected. Physical examination revealed normal pulse, blood pressure and respiratory rate and a reduced sour vesicular murmur on chest auscultation. Quantiferon TB Gold (Cellestis, Ltd., Carnegie, Australia) was negative. Intra-dermal Mantoux testing was performed obtaining an induration of eight millimetres in diameter after 72 hours. A CT scan of the thorax, abdomen and pelvi was arranged according to the current guidelines and showed extensive nodularity throughout both lung fields with miliary pattern close to ground glass and consolidative areas in the right medium lobe and in the left upper lobe, with right pleural effusion and mediastinal lymph nodes enlargement. Prostate resulted abnormal, not homogeneous, enlarged and with colliquative zones mostly in the left lobe. Mycobacteria were searched on sputum samples, Broncho-Alveolar Lavage (BAL) and urine over and over again during hospitalization using Ziehl-Neelsen staining and cultures but always resulted negative. Polymerase Chain Reaction (PCR) on urine resulted positive for mycobacteria. Isoniazid (300 mg once

daily), rifampicin (600 mg/once daily) and ethambutol (1200 mg once daily) were commenced and continued for thirty- three days till an GOT/GPT (498/419 UI/L), gamma- GT (209 UI/L) and LDH (328 UI/L) increase occurred. Isoniazid and rifampicin were stopped while ethambutol was continued together with moxifloxacin 400 mg once daily for six months. Patient improved and a new thorax CT scan on September 2019 showed a clear reduction in number and size of the nodularity previously observed.

Case 3

A 69-year-old patient came to the Infectious Diseases Unit of A.O.U.P. "P. Giaccone" of Palermo, Italy, because of painful and progressive monolateral scrotal swelling. Two years earlier the patient underwent transurethral resection of a papillary carcinoma of the bladder followed by a course of intravesical BCG therapy (six instillations weekly). The following year a second course of BCG intravesical therapy was required due to a doubtful recurrence from the cistoscopy follow up. After about six weeks from the end of the second course of therapy, a control cystoscopy and an irrigation cytology were performed showing no evidence of bladder carcinoma and normal urine cytology (Papanicolaou II). Nevertheless, a maintenance dose of intravesical BCG therapy (one instillation every three weeks for about three years) was given. One month after the last instillation the patient began to complain of scrotal swelling. He had a medical history of type 2 diabetes and ulcerative rectocolitis on treatment with metformin and mesalazine, respectively. On admission, body temperature was 36.9°C, blood pressure 130/70 mmHg, pulse 100 beats per minute, respiratory rate 18 breaths per minute and oxygen saturation 96% while breathing ambient air. The urine culture was negative. The left testis contained a soft painful mass with a cutaneous fistula; an ultrasound scan showed the presence of an abscess and surgery was required. Left orchiectomy and funiculus spermaticus ligature were performed and specimens were sent for histopathology and microbiological tests (bacterial culture). Histological investigation on testis showed a chronic granulomatous disease presenting with giant cells Langhans type, caseous necrosis and purulent abscess overlap. A chest CT scan showed bilateral apical fibrosis and millimetric nodules in the right pulmonary lobe. An interferon-gamma release assay (Quantiferon-TB Gold, Cellestis, Ltd., Carnegie, Australia) was performed and immune reactivity to *M. tuberculosis* was proved whereas three sputum and urine sample examination with the Ziehl Neelsen (ZN) technique and Polymerase Chain Reaction (PCR) for *M. tuberculosis* were negative. Because of the BCG instillations therapy history, treatment with rifampicin 600 mg daily, isoniazid 300 mg daily and ethambutol 1200 mg daily was started. The patient was discharged with indication to continue antitubercular therapy for six months of isoniazid plus rifampicin with a two month of intensive phase including ethambutol.

Case 4

A 61-year-old patient came to our Infectious Diseases Unit on September 2019 complaining of frequent and scanty urinations, strangury, macroscopic haematuria and perineal pain. His physical examination at the admission was normal and he had regular alvo. His medical history was remarkable for recurrent polymicrobial urinary tract infections and a Transurethral Resection of the Bladder (TURB) because of a relapsing, high grade urothelial bladder cancer invading submucosa and muscle. He received the 6 weekly induction schedule of BCG intravesical instillation between January and February 2019 without side effects and continued the maintenance schedule till the admission. Intra-dermal Mantoux testing was performed obtaining an induration of nine millimetres in diameter after 72 hours and interferon – gamma release assays resulted negative. Urine analysis showed acid fast bacilli and a biopsy performed during cystoscopy showed a granulomatous chronic cystitis with giant cells Langhans type. Due to this finding a therapy with rifampicin 600 mg daily, isoniazid 300 mg daily and ethambutol 1200 mg daily was started, ethambutol was stopped after the induction phase of two months and isoniazid and rifampin were continued for other four months. A good clinical response was obtained.

Case 5

An 82-year-old patient with a history of poorly differentiated urothelial carcinoma and subjected to Transurethral Resection of the Bladder (TURB) and BCG intravesical instillation on August 2019

came to our attention two months later because of high grade fever and dysuria. At the admission Tuberculin Skin Test (TST) and Quantiferon TB Gold were performed and resulted both positive. Due to urine culture showing an ESBL-producing *Klebsiella oxytoca*, amikacin (15 mg/kg every 24 hours) and colistin (4.5 millions IU every twelve hours after loading dose) was started with only slight improvement in symptoms. Mycobacteria were searched in sputum and urine and both PCR and cultures resulted negative. A urine culture was repeated and resulted negative. The patient continued to complain of dysuria and persisted with fever so a high-resolution chest CT scan was also performed and showed a large cavity lesion with satellite nodules in the right upper lobe. Bronchoalveolar lavage failed at first to demonstrate both mycobacteria and atypical cells in collected specimens. In consideration of clinical history and of laboratory results a therapy with isoniazid, rifampin, ethambutol and pyrazinamide was started and fever and dysuria disappeared after about 12 days. The patient was discharged and a urological re-evaluation and a bronchoscopy were advised. Forty days later bronchoalveolar lavage culture showed growth of *M. tuberculosis*. The patient, in poor clinical condition, is still in follow up at our outpatient clinic, his pulmonary radiological picture slightly improved and the BCG intravesical instillations were stopped. A micropapillary area in a posterior wall of the bladder appeared to a follow up TURB and oncologist prescribed intravesical epirubicin instillations.

DISCUSSION

When a patient with a history of BCG immunotherapy or vaccination presents symptoms possibly related to BCG infection, the clinical suspicion of a BCG-related-disease must arise.

BCG post-vaccination-disease in the pediatric patient has its onset weeks or months after the vaccination with various patterns classified in regional (persistent ulcer, abscess, fistula, or lymphadenopathy limited to the region of inoculation), extra-regional (osteitis or cutaneous abscess) and disseminated disease [22, 23].

Our case series include 5 patients: two patients with systemic disease (one case after BCG vaccination, the other one after BCG instillations) and three cases of a localized infection.

We diagnosed a BCG-related disease with the following criteria: contact with BCG in the patient's medical history and at least a compatible clinical syndrome not otherwise explained. For each patient we recorded the medical history and performed microbiological and immunological tests. To prove the mycobacterial infection we obtained specimens for staining for acid-fast bacilli, culture and Polymerase Chain Reaction (PCR) testing for mycobacterial DNA. The diagnosis was presumptive if there was a positive staining for acid-fast bacilli (Ziehl-Neelsen) or a PCR or granulomatous inflammation with or without caseous necrosis; if BCG was directly isolated in any specimen the diagnosis was certain. All five patients provided an informed consent.

Patient 1 at the beginning presented only a localized form but later developed a disseminated disease involving lungs and skeleton because she suffered from a severe immunodeficiency consisting in an innate defect of interferon-γ receptor R1 with an autosomal recessive inheritance. Multiple types of mutations in four genes (IFNGR1, IFNGR2, IL12 p40, IL12Rβ1) result in disorders of different severity. Complete IFNGR1 and IFNGR2 deficiencies predispose to overwhelming infection in early childhood which may respond to antibiotics but relapse at discontinuation [24]. In our case the dissemination of infection was evidenced by acid-fast bacilli and BCG strain isolated from different body districts.

The immunotherapy with BCG for NMIBC is generally considered safe but a post-instillation disease could occur and could be both localized or disseminated [13-19].

The predisposing factors for developing BCG infection after intravesical instillation are still to be cleared, but it is supposed that trauma on the bladder mucosa during the procedure and its previous state, concurrent urinary infections and catheterization are more likely to play a major role rather than other factors such as the BCG dose, the number of treatment courses or the interval elapsed since the last preceding Trans-Urethral Resection (TUR) [17]. The gold-standard BCG intravesical therapy entails an induction schedule of 6 weekly instillations starting a few weeks after tumor resection, which is followed by a maintenance schedule of additional BCG instillations every 3–6 months over 1-3 years [8-10].

The mechanism by which BCG immunotherapy works is still unknown. We do not know whether BCG directly induces an antitumoural response specific to neoplastic cells or whether the general BCG-induced immune response is responsible for the antitumoral activity. Four steps have

Table 1 - Patients with BCG related diseases observed in the Infectious Disease Department of university of Palermo.

Patient	*Sex*	*Age*	*Comorbidites*	*BCG-related-disease*	*Clinical features*	*Diagnosis*					*Treatment*	*Outcome*
						ZN	*CRP*	*Culture*	*TST*	*QTF*		
1	F	30	IFNGR1 mutation	Post-BCG vaccination	Lymphonodal abscess Pneumonia Lacrimal gland abscess Osteomyelitis	+	–	+	+	–	Rifampin 600 mg/die Isoniazid 300 mg/die Ethambutol 1200 mg/die for two months; isoniazid and rifampin for 36 months (still ongoing at the time the manuscript is being drafted).	Recovered
2	M	72		Post-instillation	Persistent fever Miliary pattern pneumonia Urinary tract symptoms	-	+ (urine)	-	+	–	Rifampin 600 mg/die Isoniazid 300 mg/die Ethambutol 1200 mg /die for thirty three days then ethambutol together with moxifloxacin for six months.	Recovered
3	M	69	DMT2 UC	Post-instillation	Scrotal abscess	-	–	–	+	+	Rifampin 600 mg/die Isoniazid 300 mg/die Ethambutol 1200 mg/die for two months then rifampin and isoniazid for additional four months.	Recovered
4	M	61		Post-instillation	Chronic ulcerative and granoulatous cystitis	+	–	–	+	–	Rifampin 600 mg/die Isoniazid 300 mg/die Ethambutol 1200 mg/die for two months then rifampin and isoniazid for additional four months.	Recovered
5	M	82	–	Post-instillation	Cystitis Fever	–	–	–	+	+	Rifampin 600 mg/die Isoniazid 300 mg/die Ethambutol 1200 mg/die Pyrazinamide 1500 mg/die for two months then rifampin and isoniazid for additional seven months.	Recovered

CRP: chain reaction polymerase, DMT2: diabetes mellitus 2, IFNGR: interferon gamma receptor, QTF: quantiferon, TST: tuberculin skin test, UC: ulcerative colitis, ZN: Ziehl Neelsen.

been supposed: attachment of BCG to urothelium, internalization, induction of innate immune responses (urothelial cells and APC produce cytokines and chemokines in order to recall granulocytes and mononuclear cells) and induction of acquired immune response. This kind of innate response is necessary to activate an adaptive Th1-mediated response. Induction of a TH1 cell response, characterized by the production of IL-2, IL-12, IFN-γ, TNF-α and TNF-β, is associated with successful BCG immunotherapy, whereas a TH2 cell response, characterized by the production of IL-4, IL-5, IL-6 and IL-10, has been correlated with BCG non-responsiveness [9, 10].

The clinical presentation of the local disease is usually non-specific and differential diagnosis is often a problem because 20% of patients receiving BCG immunotherapy develops urinary infections sustained by other microbiological agents [16]. In the systemic disease the vascular district is invaded by bacilli from urothelial breaches through which they reach a wide number of distant organs, therefore, usually it has an early presentation (less than 3 months since the instillation); only rarely it can have a late onset, expression of the BCG reactivation due to immunocompromission [16].

BCG-related-disease can be a tricky diagnosis due to the low sensitivity and most importantly specificity of the available tests. Acid-fast bacilli staining, mycobacterial culture and PCR are often negative and tissue biopsies are required to culture specimens and to evaluate non caseating granuloma formation [14].

Differential diagnosis with *M. tuberculosis* infection can be challenging, since sometimes they can also co-exist. Tuberculin Skin Test (TST) is made by mycobacterial antigens coming from different mycobacteria including *M. tuberculosis* and *BCG*, so it cannot discern between BCG and *M. tuberculosis* [25, 26]. IGRA tests are useful to bypass this problem because they measure INF-γ produced by T-lymphocytes when exposed to mycobacterial antigens (ESAT-6 and CFP-10) that are exclusively in *M. tuberculosis*, because in BCG ESAT-6 and CFP-10 (BCG region 1) genes are deleted. In conclusion BCG infection is characterized by a positive TST and a negative IGRA-test [26, 27]. Diagnosis of BCGitis should be based on microbiological and histological features.

In our case series, all five patients had a positive TST (Tuberculin Skin Test); IGRA (Interferon Gamma Release Assay)-tests, intrinsically negative for a BCG contact, was positive only in case 3 and 5 (Table 1). In the case 3, the positivity was related to a concomitant latent tuberculosis; the case 5 had a pulmonary active tuberculosis in addition to symptoms of the localized disease after he had undergone to BCG instillations.

Regarding the management of BCG related disease there are no randomized clinical trials demonstrating the optimal treatment and duration regimen. BCG strain is susceptible to most antitubercular drugs except pyrazinamide and cycloserine [1, 10, 11, 15]. The approach to treatment depends on clinical presentation [6]. The basic treatment relies on a course of 6-9 months, with ethambutol, isoniazid, rifampin for 2 months and then isoniazid and rifampin for the remaining 4 months. In localized disease, surgical evaluation is warranted in the setting of abscess, genitourinary tract obstruction, vascular infection, or prosthetic device infection [6]. In the event of hypersensitivity-reaction (arthritis, uveitis, miliary lung pattern or respiratory failure) corticosteroids and NSAIDs can play an important role, due to one of the disease pathogenetic hypothesis [15]. Treatment outcome has generally been reported to be good.

All of our patients were treated with antitubercular treatment (rifampin, isoniazid, ethambutol) excluding pyrazinamide, to which BCG is intrinsically resistant, except the case 5 because of the different final diagnosis (the patient was affected also by pulmonary tuberculosis).

In conclusion the risk awareness in administer a live attenuated vaccine in immunosuppressed subjects can reduce the cases of post-vaccination BCG pathology; more early recognition of complication during bladder BCG instillation can help establish the best therapeutic approach to infectious complications.

Conflicts of interest
None.

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