Clinical Infectious Diseases

MAJOR ARTICLE

Efficacy of BCG Vaccination Against Respiratory Tract Infections in Older Adults During the Coronavirus Disease 2019 Pandemic

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Background. Older age is associated with increased severity and death from respiratory infections, including coronavirus disease 2019 (COVID-19). The tuberculosis BCG vaccine may provide heterologous protection against nontuberculous infections and has been proposed as a potential preventive strategy against COVID-19.

Methods. In this multicenter, placebo-controlled trial, we randomly assigned older adults (aged ≥60 years; n = 2014) to intracutaneous vaccination with BCG vaccine (n = 1008) or placebo (n = 1006). The primary end point was the cumulative incidence of respiratory tract infections (RTIs) that required medical intervention, during 12 months of follow-up. Secondary end points included the incidence of COVID-19, and the effect of BCG vaccination on the cellular and humoral immune responses.

Results. The cumulative incidence of RTIs requiring medical intervention was 0.029 in the BCG-vaccinated group and 0.024 in the control group (subdistribution hazard ratio, 1.26 [98.2% confidence interval, .65–2.44]). In the BCG vaccine and placebo groups, 51 and 48 individuals, respectively tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with polymerase chain reaction (subdistribution hazard ratio, 1.053 [95% confidence interval, .71–1.56]). No difference was observed in the frequency of adverse events. BCG vaccination was associated with enhanced cytokine responses after influenza, and also partially associated after SARS-CoV-2 stimulation. In patients diagnosed with COVID-19, antibody responses after infection were significantly stronger if the volunteers had previously received BCG vaccine.

Conclusions. BCG vaccination had no effect on the incidence of RTIs, including SARS-CoV-2 infection, in older adult volunteers. However, it improved cytokine responses stimulated by influenza and SARS-CoV-2 and induced stronger antibody titers after COVID-19 infection.

Clinical Trials Registration. EU Clinical Trials Register 2020-001591-15 ClinicalTrials.gov NCT04417335.

Keywords. BCG vaccination; trained immunity; COVID-19; SARS-CoV-2.

Older adults are at high risk of severe respiratory tract infections (RTIs). Protection against respiratory disease by vaccination is associated with a decreased risk of infection and death in older adults, but protection is frequently incomplete owing to age-associated decline in immune function, also termed "immunosenescence" [1]. Older adults also account for the majority of severe coronavirus disease 2019 (COVID-19) cases and associated deaths [2]. Although specific vaccines give the best chance as preventive strategy against infection, they are not immediately available when a new pathogen emerges. Therefore, additional strategies are needed to lower infection-related morbidity and mortality rates in a new outbreak.

The BCG vaccine not only protects against tuberculosis but also induces broad effects on host defense, offering protection against a wide range of other infections [3]. In countries with high infection pressure, BCG vaccination in infants is associated with a reduction in all-cause neonatal mortality rate, mainly attributed to a reduced incidence of RTIs and sepsis [4]. These effects are thought to be mediated by heterologous lymphocyte activation and induction of trained immunity, a

Received 11 November 2021; editorial decision 24 February 2022; published online 5 March 2022.

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Clinical Infectious Diseases® 2022;75(1):e938–46

https://doi.org/10.1093/cid/ciac182

de facto immunological memory of innate immune cells [5]. A randomized clinical trial suggested a protective effect of revaccination with BCG vaccine on the incidence of RTIs in hospitalized older adults from Greece [6], but it is unknown whether similar effects occur in BCG vaccine–naive persons in this age group. Here we present the results of a multicenter, randomized, placebo-controlled trial to evaluate the safety and efficacy of BCG vaccination against RTIs, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, in individuals aged ≥60 years from a Western European country in which BCG vaccination has never been part of the standard immunization program.

METHODS

Study Design

The study was a double blind, placebo-controlled, randomized clinical trial conducted in 2 university hospitals in the Netherlands from 16 April 2020 to 14 May 2021. It was approved by the Arnhem-Nijmegen Ethical Committee (no. NL73430.091.20) and conducted according to the principles expressed in the Declaration of Helsinki and the Guidelines for Good Clinical Practice. All individuals provided written informed consent. See the Supplementary Materials for inclusion and exclusion criteria.

Trial Procedures

Participants were informed about the study by advertisements and could self-register. Eligible participants were randomly assigned in a 1:1 ratio to receive either 0.1 mL of BCG vaccine (Danish strain 1331; SSI) or 0.1 mL of saline placebo via intradermal injection, using a computer-generated dynamic randomization algorithm, stratified for hospital and age at randomization.

During the 12-month follow-up, participants used a mobile application (Research Follow App; Your Research) to fill in a daily questionnaire regarding symptoms and adverse events and a weekly questionnaire regarding COVID-19 testing, COVID-19 exposure, and visits to healthcare professionals (Supplementary Table 1). Participants unable to use a smartphone completed paper questionnaires and were called monthly and asked to contact the study team if they had contacted a healthcare professional. Adherence to questionnaires was monitored and quantified. Participants were unblinded at the end of follow-up.

Efficacy Assessments

The initial primary end point—"cumulative incidence of SARS-CoV-2–related hospital admission"—was changed on approval by the ethical committee (before data were unblinded), owing to the almost complete absence of outcomes consistent with the primary end point after 4 months of follow-up. The primary end point was changed to the cumulative incidence of clinically relevant RTIs, defined as onset or sudden aggravation of pre-existing symptoms, as reported by the participant, including at least 1 respiratory symptom (cough, throat ache, rhinorrhea or dyspnea) and 1 systemic symptom (fever, muscle ache, chills, fatigue) that required medical intervention within 5 days of the onset of symptoms. After medical assessment in primary or secondary care, an intervention was defined as initiation of antibiotic, antiviral, or corticosteroid treatment, adaptation of pulmonary maintenance medication, or hospitalization.

During the follow-up period, individuals in the Netherlands with symptoms suggestive of COVID-19 were encouraged to be tested for SARS CoV-2 in a publicly available testing facility using a polymerase chain reaction (PCR) test and nasopharyngeal swab samples. The end point of documented SARS-CoV-2 infection required a self-reported positive PCR result. Participants reporting any infection or positive PCR result were contacted for confirmation and follow-up. An overview of secondary end points is listed in the Supplementary Materials.

Cellular and Humoral Immune Assays

A subgroup of participants who had not received a COVID-19–specific vaccine were asked to donate blood for cellular and humoral immune assays at the end of follow-up (month 12). Isolation and stimulation of peripheral blood mononuclear cells were performed as described elsewhere [7]. Cytokine levels were measured using an enzyme-linked immunosorbent assay (R&D systems). A SARS-CoV-2 fluorescent microsphere–based multiplex immunoassay was used to quantify antibody responses, as described elsewhere [8, 9]. For experiment details see the Supplementary Materials.

Statistical Analysis

The study was designed as an end point–driven trial with a symmetrical group sequential design. The sample size was calculated using R package gsDesign software, version 3.0.1. We used a 2-sided α value of .05 and aimed for 90% power. Hence, we aimed to enroll 1000 subjects per arm.

The primary end point was reported as the cumulative incidence by treatment arm and analyzed using a competing events analysis (Fine–Gray model) with time to event as dependent outcome, study arm as independent variable, and death as potential competing event. Stratification variables and moderate to-strong predictors of the outcome were included as covariates. These included site of enrollment, age (categorized as 60–69, 70–79 or ≥80 years), and the comorbid conditions cardiovascular disease (including hypertension), diabetes mellitus (type 1 or 2), and chronic pulmonary disease. The effect was reported as a hazard ratio with 98.2% confidence interval (CI) for the primary end point and 95% CI for other end points. Data was analyzed using R software, version 4.0.3 [10]. Ex vivo stimulation and serology experiments were analyzed using the Mann–Whitney *U* test. Calculations were performed with GraphPad

Prism 8.3.0 software. For more details on the statistical analysis, see the Supplementary Materials.

RESULTS

Between 16 April and 14 May 2020, participants were randomized to vaccination with BCG vaccine (n = 1008) or placebo (n = 1006) (Figure 1). The enrollment coincided with the first COVID-19 wave in the Netherlands. The median age of the study population (interquartile range [IQR]) was 67 (64–72 years), and 47.5% of participants identified as female. Baseline characteristics were similar between the 2 arms (Table 1); 29.2% of the BCG-vaccinated individuals

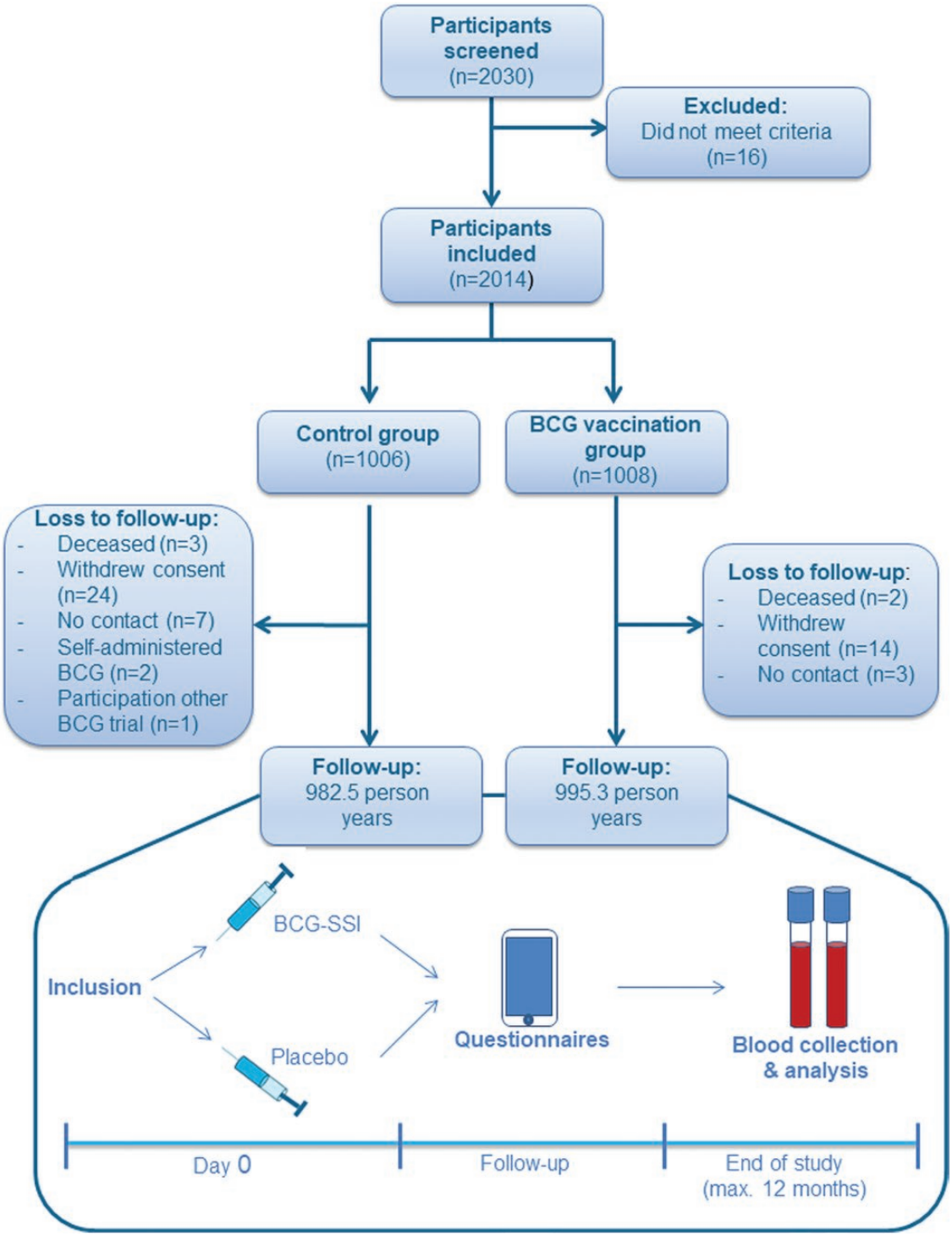

Figure 1. Enrollment of participants, randomization, and follow-up.

Table 1. Demographic Characteristics of Study Participants

Variable	Participants, No. (%)[a]	
	Placebo Group (n = 1006)	BCG Vaccine Group (n = 1008)
Demographic characteristics		
Age, median (IQR), y	67 (64–72)	67 (64–72)
Age category		
60–69 y	644 (64.0)	646 (64.1)
70–79 y	304 (30.2)	304 (30.2)
≥80 y	58 (5.8)	58 (5.8)
Sex		
Male	542 (53.9)	516 (51.2)
Female	464 (46.1)	492 (48.8)
BMI, median (IQR)[b]	25 (23.2–27.5)	25.1 (23.1–27.6)
Medical history		
Comorbid conditions		
Cardiovascular disease	194 (19.3)	176 (17.5)
Hypertension	313 (31.1)	303 (30.1)
Diabetes	66 (6.6)	71 (7.0)
Asthma	64 (6.4)	56 (5.6)
Other pulmonary disease	36 (3.6)	30 (3.0)
Renal disease	21 (2.1)	15 (1.5)
Allergic rhinitis	232 (23.1)	226 (22.5)
Medication use		
Any medication use	702 (69.8)	696 (69.1)
No. of medications used daily, median (IQR)	3.0 (1.0–5.0)	2.0 (1.0–4.0)
Smoking history		
Never smoked	358 (35.6)	350 (34.7)
Past smoking	594 (59.1)	602 (59.7)
Current smoking	49 (4.9)	53 (5.3)
Secondhand smoke exposure	4 (0.4)	3 (0.3)
BCG vaccination history		
Unknown	207 (20.6)	173 (17.2)
No	543 (54.0)	540 (53.6)
Yes	256 (25.4)	294 (29.2)
Time since vaccination, median (IQR), y	49 (44.0–57.0)	49 (44.0–54.0)
Vaccines in past year, no.		
Total	756	719
Live	5	4
Nonlive	751	715
Social characteristics		
Employment status		
Employed	341 (33.9)	324 (32.1)
Healthcare personnel	62 (6.2)	72 (7.1)
Retired	620 (61.6)	644 (63.9)
Unemployed	45 (4.5)	40 (4.0)

Abbreviations: BMI, body mass index; CI, confidence interval; IQR, interquartile range.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]BMI calculated as weight in kilograms divided by height in meters squared.

and 25.4% of the control group had a history of BCG vaccination before study enrollment. None of the participants had documented SARS-CoV-2 infection before randomization. In total, 95.8% of all participants completed the entire follow-up, resulting in 995.3 and 982.5 total person-years of follow-up in the BCG vaccine and placebo groups, respectively (Figure 1). Between month 7 of follow-up and the end of the study, 1630 participants received ≥1 dose of a COVID-19–specific vaccine (Supplementary Figure 1).

The cumulative incidence of clinically relevant RTIs requiring medical intervention was 0.029 in the BCG vaccine group (29 cases) and 0.024 in the placebo group (24 cases) during the 12-month follow-up (subdistribution hazard ratio [SHR], 1.26 [98.2% CI: .65–2.44]) (Figure 2A and Supplementary Figure 2). In addition, the incidence of SARS-CoV-2 infection was 5.1% in the BCG vaccine (n = 51) and 4.8% in the control (n = 48) group (SHR, 1.053 [adjusted 95% CI: .71–1.56]) (Figure 2B). No differences were observed in the incidence of RTIs irrespective of medical intervention (n = 351 in the BCG vaccine group and n = 338 in the placebo group; SHR, 1.055 [95% CI: .91–1.23]) (Table 2). Only 3 participants were hospitalized for COVID-19, and 1 participant died of COVID-19 (Table 2).

First episodes of self-reported symptoms associated with infection (severity score, ≥3) occurred in 647 (64.2%) of the participants in the BCG vaccine group and 611 (60.7%) in the control group (SHR, 1.11 [95% CI: .994–1.239]). No significant differences were observed between the BCG vaccine and placebo groups regarding the cumulative incidence of any of the self-reported symptoms. The total number of days on which participants reported symptoms (severity score, ≥3) was 9183 days in the BCG vaccine and 9630 days in the placebo group (risk ratio, 0.99 [95% CI: .84–1.17]). The total number of days of dyspnea was significantly lower in the BCG vaccine group (risk ratio, 0.48 [95% CI: .26–.88]). No differences were found in the total number of days of any other self-reported symptom (Table 3). In a post hoc analysis of the effect of BCG vaccine on the primary end point, the incidence of SARS-CoV-2 infection, and the incidence and duration of cough and dyspnea, no significant differences were observed among subgroups defined according to age, sex, prior BCG vaccination, or the presence of comorbid conditions (Supplementary Tables 2–7).

Safety Results

Injection site reactions, fatigue, myalgia, fever, and headache were more common in the BCG vaccine group than in the placebo group on days 7 and 14 after vaccination. In 2 participants, a local abscess developed at the injection site; a conservative approach was followed, and the abscesses resolved without any treatment. The incidences of serious adverse events were similar in the BCG vaccine and placebo groups (Supplementary Table 8), and these events were considered to be unrelated to the vaccine.

Immune Cellular Response

In a subgroup of participants (55 placebo and 50 BCG vaccine recipients), we assessed ex vivo cytokine responses to influenza

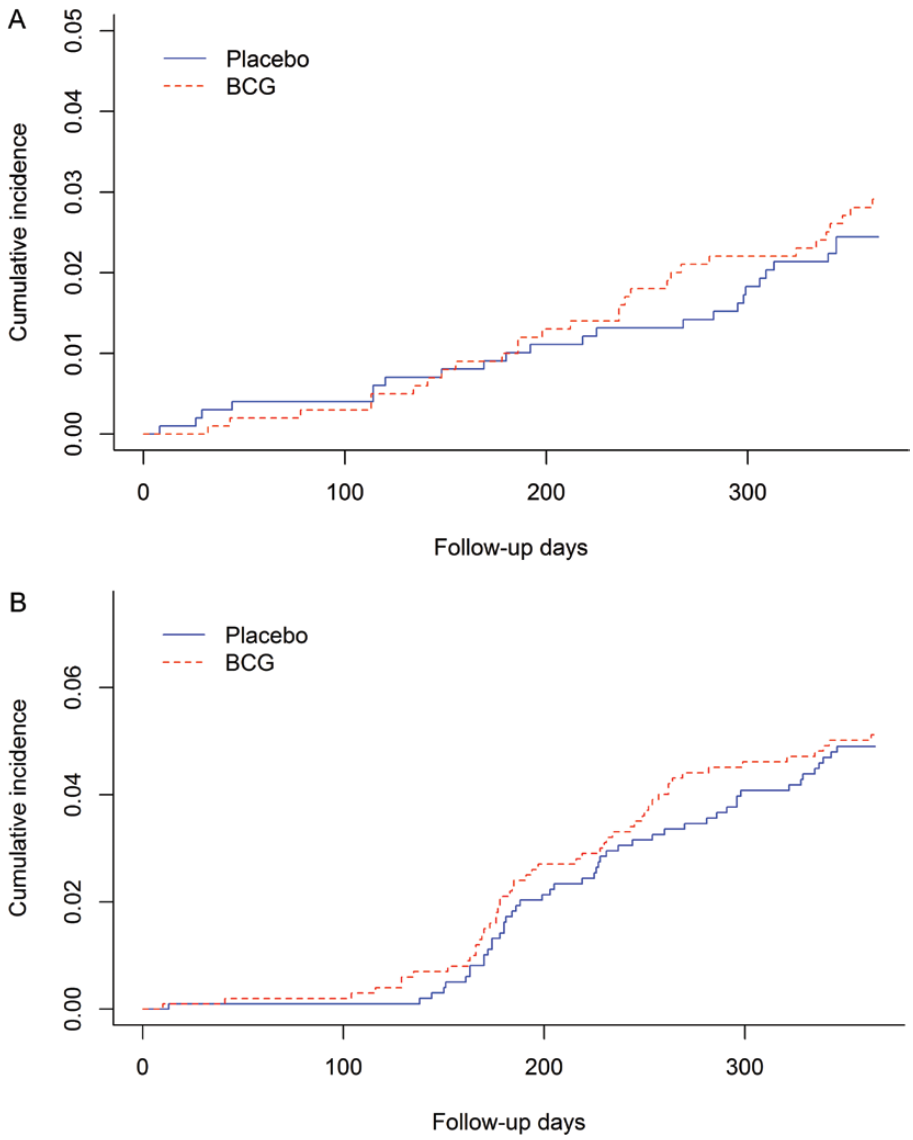

Figure 2. Cumulative incidence of respiratory tract infections (RTIs) requiring medical intervention and of documented severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. *A,* Cumulative incidence of clinically relevant RTIs, defined as new onset or sudden aggravation of preexisting symptoms, as reported by the participant or documented in medical records, including ≥1 respiratory symptom and ≥1 systemic symptom that required medical intervention within a span of 5 days. After medical assessment in primary or secondary care, intervention was defined as initiation of antibiotic, antiviral or corticosteroid treatment, adaptation of pulmonary maintenance medication, or hospitalization. *B,* Cumulative incidence of documented SARS-CoV-2 infections diagnosed using polymerase chain reaction test and nasopharyngeal swab samples.

A H1N1 California strain and SARS-CoV-2 Wuhan Hu-1 strain at the end of the study (month 12). The production of proinflammatory cytokines by peripheral blood mononuclear cells stimulated with influenza was significantly lower in the placebo than in the BCG vaccine group, with median (IQR) values as follows: interleukin 6 (IL-6), 12 550 (8396–20 237) and 17 285 (10 626–30 667) pg/mL in the placebo and BCG vaccine groups respectively ($P = .04$); interleukin 1β (IL-1β), 307.1 (144.9–553.8) and 467.4 (303.5–720.0) pg/mL ($P = .03$); and tumor necrosis factor (TNF) α, 146.5 (106.3–244.1) and 196.2 (154.7–256.8) pg/mL ($P = .02$) (Figure 3A). Furthermore, in participants without any documented SARS-CoV-2 infection (40 in the placebo and 36 in the BCG vaccine group), the production of IL-6 was significantly lower on exposure to SARS-CoV-2 in the placebo than in the BCG vaccine group (median [IQR], 1196 [782.9–1560] in the placebo vs 1646 [1109–3121] pg/mL in the BCG vaccine group; $P = .048$) (Figure 3B). In contrast, no difference was observed between placebo and BCG vaccine groups in the production of TNF-α, IL-1β, or interferon (IFN) γ after stimulation with SARS-CoV-2 (Figure 3A and 3B).

Antibody Responses

In 30 participants (15 placebo and 15 BCG vaccine recipients) who tested positive for SARS-CoV-2 between September 2020

Table 2. Secondary End Points

Secondary End Points	Placebo Group		BCG Vaccine Group		SHR (95% CI)[a]
	Events, No.	Cumulative Incidence (95% CI)	Events, No.	Cumulative Incidence (95% CI)	
Documented SARS-CoV-2 infection	48	0.049 (.037–.064)	51	0.051 (.039–.066)	1.053 (.710–1.562)
Resulting in death	0	0.000	1	0.001 (.000–.006)	NA
Resulting in ICU admission	1	0.001 (.000–.006)	0	0.000	NA
Resulting in hospital stay	1	0.001 (.000–.006)	2	0.002 (.000–.007)	1.978 (.165–23.773)
Self-reported RTI irrespective of medical intervention	338	0.345 (.315–.376)	351	0.355 (.325–.386)	1.055 (.909–1.225)

Abbreviations: CI, confidence interval; ICU, intensive care unit; NA, not applicable; RTI, respiratory tract infection; SARS, severe acute respiratory syndrome coronavirus 2; SHR, subdistribution hazard ratio.

[a]SHRs are adjusted for stratification variables (site and age category) and for cardiovascular disease (including hypertension), diabetes, and chronic pulmonary disease.

and February 2021, immunoglobulin (Ig) responses against SARS-CoV-2–stabilized trimeric spike glycoprotein, the nucleocapsid protein, and the receptor-binding domain were quantified at the end of the study. Age, sex, and time between positive test result and antibody measurement were balanced between BCG vaccine and placebo groups ($P = .52$, $P > .99$, and $P = .27$, respectively). Compared with placebo recipients, BCG-vaccinated participants had significantly higher concentrations of IgG antibodies against spike protein and receptor-binding domain (Figure 3C) and IgM antibodies against spike protein (Supplementary Figure 3) (both $P < .05$; Mann–Whitney U test.)

DISCUSSION

The data presented in this study do not support the hypothesis that BCG vaccination reduces the incidence of RTIs after RTIs please insert: during the COVID-19 pandemic in older adults living in a Western European country. Similarly, no effect was observed on the incidence of SARS-CoV-2 infection or any of the other secondary clinical end points. Exaggerated inflammatory reactions, characterized by an increased concentration of circulating cytokines, have been reported to contribute to COVID-19 severity [11]. We argue that when BCG vaccine is administered to a healthy individual it will elicit a fast and robust immune response that reduces viremia and levels of systemic inflammation, as has been demonstrated in a previous BCG vaccination study involving young adults [12]. In support of this, results of the current study indicate that BCG vaccination is safe in older adults, as no differences in the incidence of serious adverse events was observed between the BCG vaccine and placebo groups. BCG-vaccinated individuals were not more frequently admitted to the hospital because of SARS-CoV-2 infection, indicating that recent BCG vaccination is not associated with increased COVID-19–related morbidity in older adults.

In contrast to our current findings, several smaller studies performed in Indonesia, Japan, South Africa, and Greece observed a protective effect of BCG vaccine on the incidence of RTIs in adults [6, 13–16]. The difference in effects between our study and others may have several causes. One could have been the difference between first vaccination with BCG vaccine and

Table 3. Exploratory End Points

Symptom	No. of Symptomatic Days per 1000 Follow-up Days		RR (95% CI)
	Placebo Group	BCG Vaccine Group	
Sickness	12.76	13.07	1.08 (.92–1.26)
Coughing	4.54	5.82	1.29 (.94–1.75)
Sore throat	2.86	3.19	1.18 (.88–1.58)
Rhinorrhea	6.70	6.57	1.01 (.79–1.28)
Dyspnea	5.66	4.04	0.48 (.26–.88)
Loss of smell and taste	2.67	1.36	0.51 (.20–1.51)
Fever	0.92	0.96	1.17 (.83–1.65)
Myalgia	4.70	3.06	0.79 (.56–1.10)
Shivering	1.03	1.07	1.00 (.70–1.42)
Fatigue	10.44	8.57	0.94 (.72–1.22)
Headache	5.01	4.97	1.08 (.84–1.38)
Diarrhea	2.01	1.71	1.04 (.76–1.41)

Abbreviations: CI, confidence interval; RR, risk ratio.

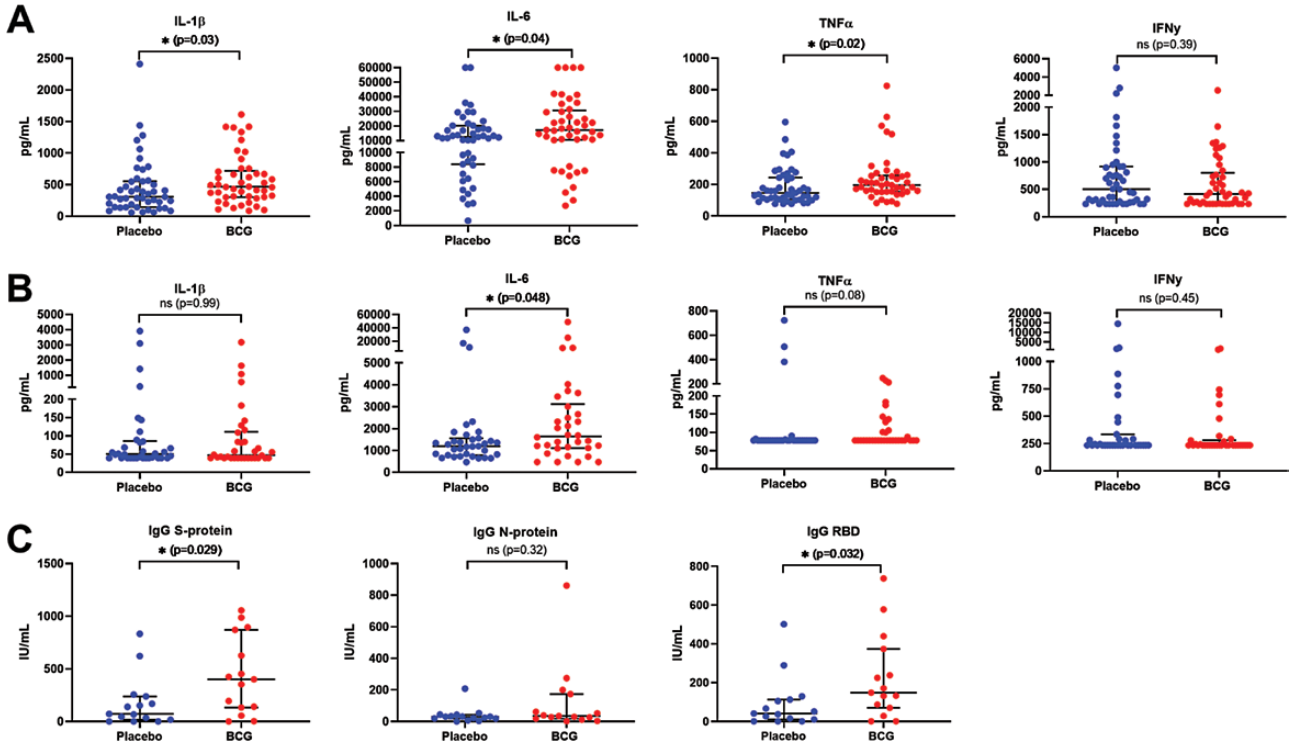

Figure 3. Innate cytokine and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody responses. *A,* Blood samples were obtained from participants who had received BCG vaccine (n = 50) or placebo (n = 55) 12 months earlier. Peripheral blood mononuclear cells (PBMCs) were isolated and stimulated ex vivo with influenza for 24 hours or 7 days. Values represent production by PBMCs of interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF) α after 24-hour stimulation and interferon (IFN) γ after 7-day stimulation with influenza H1N1 California strain. *A–C,* Values represent medians with interquartile ranges. *$P < .05$ (Mann–Whitney *U* test); NS, not significant. *B,* PBMCs from participants without any previous documented SARS-CoV-2 infection (40 in the placebo and 36 in the BCG vaccine group) were stimulated ex vivo with SARS-CoV-2 Wuhan Hu-1 strain. IL-1β, IL-6, and TNF-α levels were determined after 24 hours and IFN-γ after 7 days of stimulation. *C,* Immunoglobulin (Ig) G responses against SARS-CoV-2 spike (S) glycoprotein, the nucleocapsid (N) protein, and the receptor-binding domain (RBD) from participants (15 each in the placebo and BCG vaccine groups) who tested positive for SARS-CoV-2 infection with polymerase chain reaction between September 2020 and February 2021 and had received BCG vaccine or placebo 12 months earlier.

revaccination; however, we found no indication of an effect of previous BCG vaccination in our study, although only about 25% in our BCG vaccine group had been vaccinated previously. Another possible explanation for these differences might be the dose of the vaccine; we used the standard dose, while Wardhana et al [13] vaccinated volunteers with BCG vaccine once a month for 3 consecutive months, with a resulting reduction in RTIs, compared with placebo recipients [13]. In addition, improved immune responses have been reported with repeated doses of BCG vaccine, compared with a single dose [17].

Furthermore, it is known that the immunogenicity of BCG vaccine is significantly influenced by the vaccine strain used [18]. Other causes may include the different genetic backgrounds of the populations or differences in the epidemiology of RTIs [19] during the lockdowns caused by the pandemic. Indeed, BCG vaccine has previously been shown to strongly protect against influenza in mice [20], while the incidence of influenza was extremely low during the study period. The most likely explanation, however, for the differences observed between earlier trials and the current study is that the immune pathways activated by BCG vaccine–induced trained immunity may be involved to a lesser extent in host defense against COVID-19; indeed, the cellular assays performed in this study demonstrated a stronger amplification of the cytokine response to influenza virus in BCG-vaccinated volunteers, compared with the modestly improved response to SARS-CoV-2 (Figure 3). The lack of effect of BCG vaccine on the incidence of COVID-19 in a developed country is supported by results of the BCG-CORONA study, which showed no effect of BCG on the incidence of COVID-19 among healthcare workers (ten Doesschate T et al, submitted). It has to be underlined that no conclusions can be drawn on the impact of BCG vaccine on severity of COVID-19, for which larger studies or meta-analyses will be needed.

BCG vaccination has been reported to boost the function of innate immune cells, which is correlated with a decrease in experimental human viremia [12]. Studies on the use of intravesical BCG vaccine instillations for bladder cancer also found an increase in IL-6 production capacity and increased protection against viral infections [21–23]. In line with this, BCG-vaccinated volunteers in this study reacted to influenza stimulation with stronger monocyte-derived cytokine production (a hallmark of trained immunity) than to

placebo-vaccinated individuals, while T-cell–derived IFN-γ production was similar. In contrast, BCG vaccination resulted only in an increased IL-6 response after SARS-CoV-2 stimulation, with no significant increase observed in the production of IL-1β, TNF-α, or IFN-γ. The absence of a strong trained immunity effect of BCG vaccination on stimulation with SARS-CoV-2 (in contrast to influenza stimulation) may partially explain the lack of effect on susceptibility to COVID-19. It is important to point out that differences in cytokine production induced by BCG vaccination in the current study are in line with findings of earlier investigations [12, 24] and on the same order of magnitude as other conditions in which inflammatory processes play an important role [25].

Interestingly, among BCG-vaccinated participants with COVID-19 diagnosed, we observed higher concentrations of IgG antibodies than in placebo-vaccinated volunteers. These findings are in line with previous reports suggesting that BCG vaccine modulates humoral responses to vaccinations: BCG vaccination has been associated with significantly higher concentrations of antibodies in response to various vaccines directed against viral infections, including hepatitis B and influenza [26–28]. Such induction of high antibody responses may extend the duration of detectable titers and protection [29]; neutralizing antibody concentrations are correlated with protection against viral infections [30], and a 2021 study indicated that neutralizing antibody concentrations may be highly predictive of protection against SARS-CoV-2 [31]. It is therefore possible that BCG vaccine may enhance the host's immune response against SARS-CoV-2 infection and improve the duration of protection in older adults. The mechanism leading to this effect of BCG vaccine on antibody responses is likely to include the increased IL-6 release on SARS-CoV-2 stimulation: IL-6 increases antibody production by promoting B-cell helper capabilities of $CD4^+$ T cells through increased interleukin 21 production [32, 33]. Future studies are warranted to investigate the effect of BCG vaccine on SARS-CoV-2 vaccines and strategies to optimally exploit the vaccine's immunomodulatory effects.

There are also limitations of the current study. First, it was not possible to completely blind participants, because the local scar often produced by BCG vaccination is different from the response to placebo. However, no differences in loss to follow-up or administration of COVID-19–specific vaccines were observed between the groups. Second, we were not able to perform immune measurements and serology at baseline, owing to the crisis situation of the first wave of COVID-19 when recruitment was initiated, and future studies should include prevaccination immune assays. However, we expect that the number of participants with a previous nondocumented SARS-CoV-2 infection was low and equally distributed between the BCG vaccine and placebo groups at study enrollment. Third, at the start of the study, COVID-19 testing was available only for healthcare workers and hospitalized patients, and it is known that asymptomatic infections occur. Therefore, the reported number of COVID-19 cases may be lower than the actual value, although it is presumed to be similar for the BCG vaccine and placebo groups. Finally, during the last 4 months of follow-up, the majority of participants received ≥1 COVID-19–specific vaccine, which may have had an effect on the number of RTIs due to SARS-CoV-2 infections. COVID-19–specific vaccination was balanced between the BCG vaccine and placebo arms.

In conclusion, BCG vaccination did not protect against RTIs and COVID-19 in a Western European population of older adults during the COVID-19 pandemic. While BCG vaccination was safe, only subtle (albeit statistically significant) improvements in ex vivo cytokine responses to influenza and SARS-CoV-2 were observed. However, compared with placebo, BCG vaccination improved IL-6 and humoral responses against SARS-CoV-2 after COVID-19. Future studies should investigate whether similar effects are found in populations with different backgrounds and identify the implications for vaccination strategies in older adults.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank our participants for their dedication to the study; the members of the data and safety monitoring committee (Miquel Ekkelenkamp, Maarten van Smeden, Jan-Jelrik Oosterheert); the students (Stijn Donker, Anna Hartman, and Lucas Huijs) for assisting with the execution of the trial; members of the Radboud Technology Center Clinical Studies (Karin Saini, Emma Lenssen, Demi Schaminee, Esther Eggenhuizen, Marjolein Eybergen, Sanne Houba, Eline Reuvers, Eveline Otters, and Marlon Schimmel) for their work during the inclusion and vaccination of all participants; Katharina Goessling and Ortwin Adams for kindly helping for production of the viral stimuli; the other PhD students (Katrin Rabold, Julia van Tuijl, Freek van de Schoor, Viola Kluck, Özlem Bulut, Priya Debisarun, Gizem Kilic, and Büsranur Geckin), technicians, and colleagues from the Radboud Department of Internal Medicine, who helped in various ways during this trial; and the senior union KBO-Brabant.

Financial support. This work was investigator initiated and supported by the Radboud University Medical Center and the University Medical Center (UMC) Utrecht, Emergent Ventures (unconditional fast grant), the Mercator Center, George Mason University, Willem Bakhuys Roozeboomstichting, the European Research Council (advanced grant number 833247), and the Netherlands Organization for Scientific Research (Spinoza grant to M. G. N.).

Potential conflicts of interest. M. J. B. is principal investigator of the HERALD study, sponsored by CureVac (no personal payments) and coordinated in the Netherlands by UMC Utrecht ("A Phase 2b/3, Randomized, Observer-Blinded, Placebo-Controlled, Multicenter Clinical Study Evaluating the Efficacy and Safety of Investigational SARS-CoV-2 mRNA Vaccine CVnCoV in Adults 18 Years of Age and Older"). M. J. B. is principal investigator for the Netherlands (2020–; no personal payments) and UMC Utrecht is a study site in the VAC31518COV2001 study, a phase 2 randomized double-blind placebo-controlled multicenter study of the

coronavirus disease 2019 (COVID-19) vaccine, sponsored by Janssen Vaccines. M. J. B. is principal investigator for UMC Utrecht (2020–; no personal payments) and chair of the international steering committee for the EMBRACE study (sponsored by Janssen Vaccines, with payments to UMC Utrecht; 2017–). M. J. B. also serves on a pneumococcal vaccination (international) advisory board for Merck Sharp & Dohme (payments to UMC Utrecht; 2020–), a COVID-19 treatment advisory board (national) for AstraZeneca (payments to UMC Utrecht; 2021), a pneumococcal vaccination (international) advisory board for Pfizer (payments to UMC Utrecht; 2021), and an independent data monitoring committee for the PSK008 and PSK009 studies on pneumococcal vaccination (sponsored by Sanofi Pasteur, with payments to UMC Utrecht; 2019–). Finally, M. J. B. received payment or honoraria from Takeda (November 2019). C. H. v. W. reports payment to their institution, outside the submitted work, from Pfizer, Biomerieux, and Da Volterra; consulting fees paid to their institution from MSD/Merck and Sanofi-Pasteur; European patent application 19 306720.4 with Da Volterrra, University Antwerp, and UMC Utrecht Holding BV; in-kind contribution and test equipment to institution from Biomerieux; and in-kind contribution from Da Volterra. M. G. N. is scientific founder and on the scientific advisory board of TTxD, is scientific founder of Lemba Therapeutics and has obtained research grants from GSK Biologicals, TTxD, and Ono Pharma and consultancy fees from TTxD. The UMC Utrecht has obtained research grants from Pfizer, Biomerieux, and Da Volterra. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Pera A, Campos C, López N, et al. Immunosenescence: implications for response to infection and vaccination in older people. Maturitas **2015**; 82:50–5.
2. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature **2020**; 584:430–6.
3. Moorlag S, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect **2019**; 25:1473–8.
4. Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis **2011**; 204:245–52.
5. Netea MG, Dominguez-Andres J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. Nat Rev Immunol **2020; 20:375–88**.
6. Giamarellos-Bourboulis EJ, Tsilika M, Moorlag S, et al. Activate: randomized clinical trial of BCG vaccination against infection in the elderly. Cell **2020**; 183:315–323.e9.
7. Kleinnijenhuis J, Quintin J, Preijers F, et al. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci USA **2012**; 109:17537–42.
8. Fröberg J, Gillard J, Philipsen R, et al. Elevated mucosal antibody responses against SARS-CoV-2 are correlated with lower viral load and faster decrease in systemic COVID-19 symptoms. medRxiv [Preprint: not peer reviewed]. 3 February 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.02.02.21250910v1.full.
9. den Hartog G, Schepp RM, Kuijer M, et al. SARS-CoV-2-specific antibody detection for seroepidemiology: a multiplex analysis approach accounting for accurate seroprevalence. J Infect Dis **2020**; 222:1452–61.
10. R Core Team. R: a language and environment for statistical computing. Available at: https://www.R-project.org/. Accessed 8 August 2021.
11. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet **2020**; 395:497–506.
12. Arts RJW, Moorlag S, Novakovic B, et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe **2018**; 23:89–100.e5.
13. Wardhana EA, Datau EA, Sultana A, Mandang VV, Jim E. The efficacy of bacillus Calmette-Guerin vaccinations for the prevention of acute upper respiratory tract infection in the elderly. Acta Med Indones **2011**; 43:185–90.
14. Ohrui T, Nakayama K, Fukushima T, Chiba H, Sasaki H. Prevention of elderly pneumonia by pneumococcal, influenza and BCG vaccinations [in Japanese]. Nihon Ronen Igakkai Zasshi **2005**; 42:34–6.
15. Nemes E, Geldenhuys H, Rozot V, et al. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. N Engl J Med **2018**; 379:138–49.
16. Tsilika M, Taks E, Dolianitis K, et al. ACTIVATE-2: a double-blind randomized trial of BCG vaccination against COVID19 in individuals at risk. medRxiv [Preprint: not peer reviewed]. 24 May 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.05.20.21257520v1.
17. Husain AA, Kashyap RS, Kalorey DR, et al. Effect of repeat dose of BCG vaccination on humoral response in mice model. Indian J Exp Biol **2011**; 49:7–10.
18. Ritz N, Hanekom WA, Robins-Browne R, Britton WJ, Curtis N. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. FEMS Microbiol Rev **2008**; 32:821–41.
19. Jones N. How COVID-19 is changing the cold and flu season. Nature **2020**; 588:388–90.
20. Spencer JC, Ganguly R, Waldman RH. Nonspecific protection of mice against influenza virus infection by local or systemic immunization with bacille Calmette-Guerin. J Infect Dis **1977**; 136:171–5.
21. De Boer EC, De Jong WH, Steerenberg PA, et al. Induction of urinary interleukin-1 (IL-1), IL-2, IL-6, and tumour necrosis factor during intravesical immunotherapy with bacillus Calmette-Guérin in superficial bladder cancer. Cancer Immunol Immunother **1992**; 34:306–12.
22. Chen FH, Crist SA, Zhang GJ, Iwamoto Y, See WA. Interleukin-6 production by human bladder tumor cell lines is up-regulated by bacillus Calmette-Guérin through nuclear factor-κB and Ap-1 via an immediate early pathway. J Urol **2002**; 168:786–97.
23. Gallegos H, Rojas PA, Sepúlveda F, Zúñiga A, San Francisco IF. Protective role of intravesical BCG in COVID-19 severity. BMC Urol **2021**; 21:50.
24. Koeken VACM, de Bree LCJ, Mourits VP, et al. BCG vaccination in humans inhibits systemic inflammation in a sex-dependent manner. J Clin Invest **2020**; 130:5591–602.
25. Bekkering S, van den Munckhof I, Nielen T, et al. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. Atherosclerosis **2016**; 254:228–36.
26. Zimmermann P, Curtis N. The influence of BCG on vaccine responses—a systematic review. Expert Rev Vaccines **2018**; 17:547–54.
27. Leentjens J, Kox M, Stokman R, et al. BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. J Infect Dis **2015**; 212:1930–8.
28. Zimmermann P, Donath S, Perrett KP, et al. The influence of neonatal bacille Calmette-Guérin (BCG) immunisation on heterologous vaccine responses in infants. Vaccine **2019**; 37:3735–44.
29. Van Damme P. Long-term protection after hepatitis B vaccine. J Infect Dis **2016**; 214:1–3.
30. Tsang JS. Utilizing population variation, vaccination, and systems biology to study human immunology. Trends Immunol **2015**; 36:479–93.
31. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med **2021; 27:1205–11**.
32. Maeda K, Mehta H, Drevets DA, Coggeshall KM. IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood **2010**; 115:4699–706.
33. Dienz O, Eaton SM, Bond JP, et al. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by $CD4^+$ T cells. J Exp Med **2009**; 206:69–78.