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BRIEF REPORT

Nosocomial Mycobacterium bovis–Bacille Calmette-Guérin Infections Due to Contamination of Chemotherapeutics: Case Finding and Route of Transmission

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We studied nosocomial infections due to Mycobacterium bovis bacille Calmette-Guérin (BCG) Onco-TICE bacteria, transmitted by contamination of medication prepared in BCG Onco-TICE–contaminated hoods in the pharmacy, in 5 immunocompromised patients at 3 hospitals. The BCG strains cultured from the patients had the same DNA profile as the BCG Onco-TICE strain used for bladder instillation. To prevent these infections, a change from open to closed preparation was made; strictly separated preparation in time was always prepared first, before cytotoxics. Therefore, the biological safety cabinet was disinfected between preparations, and gloves were changed between preparations.

The bacille Calmette-Guérin (BCG) vaccine consists of live attenuated Mycobacterium bovis bacteria. In 1991, it was shown that M. bovis–BCG could also be used as an adjuvant instillation-immunotherapy in patients with superficial bladder cancer. This preparation is obtainable as Onco-TICE. Although the use of BCG for vaccination or local therapy is considered to be relatively safe, case histories describing local or disseminated infectious complications have raised concern [1, 2]. A few case reports proved or suggested that M. bovis–BCG was introduced by cross-contamination of chemotherapy [3, 4].

In the Erasmus MC in Rotterdam, The Netherlands (hospital A), 3 patients received a diagnosis of disseminated M. bovis–BCG infections. The most recent patient had not been vaccinated with BCG and was admitted in the hospital for >3 months at the time of infection, which led us to investigate whether the infections were iatrogenic and to search for more patients with this iatrogenic infection. We reviewed the national reports of BCG infections and assessed how many were probably acquired in this way. We also evaluated the likelihood of contamination in the pharmacy preparation cabinet of hospital A.

Methods. In hospital A, we performed a case-finding study. Patients deemed to be at risk had received cytotoxic chemotherapy prepared on the same days (“risk days”) as did patients 1–3 (see Results). Patients who received cytotoxics prepared on risk days were identified from pharmacy records. BCG Onco-TICE was always prepared first, before cytotoxics. Therefore, all patients who received cytotoxics prepared on risk days were followed up. We looked for clinical signs and symptoms indicating M. bovis–BCG infections, such as malaise, fever, sweats, weight loss, increased erythrocyte sedimentation rate, or liver and/or lung dysfunction. Symptoms that raised the possibility of M. bovis–BCG meningitis were fever, headache, irritability, disturbance in consciousness, or chemical and cellular abnormalities in cerebrospinal fluid (CSF). The incubation period was taken to be <1 year. Suspected case patients were defined as having ≥1 of the clinical signs, not explained by other conditions, that developed after having received cytotoxics prepared on risk days. From suspected case patients, relevant clinical samples were retested or obtained, if possible. A suspected case turned into a proven case if M. bovis–BCG identical to Onco-TICE BCG was isolated.

All mycobacteria strains isolated from patients in The Netherlands are routinely sent to the National Institute of Public Health and the Environment. Strains were available from July 1989 to May 1996. Clinical symptoms for selecting a suspected case were those used in the case-finding study in hospital A (see above). Clinical data were obtained by sending a questionnaire to the clinician. Suspected patients had a positive M. bovis–BCG culture and ≥1 clinical signs after receiving cytotoxic agents not otherwise explained. A suspected case turned
into a proven case if the patient’s *M. bovis*–BCG strain was identical to *M. bovis*–BCG Onco-TICE.

*M. bovis*–BCG strains were identified as described elsewhere [5] and were further analyzed by restriction fragment–length polymorphism typing (figure 1). Hybridization with the B9 probe is specific for BCG Onco-TICE strains [6].

Here, “*M. bovis*–BCG” refers to a strain not further typed. Strains typed and found to be identical to *M. bovis*–BCG Onco-TICE, the strain used for bladder instillation, were indicated as BCG Onco-TICE.

In hospital A, we performed an inspection during work at the pharmacy. We tested the work environment for the presence of *M. bovis*–BCG bacteria. During preparation of BCG Onco-TICE suspension, 6 petri dishes (16 cm in diameter) filled with sterile water were placed in the cabinet. The gloves we used were rinsed in sterile water, which was then tested for mycobacteria by the Mycobacterium Direct Test (Gen-Probe; BioMerieux) and IS6110 polymerase chain reaction (PCR) [7] and by dot-blot hybridization [8]. A sample of dust was obtained from the spill trough under the grid cover of the biological safety cabinet and examined for *M. tuberculosis* complex DNA.

**Results.**

Patient 1 was an 11-year-old girl who had acute lymphatic leukemia and was treated with intravenous chemotherapy and intrathecal instillation. She presented with malaise, anorexia, and vomiting due to progressive ventriculitis. Cultures of her CSF grew *M. bovis*–BCG. Her purified protein derivative skin reaction was negative. Despite treatment with antituberculosis agents, she died 9 months later. Autopsy revealed a recurrence of leukemia, and PCR of the CSF was positive for *M. tuberculosis* complex. The patient was not BCG vaccinated and had not been exposed to recently vaccinated persons.

Patient 2, a 13-year-old girl with Hodgkin disease, was treated with intravenous chemotherapy and was readmitted because of malaise, headache, sweating, and weight loss. Analysis of lung tissue obtained via biopsy revealed granulomatous lesions and grew *M. bovis*–BCG, at that time thought to be a reactivation of the BCG vaccination strain. She recovered completely.

Patient 3 was a 39-year-old man who developed diarrhea, vomiting, and fever after treatment with intravenous cytotoxic chemotherapy. Multiple nodules were seen on the chest X-ray. Cultures from transbronchial biopsy samples, bone marrow, and gastric aspirates grew *M. bovis*–BCG, at that time assumed to be an endogenous reactivation of his vaccine strain.

Patients 1–3 were treated at hospital A. Patient 4, treated at hospital B (Amsterdam), was a 30-year-old man, previously described as a fatal case of reactivation 12 years after vaccination [9]. He had AIDS and a Burkitt-like non-Hodgkin lymphoma. He was treated intravenously and intrathecally with cytotoxic chemotherapy. He presented with fever, headache, and neck stiffness. *M. bovis*–BCG was isolated from CSF. He died 11 days, later despite undergoing therapy for tuberculosis.

Patient 5, treated at hospital C, was a 4-year-old girl with pre–B cell acute lymphatic leukemia. She was treated with systemic chemotherapy. She was not BCG vaccinated. More than 1 year later, a relapse of leukemia was diagnosed, and she was again treated both systemically and intrathecally. Eight months later, she presented with fever, coughing, malaise, and headache. Bronchoalveolar lavage revealed *M. bovis*–BCG bacteria. Analysis of bone marrow sample obtained at biopsy revealed a granulomatous reaction, but no mycobacteria were cultured. Later, osteomyelitis was diagnosed, and analysis of biopsy samples revealed acid-fast bacilli, although they were not cultured, probably because the patient was undergoing therapy for tuberculosis. PCR results were negative.

Patients 1–3 from hospital A all received cytotoxic agents prepared in the hospital pharmacy after the preparation of an BCG Onco-TICE suspension on 2 (patient 1), 6 (patient 2), and 13 (patient 3) occasions. Thus, 21 risk days could be selected. A total of 296 patients had received cytotoxics prepared on a risk day, and clinical data were obtained. Twenty-eight suspected patients were selected; 18 suspected patients had died, and 10 were still alive. From 8 of the suspected patients who had already died, 8 samples (2 from lung, 2 from lymph node, 1 from tumor, 1 from granuloma, 1 from liver, and 1 from testis) obtained by autopsy or biopsy were available and were found to be PCR negative. From 10 suspected patients who had already died, no materials were available. From 2 suspected patients still alive,
samples were obtained for histologic testing and culture for mycobacteria or PCR. All cultures and PCRs to detect \textit{M. tuberculosis} complex bacteria or DNA were negative. In the other 8 suspected patients, symptoms resolved spontaneously. One patient showed no symptoms during this survey but later died from an unexplained infectious disease 13 months after receiving cytotoxics prepared on one of the risk days. Unfortunately, no materials were available to test for BCG Onco-TICE infection. Thus, no proven cases could be identified.

From 77 patients nationwide, \textit{M. bovis}–BCG strains were sent to the national reference laboratory (Bilthoven) between July 1989 and May 1996. From all patients, clinical data were collected. Two patients were suspected cases and were found to be proven cases when isolates were typed and were found to be identical to BCG Onco-TICE (patients 4 and 5). The patients had not undergone BCG Onco-TICE therapy, nor had personnel or patients in their departments handled or received this agent. In both hospital pharmacies, BCG Onco-TICE was prepared.

The BCG isolates of patients 1–5 were strains with a single IS6110 copy and showed a positive hybridization with the B9 probe (figure 1). This proves that these bacteria did not represent the Dutch vaccine strain, because the latter yielded a negative hybridization. Patient 4 was reported earlier with a fatal reactivation of \textit{M. bovis}–BCG bacteria 12 years after BCG vaccination [9]. Retyping of the respective BCG isolate with the B9 probe indicated that the isolated BCG bacteria did not represent the Dutch vaccine strain, but rather BCG Onco-TICE. Therefore, this infection was nosocomial and not an endogenous reactivation.

In hospital A, at the time of the incidents, the same biosafety cabinet was used for preparing BCG Onco-TICE and cytotoxic agents. BCG Onco-TICE was prepared first. Gloves were not routinely changed, and the safety cabinet was not routinely cleaned or disinfected after each preparation of BCG Onco-TICE. In case of macroscopic spill, tap water was used. At that time, the BCG Onco-TICE came freeze-dried in a glass ampoule (5 × 10⁷ cfu). For preparation, the ampoule was broken, sterile water was injected, and the content was suspended by swirling the open ampoule. PCR techniques demonstrated the presence of \textit{M. tuberculosis} complex DNA in 2 of the 6 petri dishes and in the dust collected from the spill trough of the safety cabinet. Both gloves of the pharmacy technician were contaminated with \textit{M. tuberculosis} complex DNA.

In all hospitals, BCG Onco-TICE suspensions were prepared in the same biosafety cabinet as the cytotoxics. All patients received medication prepared in the same biological safety cabinet on the same days as BCG Onco-TICE was prepared. However, in hospital C, a guideline was implemented that described cleaning and disinfection after the preparation of BCG Onco-TICE suspension and that in case of spill, rigorous disinfection was to take place.

**Discussion.** We found that 5 patients from 3 different hospitals in the Netherlands had nosocomial infections caused by BCG Onco-TICE in the last 7 years. In all 3 hospitals, BCG Onco-TICE was prepared in the same biological safety cabinet as the cytotoxic chemotherapy in 2 hospitals (A and B), even on the same day.

After the incident in hospital A, an advisory group of Dutch hospital pharmacists recommended strict separation in time of the preparation of BCG Onco-TICE instillations and the preparation of other medications by disinfecting the safety cabinet and changing gloves between preparations. These recommendations were in addition to standard good manufacturing practice procedures for the validation and monitoring of biohazard safety cabinets, as currently described in the European standard [10]. This guideline has been implemented nationally. Pharmacy personnel were informed about the infectious nature of preparation. In hospital A, we could use a specifically dedicated safety cabinet for this type of preparation. We informed the manufacturer of BCG Onco-TICE about the dangers of breaking the glass ampoule and of contamination during dilution of the mycobacteria. Recently, vials with rubber stoppers have been introduced, and the instructions now carry a warning to handle the product strictly separated. The national working party on hospital infections was alerted of the dangers of transmission. In hospital C, in the pharmacy, hygienic measures to prevent cross-contamination were already in force when patient 5 presented. However, in the pediatric ward of patient 5, it was ruled out that a patient was treated with BCG Onco-TICE or that personnel had handled BCG Onco-TICE.

Cross-contamination in the pharmacy has also been postulated by others [3, 4, 11]. More et al. [12] described 4 patients with \textit{M. bovis}–BCG infections by strains identical to those used for bladder instillation. The same personnel gave the BCG instillation therapy and looked after these patients. The real number of iatrogenic BCG infections is probably underestimated, because many clinicians do not consider this route of infection, and laboratory identification is complicated. We strongly recommend that all \textit{M. tuberculosis} complex isolates should be identified to the species or subspecies level and tested for endogenous reactivation or nosocomial transmission. We used the probe B9 [6], which reacted with \textit{M. bovis}–BCG strains with a single copy of IS6110, but not with the Pasteur strain (BCG-Bilthoven), which was used in the Netherlands for vaccination before 1998. There are, however, many more genetic characteristics that can be used [13]. Furthermore, because it was shown that transmission is possible by personnel [12], we recommend that clinical departments develop guidelines describing hygienic measures to prevent transmission of BCG Onco-TICE or BCG Connaught to other patients. This guideline should include education of personnel about the high bio-burden of the instillation therapy, strict disinfection of hands.
and all materials used, and, if possible, the use of separate rooms for preparation.

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