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Research Report

No Association Between BCG Instillations and COVID-19 Incidence in a Dutch Non-Muscle Invasive Bladder Cancer Cohort

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Abstract.
BACKGROUND: Randomized controlled trials of BCG vaccination did not show a protective effect against COVID-19 infections, although an impact on severity has been suggested, especially by repetitive BCG administrations. Studies into multiple BCG instillations in patients with non-muscle invasive bladder cancer (NMIBC) and COVID-19 are however scant, observational, and small.
OBJECTIVE: To study the association between BCG instillations and the occurrence and severity of a COVID-19 infection in a large, well-phenotyped cohort of patients with NMIBC.
METHODS: Patients from the BlaZIB and UroLife studies with date of primary NMIBC diagnosis between May 2014 and April 2020 and their life partners were invited. Data on COVID-19 (hospitalization, positive test, physician-diagnosis, self-diagnosis) in the period between February 2020 and May 2021, and on relevant covariables, were obtained via repeated web-based questionnaires. Information on BCG treatment was collected from medical records. We defined subgroups of NMIBC patients that were fully, partially, or not exposed to BCG during the COVID-19 assessment period. Proportional hazard models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs).
RESULTS: Out of the 685 included NMIBC patients, 399 were treated with BCG (130 fully, 269 partially exposed) and 286 were not. During a median (IQR) follow-up time of 68 (51–68) weeks, 115 patients reported a COVID-19 infection (17%). BCG exposure was not associated with risk of COVID-19 infection (adjusted HR 1.03 (95%CI 0.70–1.50)). Ten patients (1.5%) were hospitalized for COVID-19; interestingly, none of them were in the fully exposed BCG group. Censuring at first COVID-19 vaccination did not change the results. The rate of affected partner-non-affected patient-pairs was higher in the BCG-exposed (42%) compared to the BCG-unexposed pairs (36%), but not at a level of statistical significance ($p = 0.67$).

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CONCLUSIONS: We found no association between BCG bladder instillations and the occurrence of COVID-19 in bladder cancer patients.

Keywords: Bacillus Calmette-Guérin (BCG), SARS-CoV-2, COVID-19, non-muscle invasive bladder cancer, cohort study

INTRODUCTION

Bacillus Calmette-Guérin (BCG) is well-known for its use as vaccine against tuberculosis. BCG vaccination also induces a non-specific (heterologous) response to a wide range of (unrelated) pathogens and a reduced incidence of respiratory infections and sepsis [1]. These non-specific effects are at least in part due to the induction of trained immunity, a de-facto immunological memory of innate immune cells. Trained immunity involves the long-term epigenetic and transcriptomic reprogramming of innate immune cells which leads to a more effective response towards a second, unrelated, challenge [2]. We recently showed that repeated bladder instillations with BCG in the treatment of non-muscle invasive bladder cancer (NMIBC) induces trained immunity as well. We also observed a reduced incidence of respiratory tract infections, in particular pneumonia, in BCG-exposed versus BCG-unexposed NMIBC patients [3].

The coronavirus disease-19 (COVID-19) pandemic led to an interest in BCG vaccination as preventive measure against SARS-CoV-2 infection. However, randomized controlled trials mostly showed no reduction in COVID-19 incidence after BCG vaccination [4–12], despite an increased serologic response and reduced SARS-CoV-2 immunopathology [8, 13]. None of these studies had enough power to assess the effect of BCG vaccination on COVID-19 severity, although summary statistics of published trials suggested a decreased mortality due to COVID-19 in individuals vaccinated with BCG [14]. In addition, repeated BCG vaccination in type 1 diabetes patients showed remarkable protection against COVID-19 in a randomized phase 2/3 trial [7].

Recently, Pichler et al., studied PBMCs that were isolated during BCG treatment from eleven patients with NMIBC and found that repeated BCG bladder instillations also induced a trained immunity-related innate immune response against SARS-CoV-2 [15]. The association between multiple BCG instillations in patients with NMIBC and COVID-19 incidence has been evaluated in a few observational studies with some limitations in study design and analyses, i.e. small study size, lack of proper comparison group and lack of data on relevant covariables. Results of these observational studies suggested no association between BCG instillations and COVID-19 occurrence [16–21] but some suggested an inverse association with severity [18, 21]. Here, we extend the evidence base on the association between multiple BCG instillations and occurrence and severity of symptomatic COVID-19 infection in a large and well-phenotyped cohort of patients with NMIBC and their partners.

MATERIALS AND METHODS

Study population

We performed the current study within the BlaZIB [22] and UroLife [23] studies. BlaZIB is a nationwide prospective cohort study including all patients with a primary diagnosis of high-risk NMIBC (Tis and/or T1, N0, M0/x) or non-metastatic muscle invasive bladder cancer in the Netherlands between November 2017 and November 2019. UroLife is a multicentre prospective cohort study among patients who were newly diagnosed with NMIBC in the years 2014 to 2021 and recruited from 22 hospitals in the Netherlands. Patients participating in BlaZIB or Urolife with date of primary NMIBC diagnosis between May 2014 and April 2020, who provided consent to be approached for additional research, and for whom an email address was available, were invited in May 2020 to participate in this substudy. Patients were invited via email and were also asked to invite their potential life partner/spouse (to allow for evaluation of risk of transmission). A total of 1,232 patients were invited of which 688 (56%) consented and filled out at least one web-based questionnaire ($n = 186$ BlaZIB; $n = 502$ UroLife); 399 partners filled out a questionnaire as well. BlaZIB was approved by the Privacy Review Board of the Netherlands Cancer Registry (reference number K22.029). UroLife has received approval by a local Institutional Review Board (approval number CMO 2013-494). Informed signed consent has been obtained from all participants.

Outcome assessment

Information on COVID-19 infection and severity was collected from participating patients and partners using multiple web-based questionnaires: a first questionnaire was sent on May 14 2020 (incomplete for 10 patients and 14 partners), short biweekly questionnaires were sent between June 3 2020 and April 7 2021, and a final questionnaire was sent on May 27 2021 (incomplete for 109 patients and 87 partners). Participants reported if they were 1) tested on COVID-19 with accompanying test result, 2) physician-diagnosed with probable COVID-19, or 3) self-diagnosed with COVID-19. A positive COVID-19 test, a physician-diagnosis, or a self-diagnosis of COVID-19 were all included as incidence of a COVID-19 infection, as at the start of the pandemic COVID-19 testing was only available in the Netherlands for healthcare workers and hospitalized patients ((symptom-driven) SARS-CoV-2 PCR tests available from June 1 2020, SARS-CoV-2 antigen self-tests from March 31 2021). Severity of COVID-19 was evaluated by 1) hospitalization because of COVID-19, 2) oxygen administration during hospitalization for COVID-19, 3) artificial ventilation during hospitalization for COVID-19, and 4) intensive care unit (ICU) admittance during hospitalization for COVID-19. The first questionnaire (sent May 14 2020) retrieved information on COVID-19 infection and severity between the start of the pandemic (February 1 2020) to the end of May 2020. The COVID-19 assessment period for the biweekly questionnaires (sent from June 3 2020 to April 7 2021) was the two weeks prior to receiving the questionnaire. The final questionnaire (sent May 27 2021) was sent to all participants, including those who stopped responding to the biweekly questionnaires, with an assessment period of the start of the pandemic to the date of filling out the questionnaire.

Exposure assessment

BCG treatment in the Netherlands is generally in compliance with the guidelines of the European Association of Urology (i.e. an induction cycle followed by 1–3 years of BCG maintenance therapy). Information on BCG treatment within BlaZIB and UroLife was collected from medical records by trained registrars from the Netherlands Cancer Registry (NCR). These NCR data were used to define the timing of BCG exposure for the patients. Given the period of outcome assessment of February 2020 (first COVID-19 patient in the Netherlands) to May 2021 (date of final questionnaire), we defined subgroups of BCG-treated NMIBC patients that we considered as fully or partially exposed to BCG during this outcome assessment period. The immunological phenotype of BCG-induced trained immunity has been shown to last at least up to 1 year and longer-term heterologous protection of BCG vaccination against infections has been described [24, 25]. We therefore assumed that a trained immunity phenotype is fully induced two weeks after the first instillation and will be present up to 1.5 year after the last BCG instillation. Those patients with the first BCG instillation prior to January 15 2020 and the final BCG instillation after November 15 2019 were hence considered fully exposed. The other BCG-treated patients were considered to be partially exposed. NCR follow-up was completed until at least February 2020 for 670 patients (98%); for the remaining 18 patients self-reported data on BCG treatment were used to define the timing of BCG treatment and three patients were excluded due to missing data on BCG treatment.

Covariate assessment

The first questionnaire contained questions on age, sex, height, smoking, current body weight, BCG vaccination status, flu vaccination status, and comorbidities. Furthermore, it was queried if the participant or one of their housemates worked in a 'vital profession' (as defined by the Dutch government during the pandemic). If the participant answered yes to this question, we categorized this patient and/or life partner/spouse employed as 'healthcare personnel'. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). The final questionnaire included an additional question on COVID-19 vaccination status and timing.

Statistical analyses

Statistical analyses entailed the description of characteristics in cross tables for comparison of groups that differed in their exposure to BCG. Proportional hazard models (PHM) were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for association between BCG exposure status and COVID-19 infection. Follow-up time was calculated in weeks from February 1 2020 to date of questionnaire completion in which the first COVID-19 infection was reported, or the date of the last completed questionnaire in case no COVID-19 was

reported (maximum follow-up time of 68 weeks). Multivariable PHM analysis was used to allow for adjustment of factors that are known to affect the incidence of COVID-19 infection and are associated with BCG exposure or NMIBC patient status and may hence confound the association of interest. The main determinant of COVID-19 infection is exposure to SARS-CoV-2. We hence adjusted for healthcare personnel status within the household of the participants. More specifically, in analyses including patients with NMIBC only, we adjusted for age, sex, BMI, and health care personnel status. Adjustment for relevant comorbidities (heart disease, chronic lung disease, diabetes mellitus) did not change the reported HRs and these were therefore not included in the final models. In the PHM analyses that also included partners, we only adjusted for age, sex, and BMI; health care personnel status was measured on the level of the household and therefore equal for patients and partners and comorbidities did not affect HRs. If BCG exposure reduces risk of COVID-19 infection, we expect to see less transmission between partner and BCG-exposed patient compared to partner and BCG-unexposed patient. Descriptive statistics were used to compare (dis)concordance in COVID-19 infections between patients and their partners by BCG exposure status of the patients.

RESULTS

Cohort characteristics

Out of the 685 patients with NMIBC, 286 (42%) were not treated with BCG. Of the 399 patients that were treated with BCG, 130 (33%) were classified as fully exposed and 269 (67%) as partially exposed during the outcome assessment period. Characteristics of the NMIBC patients are depicted in Table 1. The frequency of males was higher in the BCG-exposed (85%) compared to BCG-unexposed patients (79%); the groups were comparable for all other characteristics. For 389 analyzed NMIBC patients, partner data were available. General characteristics of these patients and their partners are given in Supplemental Table 1. Partners were predominantly female and slightly younger, more often never-smokers, and less frequently reported a history of heart disease, in line with expectations.

Table 1
Characteristics of the study participants with NMIBC by BCG exposure

	NMIBC BCG			NMIBC no BCG	*P*-value BCG vs no BCG
	Total	Fully exposed	Partially exposed		
Total	399	130	269	286	
Male sex (%)	341 (85%)	107 (82%)	234 (87%)	225 (79%)	0.02
Age at first questionnaire (median (IQR))	71 (64–75)	71 (65–75)	71 (64–75)	70 (65–75)	0.67
Smoking history[1]					0.71
Never smoker (%)	75 (19%)	25 (19%)	50 (19%)	59 (21%)	
Previous smoker (%)	288 (72%)	95 (73%)	193 (72%)	199 (70%)	
Current smoker (%)	35 (9%)	10 (8%)	25 (9%)	28 (10%)	
Body mass index (median, IQR)[1]	26.3 (24.4–29.0)	27.2 (24.6–29.2)	26.3 (24.4–29.0)	26.3 (24.2–28.9)	0.39
Comorbid conditions[1]					
History of heart disease (including hypertension)	208 (52%)	66 (51%)	142 (53%)	156 (55%)	0.59
History of chronic lung disease (asthma, COPD, bronchitis, emphysema)	55 (14%)	19 (15%)	36 (13%)	42 (15%)	0.82
Diabetes mellitus[1]	56 (14%)	12 (9%)	44 (16%)	39 (14%)	0.91
BCG vaccination ever (%)[2]	69 (18%)	22 (17%)	47 (18%)	55 (20%)	0.55
Employment status					0.23
Employed	86 (22%)	27 (21%)	59 (22%)	61 (21%)	
Retired	289 (72%)	94 (72%)	195 (72%)	208 (73%)	
Unemployed	24 (6%)	9 (7%)	15 (6%)	17 (6%)	
Health care personnel[3]	33 (8%)	9 (7%)	24 (9%)	35 (12%)	0.09

[1]Data of 1 patient was missing. [2]Data of 10 patients were missing. [3]Data of 3 patients were missing.

Association between BCG instillations and COVID-19 infection

During a median (IQR) follow-up time of 68 (51–68) weeks, 115 COVID-19 infections (17%) were reported among the 685 NMIBC patients. More than half (56%) of COVID-19 infections were based on self-diagnosis (Table 2a). Ten patients (1.5%) were hospitalized for COVID-19, of whom two were admitted to the intensive care unit. BCG exposure was not associated with risk of COVID-19 infection; the adjusted HR for BCG exposure versus no exposure was 1.03 (95%CI 0.70–1.50) (Table 2b). Restriction to COVID-19 occurrences based on physician-diagnosis or positive test only resulted in a statistically insignificant adjusted HR of 1.32 (95%CI 0.73–2.36). None of the ten hospitalized patients were part of the fully BCG-exposed group where 2 (130/685 * 10) were expected under assumption of independence between BCG exposure status and COVID-19 hospitalization. Between January 15 2021 (week 49 of follow-up) and the end of the study, 545 NMIBC patients (and 290 partners) received ≥ 1 dose of a COVID-19 specific vaccine. Ending the follow-up time at first COVID-19 vaccination did not change the results (Table 2c). Sixty-five partners reported a COVID-19 infection. The rate of affected partner-non-affected patient pairs was higher in the BCG-exposed (42%) compared to the BCG-unexposed pairs (36%), but not at a level of a statistical significance ($p = 0.67$) (Supplemental Table 2). PHM analysis for patients and partners indicated no difference in COVID-19 risk for BCG-exposed patients compared to partners (HR 0.82, 95%CI 0.53–1.28) and for non-BCG-exposed patients compared to partners (HR 1.10, 95%CI 0.62–1.94). The direction of the effect estimates is in line with an inverse association between BCG instillations and COVID-19 but estimates were imprecise and low numbers hampered proper adjustment for covariables in a multivariable PHM analysis (Supplemental Table 3).

DISCUSSION

The potential protective role of BCG vaccination against COVID-19 has been investigated in many types of studies, including animal models, experimental infection models in humans, ecologic studies, and randomized controlled trials (RCTs). While many of these studies have suggested a potential reduction in COVID-19 incidence and/or severity, the RCTs have mostly shown lack of a protective effect against the total number of infections. In contrast, a protective effect of BCG vaccination against COVID-19-induced death has been suggested by summary statistics of available randomized trials [14]. Several unclarities remain, including the role of route of administration, dosage, timing, and number of BCG vaccinations, in relation to the risks of COVID-19 and other non-tuberculosis infectious diseases [26].

We evaluated the association between exposure to repeated BCG instillations in the bladder and occurrence of COVID-19 in a substantial population of 685 patients with NMIBC and their 389 partners and observed no association. The potential association with reduced severity of COVID-19 could not be properly studied here due to low numbers, but we observed no hospitalization among those who were fully BCG-exposed during the COVID-19 outcome assessment period.

We aimed to add to the currently limited evidence base on BCG treatment in NMIBC and COVID-19. Six observational studies among NMIBC patients have been previously published but the design and analyses of these studies showed weaknesses, including small sample size, lack of a proper comparison group, and inability to properly deal with potential confounders [16–21]. The largest study, with limited number of events though ($\ll$1% in bladder cancer patients), was performed by Fedeli et al. COVID-19 incidence up to May 14 2020 as registered in a centralized surveillance system in a region of Italy for a cohort of 2,803 NMIBC patients treated with BCG between 2015–2019 was compared to a cohort of 11,130 BCG-unexposed bladder cancer patients who were hospitalized in 2015–2019, and to the overall regional population. Standardized incidence ratios generated for both reference groups did not show a statistically significant difference for either COVID-19 infection, hospital admission, or intensive care unit admission or death [17]. Hurle et al., included 506 NMIBC patients who were treated with intravesical adjuvant therapy between January 2018 and December 2019; 67% were BCG-exposed. COVID-19 data were retrieved via two telephone interviews after the first and second COVID-19 wave in Italy and SARS-CoV-2 infection rates were optionally determined via serology tests. Results indicated no association between BCG instillations and a reduced COVID-19 and SARS-CoV-2 infection rate [19].

Our study has a number of strengths compared to the previous studies. It included a substantial number of 685 patients with NMIBC for whom data on

Table 2a
COVID-19 diagnosis, type of diagnosis, and severity by BCG exposure among patients with NMIBC

	Total ($n = 685$)	BCG ($n = 399$)			no BCG ($n = 286$)
		Total	Fully exposed	Partially exposed	
COVID-19 diagnosis	115 (17%)	66 (17%)	25 (19%)	41 (15%)	49 (17%)
Self-diagnosis	64 (56%)	33 (50%)	14 (56%)	19 (46%)	31 (63%)
Physician-diagnosis	15 (13%)	9 (14%)	4 (16%)	5 (12%)	6 (12%)
Positive test	36 (31%)	24 (36%)	7 (28%)	17 (41%)	12 (24%)
COVID-19 resulting in hospital stay	10 (9%)	4 (6%)	0 (–)	4 (9%)	6 (12%)
Including oxygen	10	4	0	4	6
Including ventilation	3	1	0	1	2
Including ICU stay	2	0	0	0	2

Table 2b
Hazard ratios (HRs) for the association of BCG exposure with risk of COVID-19 among patients with NMIBC

BCG exposure	N	Events/person-years	Crude model HR (95%CI)	Adjusted model* HR (95%CI)
No BCG	286	49/315	1.00 (ref)	1.00 (ref)
BCG	399	66/443	0.96 (0.66, 1.39)	1.03 (0.70, 1.50)
Partial	269	41/303	0.87 (0.58, 1.32)	0.94 (0.62, 1.44)
Full	130	25/140	1.14 (0.71, 1.85)	1.19 (0.73, 1.94)

*Adjusted for age, sex, BMI, and health care personnel status for $N = 682$ (–3) with 113 (–2) events due to missing data in covariates.

Table 2c
Hazard ratios (HR) for the association of BCG exposure with risk of COVID-19 among patients with NMIBC with follow-up until first COVID vaccination

BCG exposure	N	Events/person-years	Crude model HR (95%CI)	Adjusted model* HR (95%CI)
No BCG	286	45/291	1.00 (ref)	1.00 (ref)
BCG	399	62/408	0.98 (0.66, 1.43)	1.03 (0.70, 1.53)
Partial BCG	269	39/277	0.91 (0.59, 1.39)	0.97 (0.62, 1.50)
Full BCG	130	23/131	1.12 (0.68, 1.85)	1.16 (0.70, 1.93)

*Adjusted for age, sex, BMI, and health care personnel for $N = 682$ (–3) with 113 (–2) events due to missing data in covariates.

factors that are known to affect COVID-19 incidence were collected and were highly comparable for BCG-exposed and BCG-unexposed patients. The inclusion of 389 partners, who are in close physical contact with the patients, allowed for additional analysis into the association between BCG exposure and COVID-19 risk. However, the COVID-19 event rate was too low for precise estimates in analyses of the pairs. Also, data on dates of first and last BCG instillation from the Netherlands Cancer Registry allowed us to define both fully and partially exposed subgroups for BCG-exposure relative to the COVID-19 assessment period.

Although our study is a large addition to the current evidence base, given the sample size and observed COVID-19 incidence rate of 17%, it only had ample power (i.e. at least 80% at a statistical significance threshold of 0.05) to detect associations of BCG exposure with COVID-19 for hazard ratios smaller than 0.6 or larger than 1.75. Another limitation of our study was that COVID-19 status was retrieved via self-report digital questionnaires in the first year of the COVID pandemic. Participants were asked to report hospitalization for COVID-19, a test for COVID-19 with accompanying test result, a physician-diagnosis of probable COVID-19, and self-diagnosis of COVID-19. Asymptomatic SARS-CoV-2 infections could hence not be analysed. Also, for information on death due to COVID-19, which was not observed in our study, we were reliant on active report by those who were in contact with the participant and aware of this study. For the far majority of participants (85%), the outcome assessment was complete until May 2021 because they either

filled out (at least) the final questionnaire or/and reported a COVID-19 occurrence during follow-up. The frequency of the repeated questionnaires was high which limited risk of recall bias, but half of the COVID-19 reports in our study population were based on a patient's self-diagnosis which may be vulnerable to misclassification. Given that any errors in COVID-19 classification are expected to be independent of BCG-exposure status, bias, if any, resulting from this will lead to an underestimation (bias toward HR of 1) of the estimated association.

In conclusion, the current data at hand suggest no association between BCG instillations and risk of COVID-19 infection in patients with NMIBC, while future studies would need to assess BCG effects on disease severity. Novel studies in contemporary NMIBC populations can give information on the relation between BCG instillations and COVID-19 after introduction of COVID-19 vaccination programs. Our study adds to the evidence base on the association between BCG and COVID-19 disease in general and in NMIBC in specific and provides urologists with relevant information that can be used in communication with patients that are treated with BCG instillations.

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AUTHOR CONTRIBUTIONS

Conception: LALMK, JAW, LABJ, MGN, SHV; performance of work: MZ, UTHO, JSFM, KKHA, AV, SHV; interpretation of data: MZ, LALMK, JSFM, JAW, LABJ, MGN, KKHA, AV, SHV; writing the article: MZ, LALMK, JSFM, JAW, LABJ, MGN, KKHA, AV, SHV; access to data: MZ, UTHO, JSFM, KKHA, AV, SHV.

CONFLICT OF INTEREST

LALMK and JAW are Editorial Board members of this journal, but were not involved in the peer-review process nor had access to any information regarding its peer-review. LABJ and MGN are scientific founders of TTxD and Lemba. MGN is a scientific founder of Biotrip. MZ, UTHO, JSFM, KKHA, AV, and SHV have no conflict of interest to report.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/BLC-230088.

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