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ORIGINAL ARTICLE

BCG Vaccination at Birth and Rate of Hospitalization for Infection Until 15 Months of Age in Danish Children: A Randomized Clinical Multicenter Trial

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Background. The bacillus Calmette-Guérin (BCG) vaccine against tuberculosis might reduce the non–tuberculosis-related child mortality rate in low-income settings. We tested the hypothesis that BCG vaccination at birth would reduce early childhood hospitalization for infection in Denmark, a high-income setting. Hospitalization for infection was a secondary outcome in a randomized trial with the primary aim to estimate the potential non-specific effects of BCG vaccination at birth on all-cause hospitalization.

Methods. A total of 4262 children included in the Danish Calmette Study were assigned randomly to either receive the BCG vaccine or not and were followed through the Danish National Patient Register. The outcome was number of hospitalizations for infection until the age of 15 months. Data were analyzed by Cox regression in intention-to-treat (ITT) and per-protocol (PP) analyses.

Results. In the ITT analysis, we observed 588 hospitalizations for infection (mean, 0.28 hospitalization per child) among the 2129 children allocated to receive the BCG vaccine and 595 hospitalizations for infection (mean, 0.28 hospitalization per child) among the 2133 children allocated to the control group (hazard ratio [HR], 0.99 [95% confidence interval (CI), 0.85–1.15]). The PP analysis yielded an HR of 1.00 (95% CI, 0.86–1.16). Predefined interaction ITT analyses showed that among 740 children with a BCG-vaccinated mother, the HR for BCG-vaccinated children was 0.65 (95% CI, 0.45–0.94); the HR for children who had a non–BCG-vaccinated mother was 1.10 (95% CI, 0.93–1.29) ($P = .01$, test of no interaction). Cesarean delivery modified the effect of BCG vaccination (HRs, 0.73 [95% CI, 0.54–0.99] in children born by cesarean section vs 1.10 [95% CI, 0.92–1.30] in other children; $P = .02$). When the outcome was defined as time to first hospitalization, the HR for premature children after BCG vaccination was 1.81 (95% CI, 0.95–3.43), whereas the HR was 0.94 (95% CI, 0.82–1.08) for children born at term ($P = .05$).

Conclusion. BCG vaccination did not affect the rate of hospitalization for infection up to the age of 15 months in Danish children. In future studies, the role of maternal BCG-vaccination, premature birth, and cesarean delivery needs further exploration.

Keywords. Bacillus Calmette-Guérin (BCG); children; hospitalization for infection; nonspecific effect of immunization.

Randomized controlled trials from low-income high-mortality-rate settings in West Africa have found that the live attenuated bacillus Calmette-Guérin (BCG) vaccine against tuberculosis has been associated with decreased non–tuberculosis-related child death [1, 2], and a recent review of nonspecific effects of the BCG vaccine suggested possible beneficial effects on the all-cause child mortality rate in low-income settings [3]. Child deaths in low-income settings are caused, to a large degree, by infectious diseases, and the studies in West Africa found that the BCG vaccine protected particularly against neonatal sepsis and pneumonia [1, 2, 4]. This potential beneficial "nonspecific" or "heterologous" effect of BCG vaccination might be explained by training of the innate immune system, which increases its capability to respond to nonrelated pathogens [5, 6].

The Danish Calmette Study, a large randomized controlled multicenter trial described in detail elsewhere [7], tested the primary hypothesis that BCG vaccination at birth would reduce the all-cause hospitalization rate in a high-income setting but found no protective effect of BCG vaccination [8].

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Because all-cause hospitalization might not be sufficiently specific to detect a potential beneficial protection of the BCG vaccine against infections, a secondary outcome was hospitalization for infection. In our study, we examined whether BCG vaccination within 7 days after birth affected the rate of hospitalization for infection in children up to the age of 15 months.

METHODS

The Danish Calmette Study

The Danish Calmette Study included 4262 Danish newborns and allocated the children 1:1 to intradermal vaccination with 0.05 ml of Danish BCG strain 1331 or no intervention within 7 days of birth [7]. Because nonspecific effects of vaccines might be stronger among the smallest newborns [1], the allocation was stratified according to prematurity, defined as a gestational age of <37 weeks. Children from multiple births were allocated to the same randomization group based on the random assignment of the first-born child. Information on baseline characteristics was collected using structured telephone interviews.

Outcome Definition

The outcome was hospitalizations for infection between vaccination/randomization and 15 months of age, the time at which the measles, mumps, and rubella vaccine is scheduled by the Danish national childhood vaccination program [9]. Data on hospitalizations for infection were obtained from the Danish National Patient Register [10, 11], which keeps information that includes the dates of admission and discharge on all hospital contacts in Denmark categorized according to International Classification of Diseases, 10th Revision (ICD-10) diagnosis codes. Outpatient and emergency department hospital contacts that did not lead to admission and hospitalizations that started before or on the date of random assignment were excluded from our analysis. A hospitalization was defined as caused by infection if an ICD-10 code specific for infection was recorded as the primary diagnosis or among the secondary diagnoses. One child had a diagnosis of adenitis (I88). That child was allocated to receive the BCG vaccine at birth and was hospitalized with adenitis at the age of 3 months; we have no information from our interviews or clinical examinations to judge whether the adenitis was related to the BCG vaccination.

ICD-10 codes that define infectious diseases are presented in Supplementary Data.

Statistics

Cox proportional hazards models were used to estimate the hazard ratio (HR) of hospitalization for infection between the BCG and control groups. Hospitalizations for infections were analyzed as recurrent events, and hospitalization for infection ≥1 day from the latest discharge counted as a new event. In a sensitivity analysis, we tested whether the overall estimates changed if the new hospitalization was defined after >1, >3, >7, or >14 days from the latest discharge. The children were censored at migration, 15 months of age, or death, whichever came first. The analyses were stratified according to prematurity in accordance with the randomization procedure.

In the intention-to-treat analysis (ITT), hospitalizations for infection from random assignment to 15 months of age were analyzed according to randomization group. In the per-protocol (PP) analysis, 47 children (11 allocated to the BCG group, 36 allocated to the control group) who did not follow the allocation were excluded, and hospitalizations for infection from the time since vaccination for the BCG group and time since random assignment for controls were included in the analysis. The results are presented as HRs with 95% confidence intervals (CIs) and corresponding *P* values. *P* values of <.05 were considered statistically significant.

We also conducted the main analyses with the outcome defined as time to first hospitalization for infection.

Because of the randomized design, the unadjusted estimates were considered the main outcomes, but we also present the results of analyses adjusted for all baseline characteristics (ie, prematurity, sex, study site, cesarean delivery, antibiotics during delivery, multiple birth, birth weight, child age at random assignment, maternal age, parental ethnicity [≥1 parent of an ethnicity other than Danish], maternal education, parents living together, maternal BCG vaccination [according to maternal recall], siblings, maternal atopy, and maternal smoking during pregnancy). Adjustment for recurrent hospitalizations for infection for each child was made using robust standard errors for the estimated HRs. No children were excluded from the unadjusted analyses because of missing information, but children with missing information were excluded from the adjusted analyses. Curves for the mean number of hospitalizations according to randomization arm as a function of time since randomization were estimated using the Nelson–Aalen method. In addition, because the development of a scar is a marker of correct vaccine administration [12], a subanalysis compared BCG-vaccinated children with a BCG vaccine scar at 13 months of age with control children. The inclusion of children from multiple births as clusters in the analyses was tested. All analyses were performed using Stata 14 (StataCorp, College Station, Texas).

Potential Effect Modification: Analytical Strategy

In preplanned analyses using tests of no interaction, we estimated the potential effect of modification by the following baseline characteristics collected during the trial: prematurity, sex, study site, cesarean delivery, antibiotics during delivery, birth weight of <2500 g, number of siblings, parental atopy, season of randomization, maternal BCG vaccination, child age in days at randomization, and BCG vaccine batch. Because at 1 study site (Kolding, Denmark) short acute admissions were classified as outpatient consultations, and to examine if the BCG-vaccination effect might differ

according to the duration of admission, we tested the effect of BCG vaccination also for hospitalizations for infection with a duration of >1 day. Because potential nonspecific effects of a vaccine can be modified by subsequent vaccination with a different vaccine [13] and nonspecific effects of BCG vaccination on child mortality rates were found primarily in the first months of life before the children received other vaccines [1, 2], we analyzed whether the BCG-vaccination effects were similar between birth and 89 days of age, during which the children were supposed to not have received other vaccines, and between 3 and 15 months of age (90–456 days), a time in which the routine vaccination program recommends vaccination with the inactivated vaccines against diphtheria, tetanus, acellular pertussis, polio, and *Haemophilus influenzae* type b and pneumococcal conjugate vaccine at 3, 5, and 12 months of age [9]. The potential effect modifiers examined in our study were defined at birth or enrollment. Factors that changed over the follow-up period that might have been influenced by the parents' knowledge about their allocation (eg, breastfeeding) were not included in the effect-modifier analysis. The rates of breastfeeding did not differ between children allocated to the BCG group (57%) and those allocated to the control group (56%).

Ethics, Consent, and Permissions

This trial was approved by the Committees on Biomedical Research Ethics (De Videnskabsetiske Komiteer for Region Hovedstaden [see https://www.regionh.dk/vek]) (Journal number [J.no.] H-3-2010-087), the Danish Data Protection Board (J.no. 2009-41-4141), and the Danish Medicines Agency (J.no. 2612–4356; EudraCT 2010-021979-85; protocol 2009–323). This study was registered at ClinicalTrials.gov under identifier NCT01694108 and was monitored by good clinical practice units and an independent data safety monitoring board. All parents provided written informed consent.

RESULTS

Among the 4262 children included in the study, 2129 children were allocated to the BCG-vaccination group and 2133 to the control group.

Rates of Hospitalization for Infection

No overall difference between BCG-vaccinated and control children in the rates of hospitalization for infection (recurrent events) was observed in the ITT or PP analysis (Table 1, Figures 1 and 2), and adjustment for baseline characteristics essentially did not change the estimates (Table 1).

The subgroup of 1857 (87.7%) BCG-vaccinated children who developed a BCG scar had a HR of 0.98 (95% CI, 0.85–1.13) compared with control children. The inclusion of children from multiple births as clusters in the analyses did not change the estimates (data not shown).

The overall estimates remained essentially the same regardless of whether the new hospitalization for infection was defined after >1, >3, >7, or >14 days from the latest discharge (data not shown). If the outcome was defined as time to first hospitalization for infection instead of number of hospitalizations for infection, the HRs were 0.97 (95% CI, 0.85–1.11) (ITT) and 0.98 (95% CI, 0.86–1.13) (PP).

Effect Modification

Among prespecified potential effect modifiers, maternal BCG vaccination significantly modified the effect of the BCG vaccine; the HRs for BCG-vaccinated children were 0.65 (95% CI, 0.45–0.94) for those whose mother was BCG vaccinated and 1.10 (95% CI, 0.93–1.29) for those whose mother was not BCG vaccinated (P = .01, test of no interaction [ITT-analysis]) (Table 2). Adjustment did not change the estimates.

Cesarean delivery modified the effect of BCG vaccination; the HRs were 0.73 (95% CI, 0.54–0.99) for children born via cesarean delivery and 1.10 (95% CI, 0.92–1.30) in other children (P = .02). However, after adjustment for baseline characteristics, the effect was no longer significant (Table 2).

Maturity did not affect the estimates significantly (Table 2). However, when the outcome was defined as time to first hospitalization, the interaction between BCG vaccination and prematurity was borderline significant; the HR for premature children after BCG vaccination was 1.81 (95% CI, 0.95–3.43), whereas the HR was 0.94 (95% CI, 0.82–1.08) in those born at term (P = .05, test of no interaction [ITT analysis]).

Table 1. The Effect of BCG Vaccination at Birth on Number of Hospitalizations for Infection Among 4262 Danish Children Randomized to BCG Vaccination at Birth or No Intervention[a]

Analysis Type	BCG Vaccination[b]	Control[b]	HR	95% CI	Adjusted HR[c]	95% CI
Intention to treat (n = 4262)[d]	588 (2129) (0.28)	595 (2133) (0.28)	0.99	0.85–1.15	0.99	0.85–1.15
Per protocol (n = 4215)[e]	585 (2118) (0.28)	578 (2097) (0.28)	1.00	0.86–1.16	0.99	0.85–1.16

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; HR, hazard ratio.

[a]Intention to treat and per-protocol analyses with stratification according to prematurity in accordance with the randomization procedure.

[b]Values shown are the number of hospitalizations for infection, number of children included in the analysis (in parenthesis) and mean number of hospitalizations for infection per child (in parenthesis).

[c]Adjusted for base line characteristics (prematurity, sex, study site, cesarean delivery, antibiotics during delivery, multiple birth, birth weight, child age at randomization, maternal age, parental ethnicity (≥ 1 parent of other ethnicity than Danish), maternal education, parents living together, maternal BCG (by maternal recall), siblings, maternal atopy, and maternal smoking during pregnancy) using Cox regression. The analysis included n = 3953 children (93%) with full information.

[d]All children randomly assigned were followed from randomization to 15 months of age and analyzed according to randomization group using Cox regression.

[e]Children who completed the trial according to the protocol were included in the per-protocol (PP) analysis where children having received the BCG vaccine but randomized to control group, or vice versa were excluded. The remaining children were followed from BCG vaccination for the BCG group and randomization for the control group.

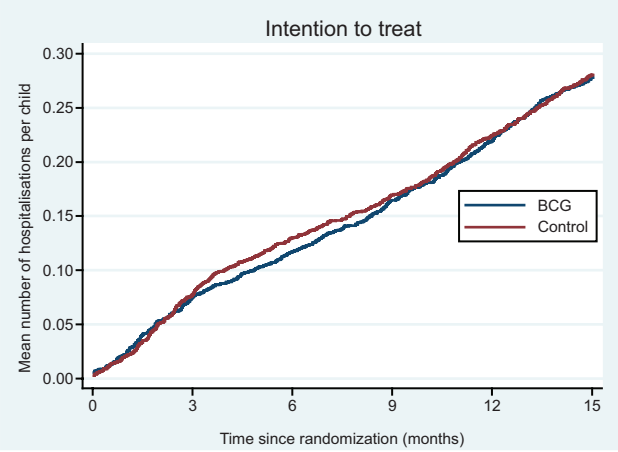

Figure 1. Mean number of hospitalizations for infection according to randomization arm as a function of time since random assignment among 4262 Danish children randomly assigned to either bacillus Calmette-Guérin vaccination at birth or no intervention (Nelson–Aalen method). Intention-to-treat analysis.

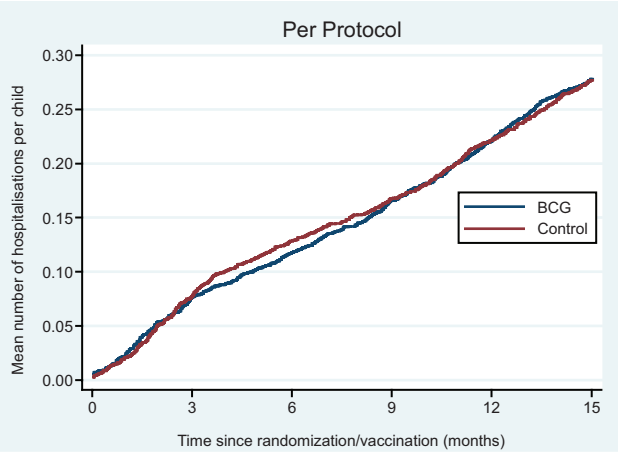

Figure 2. Mean number of hospitalizations for infection according to randomization arm as a function of time since random assignment among 4215 Danish children randomly assigned to either bacillus Calmette-Guérin vaccination at birth or no intervention (Nelson–Aalen method). Per-protocol analysis.

DISCUSSION

In line with our findings for the primary outcome of all-cause hospitalization [8], we found no beneficial effect of BCG vaccination on hospitalization for infection.

We found 3 potential effect modifiers, namely, maternal BCG vaccination, cesarean delivery, and prematurity.

Strengths and Weaknesses

The main strengths of this study were the randomized design, large sample size, and 100% follow-up (all Danish inhabitants are registered in the public registers). The results essentially remained the same regardless of whether the outcome was defined with a longer time span from the latest discharge or as time to first hospitalization.

Study weaknesses included a limited power to assess effect modification caused by small subgroups. This trial tested Danish BCG strain 1331. Because variations between strains regarding both specific and nonspecific effects have been observed [14, 15], our findings might not be generalizable to other BCG vaccine strains. Although partly addressed by the analysis according to duration of hospitalization, it is a limitation that there is no gold standard for when a child is hospitalized, which might be influenced by registration practice and, for example, the child's age, the physician's experience, routine, and attitude, and parental socioeconomic status.

Our information on maternal BCG vaccination was based on maternal recall; information on age at vaccination and BCG vaccine strain was not available. The public Danish health registers do not go back to the 1970s, which was when some mothers in this cohort received their BCG vaccination, and the recall information therefore could not be verified. The age of routine BCG vaccination in Denmark, before it was phased out beginning in 1979, was mostly at school entry, around 6 to 7 years of age, and it is most likely that these women received Danish BCG strain 1331. However, nearly 40% of BCG-vaccinated mothers in our trial were from families of an ethnicity other than Danish, and no information about maternal age at vaccination or BCG strain was available.

Because of these limitations and because the results were not corrected for multiplicity, the interaction analyses should be interpreted cautiously.

Interpretation

The trial results disproved the hypothesis that BCG vaccination reduces the overall rate of hospitalization for infection. This finding is in line with the primary study outcome [8] and is corroborated by that of another substudy based on data collected for the Danish Calmette Study on parent-reported number of episodes of infectious illness, in which no overall effect of BCG vaccination was found [16].

Although we found no overall effect, the results of 3 prespecified subgroup analyses indicated that exploring whether the effect of BCG vaccination is modified by gestational age, cesarean delivery, or maternal BCG might be worthwhile.

First, we found a signal that BCG vaccination might be associated with a shorter time to first hospitalization for infection among premature children born at 32 to 36 weeks' gestation. Only 144 premature children were included in our trial, and the signal was weak, but it merits attention as a potential danger signal.

Second, BCG vaccination might decrease the rate of hospitalization for infection in children born via cesarean delivery, although our *P* value was not significant after adjustment for baseline characteristics. This finding might be

Table 2. Secondary Analyses: Tests of Effect Modifications. Hazard Ratios of Number of Hospitalizations for Infection

Analysis Type	Allocated to BCG Group (n = 2129 [ITT] or 2118 [PP])[b]	Allocated to Control Group (n = 2133 [ITT] or 2097 [PP])[b]	HR	95% CI	*P* (Effect Modification Between Groups)	Adjusted HR[c]	95% CI	*P* (Effect Modification Between Groups)
Intention to treat analysis[d]								
Duration of hospitalization[e]					0.67			0.56
≤1 day	306 (2129) (0.14)	302 (2133) (0.14)	1.02	0.84–1.23		1.03	0.85–1.25	
>1 day	282 (2129) (0.13)	293 (2133) (0.13)	0.97	0.80–1.16		0.97	0.80–1.17	
Follow-up period					0.86			0.91
<3 mo	160 (2129) (0.08)	165 (2133) (0.08)	0.97	0.75–1.26		0.97	0.75–1.27	
≥3 mo	428 (2126) (0.20)	430 (2131) (0.20)	1.00	0.85–1.18		0.99	0.83–1.18	
Prematurity					0.67			0.09
Premature (n = 144)[f]	35 (71) (0.49)	31 (73) (0.43)	1.16	0.55–2.47		2.04	0.86–4.84	
Mature (n = 4118)	553 (2058) (0.27)	564 (2060) (27.4)	0.98	0.84–1.14		0.95	0.82–1.11	
Sex					0.53			0.68
Male (n = 2241)	339 (1104) (0.31)	338 (1137) (0.30)	1.04	0.85–1.26		1.01	0.83–1.24	
Female (n = 2021)	249 (1025) (0.24)	257 (996) (0.26)	0.94	0.76–1.17		0.95	0.76–1.19	
Study site					0.32			0.22
Rigshospitalet (n = 1567)	214 (764) (0.28)	247 (803) (0.31)	0.92	0.71–1.19		0.91	0.69–1.19	
Hvidovre (n = 1470)	257 (738) (0.35)	224 (732) (0.31)	1.14	0.91–1.42		1.15	0.91–1.45	
Kolding (n = 1225	117 (627) (0.19)	124 (598) (0.21)	0.89	0.66–1.20		0.85	0.63–1.14	
Cesarean delivery								
Yes (n = 892)	117 (428) (0.27)	172 (464) (0.37)	0.73	0.54–0.99	0.02	0.78	0.57–1.08	0.10
No (n = 3370)	471 (1701) (0.28)	423 (1669) (0.25)	1.10	0.92–1.30		1.06	0.89–1.26	
Antibiotics during delivery								
Yes (n = 712)	130 (340) (0.38)	123 (372) (0.33)	1.18	0.85–1.63	0.26	1.21	0.85–1.74	0.20
No (n = 3550)	458 (1789) (0.26)	472 (1761) (0.27)	0.95	0.81–1.12		0.94	0.79–1.11	
Birth weight < 2500 g								
Yes (n = 123)	25 (61) (0.41)	24 (62) (0.39)	1.11	0.56–2.20	0.74	1.62	0.85–3.07	0.13
No (n = 4139	563 (2068) (0.27)	571 (2071) (0.28)	0.95	0.83–1.10		0.97	0.83–1.13	
≥1 older sibling								
Yes (n = 1761)	279 (905) (0.31)	273 (856) (0.32)	0.96	0.77–1.19	0.70	0.93	0.75–1.17	0.52
No (n = 2498)	309 (1223) (0.25)	321 (1275) (0.25)	1.01	0.83–1.24		1.03	0.84–1.28	
Parental atopic disease								
Yes (n = 2558)	354 (1286) (0.28)	360 (1272) (0.28)	0.97	0.80–1.17	0.65	0.98	0.80–1.18	0.81
No (n = 1379)	184 (675) (0.27)	213 (704) (0.30)	0.90	0.69–1.17		0.94	0.72–1.22	
Season at randomization[g]								
Winter (n = 1047)	177 (522) (0.34)	182 (525) (0.35)	0.98	0.74–1.29	0.15	0.98	0.73–1.30	0.09
Spring (n = 981)	143 (488) (0.29)	107 (493) (0.22)	1.36	1.00–1.85		1.39	1.02–1.90	
Summer (n = 1057)	100 (530) (0.19)	115 (527) (0.22)	0.86	0.61–1.21		0.83	0.58–1.18	
Autumn (n = 1177)	168 (589) (0.29)	191 (588) (0.33)	0.88	0.67–1.16		0.86	0.65–1.15	
Mother BCG vaccinated								
Yes (n = 740)	84 (372) (0.23)	128 (368) (0.35)	0.65	0.45–0.94	0.01	0.66	0.45–0.97	0.02
No (n = 3453)	499 (1730) (0.29)	453 (1723) (0.26)	1.10	0.93–1.29		1.07	0.91–1.27	
Per protocol analysis[h]								
Duration of hospitalization [4]								
≤1 day	305 (2118) (0.14)	293 (2097) (0.14)	1.03	0.85–1.25	0.63	1.04	0.86–1.27	0.52
>1 day	280 (2118) (0.13)	285 (2097) (0.14)	0.97	0.81–1.18		0.97	0.81–1.18	
Follow-up period					0.87			0.92
<3 mo	161 (2118) (0.08)	162 (2097) (0.08)	0.99	0.76–1.28		0.99	0.75–1.29	
≥3 mo	424 (2115) (0.20)	416 (2095) (0.20)	1.01	0.85–1.20		1.00	0.84–1.20	
Prematurity					0.61			0.08
Premature (n = 143)[f]	35 (71) (0.49)	29 (72) (0.40)	1.22	0.56–2.69		2.21	0.87–5.63	
Mature (n = 4072)	550 (2047) (0.27)	549 (2025) (0.27)	0.99	0.85–1.15		0.96	0.82–1.12	
Sex					0.40			0.53
Male (n = 2214)	339 (1096) (0.31)	326 (1118) (0.29)	1.06	0.87–1.30		1.04	0.84–1.28	
Female (n = 2001)	246 (1022) (0.24)	252 (979) (0.26)	0.94	0.75–1.17		0.94	0.75–1.18	
Study site					0.42			0.32
Rigshospitalet (n = 1543)	214 (761) (0.28)	239 (782) (0.31)	0.93	0.72–1.21		0.92	0.70–1.20	

Table 2. Continued

Analysis Type	Allocated to BCG Group (n = 2129 [ITT] or 2118 [PP])[b]	Allocated to Control Group (n = 2133 [ITT] or 2097 [PP])[b]	HR	95% CI	*P* (Effect Modification Between Groups)	Adjusted HR[c]	95% CI	*P* (Effect Modification Between Groups)
Hvidovre (n = 1456)	254 (734) (0.35)	220 (722) (0.31)	1.13	0.91–1.42		1.14	0.90–1.44	
Kolding (n = 1216)	117 (623) (0.19)	119 (593) (0.20)	0.93	0.69–1.24		0.88	0.65–1.19	
Cesarean delivery								
Yes (n = 881)	114 (424) (0.27)	168 (457) (0.37)	0.73	0.54-0-99	0.02	0.77	0.56–1.06	0.08
No (n = 3334)	471 (1694) (0.28)	410 (1640) (0.25)	1.12	0.94–1.32		1.07	0.90–1.28	
Antibiotics during delivery								
Yes (n = 701)	130 (339) (0.38)	120 (362) (0.33)	1.18	0.85–1.64	0.29	1.21	0.84–1.74	0.24
No (n = 3514)	455 (1779) (0.26)	458 (1735) (0.26)	0.97	0.82–1.14		0.91	0.80–1.13	
Birth weight < 2500 g								
Yes (n = 122)	25 (61) (0.41)	24 (61) (0.39)	1.09	0.55–2.17	0.80	1.59	0.84–3.03	0.15
No (n = 4093)	560 (2057) (0.27)	554 (2036) (0.27)	1.00	0.86–1.17		0.98	0.84–1.15	
≥1 older sibling								
Yes (n = 1751)	279 (901) (0.31)	267 (850) (0.31)	0.98	0.78–1.21	0.77	0.95	0.76–1.18	0.57
No (n = 2461)	306 (1216) (0.25)	310 (1245) (0.25)	1.02	0.83–1.25		1.04	0.84–1.29	
Parental atopic disease								
Yes (n = 2530)	354 (1278) (0.28)	350 (1252) (0.28)	0.99	0.82–1.20	0.55	0.99	0.82–1.21	0.68
No (n = 1361)	181 (672) (0.27)	207 (689) (0.30)	0.89	0.68–1.17		0.93	0.71–1.21	
Season at randomization[f]								
Winter (n = 1035)	174 (519) (0.34)	181 (516) (0.35)	0.96	0.72–1.26	0.13	0.94	0.71–1.26	0.44
Spring (n = 970)	143 (488) (0.29)	101 (482) (0.21)	1.41	1.03–1.92		1.44	1.05–1.99	
Summer (n = 1042)	100 (525) (0.19)	112 (517) (0.22)	0.88	0.62–1.24		0.83	0.58–1.19	
Autumn (n = 1168)	168 (586) (0.29)	184 (582) (0.32)	0.91	0.69–1.20		0.89	0.67–1.19	
Mother BCG vaccinated								
Yes (n = 721)	84 (371) (0.23)	117 (350) (0.33)	0.68	0.46–0.99	0.02	0.69	0.46–1.02	0.04
No (n = 3427)	496 (1722) (0.29)	447 (1705) (0.26)	1.10	0.93–1.30		1.07	0.91–1.27	
Child ≤2 days at vaccination (n = 1775)[i]	486 (1775) (0.27)	578 (2097) (0.28)	1.00	0.85–1.17	0.73 (test of difference in the BCG effect)	1.01	0.85–1.19	0.60 (test of difference in the BCG effect)
Child >2 days at vaccination (n = 343)[i]	99 (343) (0.29)		1.03	0.78–1.38		0.92	0.68–1.26	
Batch 111046B (n = 923)[i,j]	306 (923) (0.33)	578 (2097) (0.28)	0.96	0.79–1.18	0.89 (test of difference in the BCG effect)	0.98	0.79–1.22	0.89 (test of difference in the BCG effect)
Batch 112032A (n = 1195)[i,j]	279 (1195) (0.23)		1.03	0.84–1.27		0.98	0.79–1.21	

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; HR, hazard ratio; ITT, intention to treat; PP, per protocol.

[a]Test of effect modifications and differences in BCG effects.

[b]Values shown are the number of hospitalizations for infection, number of children included in the analysis (in parenthesis) and mean number of hospitalizations for infection per child (in parenthesis).

[c]Adjusted for baseline characteristics (sex, prematurity, study site, cesarean delivery, antibiotics during delivery, multiple birth, birth weight, child age at randomization, maternal age, parental ethnicity [≥1 parent of an ethnicity other than Danish], maternal education, parents living together, maternal BCG [according to maternal recall], siblings, maternal atopy, and maternal smoking during pregnancy). The analysis included 3953 children (93%) with full information.

[d]All randomly assigned children were followed from randomization to 15 months of age and analyzed according to their randomization group using Cox regression.

[e]Competing risks; the same children are at risk for both outcomes.

[f]Prematurity was defined as birth before 37 weeks' gestation.

[g]Winter, December, January, and February; spring, March, April, and May; summer, June, July, ane August; and autumn, September, October, and November.

[h]Children who received the BCG vaccine but were randomly assigned to the control group, or vice versa, were excluded. The remaining children were followed from time since vaccination for the BCG-vaccination group and time since randomization for control children.

[i]Among vaccinated children.

[j]Adjusted for seasonality in months.

in line with studies of the hygiene hypothesis [17]. Exposure to maternal microflora during delivery has been shown to be essential for the establishment of the microbiota, which again is important for shaping and educating the immune system of newborn children [18, 19]. Thus, the potential immune training exerted by BCG vaccination might play a role in children born via cesarean delivery, who lack immune stimulation from the early exposure to the maternal vaginal microbiota.

Third, the lack of an overall beneficial effect of BCG vaccination might be related to the limited numbers of Danish mothers who had been BCG vaccinated. Studying effect modification according to maternal BCG status was not planned in our trial protocol. The observation that the nonspecific beneficial effects of measles vaccine might be stronger if children are vaccinated in the presence of maternal measles antibodies [20] was made after the protocol of our trial was approved. However, our study was prespecified to examine potential effect

modification according to maternal BCG vaccination status in the statistical analysis plan deposited at the data safety monitoring board before the randomization code was revealed. Our study represented an opportunity to test the role of maternal BCG-vaccination status, because the cohort of mothers experienced the phase-out of BCG vaccine in Denmark in the 1970s and early 1980s; thus, both BCG-vaccinated and -unvaccinated mothers were enrolled. For the primary outcome of the Danish Calmette Study, all-cause hospitalization, a tendency toward a protective effect of BCG vaccination at birth on all-cause hospitalization was observed in children of BCG-vaccinated mothers, whereas there was a tendency toward an increased risk of hospitalization among children of BCG-unvaccinated mothers [8]. When we pursued that result in our study, we found that the rate of hospitalization for infection was reduced significantly in BCG-vaccinated children compared to that in control children in the subgroup of those whose mother was BCG vaccinated, whereas a tendency for the opposite trend was found among children of BCG-unvaccinated mothers.

The importance of maternal immunity in vaccinology is well known; however, the focus has been on vaccination during pregnancy to protect the mother and/or the neonate [21] and to minimize the interference of maternal antibodies on offspring immune responses [22]. Only a few studies have examined the influence of maternal BCG status or maternal exposure to mycobacteria before pregnancy on the BCG response in the offspring. Choi et al [23] found an increased in vitro interferon-γ response to purified protein derivative when a comparison of splenocytes in BCG-vaccinated offspring was made between BCG-vaccinated and non–BCG-vaccinated mice. Two studies found maternal BCG scars to be associated with a reduced T helper cell type 2 response to mycobacterial antigens in their BCG-vaccinated children [24, 25]. These findings support the observation that maternal immunity can influence an offspring's response to BCG vaccination in mycobacterium-specific ways. A recent small study in Uganda found that a maternal BCG scar can affect the child in a nonspecific way; interferon and inflammation responses were upregulated in infants of mothers with a BCG scar at 1 and 6 weeks after BCG immunization [26].

A recent World Health Organization review, which suggested possible beneficial effects of BCG vaccination on all-cause mortality, was mainly based on studies from low-income countries, where most mothers would have been BCG vaccinated [27]. Maternal priming through maternal exposure to tuberculosis and other mycobacteria might explain why potential positive nonspecific effects of BCG vaccination were seen shortly after its introduction in 1927 in Sweden [28] and in older controlled trials of BCG in the United Kingdom and United States [29]. A recent study in Greenland, where most mothers would have been vaccinated against the Danish BCG strain 1331 (also used in our study), compared the rates of hospitalization for infection in children born in a period with neonatal BCG strain 1331 vaccination available and the rates of hospitalization of for infection in children born in a period without neonatal BCG vaccination [30]. The adjusted incidence rate ratios were 0.72 (95% CI, 0.49–1.06) in children aged 3 days to 3 months and 1.07 (95% CI, 0.96–1.20) in children aged 3 months to 3 years [30–32]. In our study and with regard to the primary study outcome [8], no overall difference in the effects of BCG vaccination before or after 3 months of age was found.

The Denmark BCG strain used in our study resulted in 5-fold more adverse effects (large and/or suppurative local reactions and suppurative lymphadenitis) than expected [33]. It might be interesting to test the effect of vaccination with a BCG strain that results in fewer adverse effects on newborns born to a BCG-vaccinated mother in a high-income setting; such a study could be performed in one of the European countries that are still offering routine BCG immunizations or have stopped them only recently.

CONCLUSION

The results of our trial rejected the hypothesis that BCG vaccination within 7 days of birth reduced the rate of hospitalization for infection until 15 months of age among Danish children. Our findings call for further studies of the role of gestational age, cesarean delivery, and maternal BCG vaccination in the nonspecific effects of vaccines.

Supplementary Data

Supplementary materials are available at *Journal of the Pediatric Infectious Diseases Society* online.

Acknowledgments

Author contributions. L. G. S. is the guarantor of the study. L. G. S. and P. A. conceived the idea of the trial. C. S. B. conceived the idea of including maternal priming with BCG among potential effect modifiers. L. G. S., G. G., P.-E. K., O. P., C. S. B., and P. A. constituted the trial steering committee; D. L. J. was the alternate for O. P. N. M. B., J. K., T. N. N., G. T. P., and L. M. T. participated in the data collection. L. G. S., H. R., and S. S. obtained the register-based data. H. R. and L. G. S. carried out data management and analyzed the data. L. G. S. drafted the first version of the manuscript and revised the manuscript. All the authors were involved in the interpretation of the results and read and approved the final manuscript.

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