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MAJOR ARTICLE

Effects of Neonatal BCG-Japan Versus BCG-Russia Vaccination on Overall Mortality and Morbidity: Randomized Controlled Trial From Guinea-Bissau (BCGSTRAIN II)

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Background. Vaccination with the Danish strain of bacille Calmette-Guérin (BCG) has been associated with pronounced reductions in all-cause neonatal mortality and morbidity. Developing a skin reaction postvaccination is associated with markedly reduced mortality risk. It is unknown whether the beneficial nonspecific effects are maintained across different BCG strains.

Methods. This was an open-label randomized controlled trial in Guinea-Bissau, comparing BCG-Japan (n = 8754) versus BCG-Russia (n = 8752) for all-cause hospital admission risk by 6 weeks of age (primary outcome) and 6 months of age. Additional secondary outcomes were in-hospital case-fatality risk (CFR), all-cause mortality, and BCG skin reaction prevalence. Participants were followed through telephone calls at 6 weeks and 6 months, with a subgroup also visited at home. We assessed admission and mortality risk in Cox models providing incidence rate ratios (IRRs) and mortality rate ratios. CFR and skin reactions were assessed by binomial regression providing risk ratios. Analyses were done overall and stratified by sex.

Results. BCG strain was not associated with admission risk, the BCG-Japan/BCG-Russia IRR being 0.92 (95% confidence interval [CI], .81–1.05) by 6 weeks and 0.92 (95% CI, .82–1.02) by 6 months. By 6 months of age, there were significantly fewer BCG-Japan infants with no skin reaction (1%) than for BCG-Russia (2%), the risk ratio being 0.36 (95% CI, .16–.81). BCG-Japan skin reactions were also larger.

Conclusions. Both vaccines induced a skin reaction in almost all participants. The BCG strains had comparable effects on morbidity and mortality, but BCG-Japan was associated with more and larger skin reactions that are indicators of lower mortality risk.

Clinical Trials Registration. NCT03400878.

Keywords. bacille Calmette-Guérin vaccine; BCG strains; early-life morbidity and mortality; nonspecific effects of vaccines.

July 2021 marked the centenary of the first vaccination with live bacille Calmette-Guérin (BCG) to a Parisian newborn heavily exposed to tuberculosis (TB) [1]. More inoculations followed and as no adverse events were recorded, BCG vaccines began to be distributed to laboratories around the world. The growth and maintenance of BCG resulted in the accumulation of mutations causing substantial genetic diversity between the BCG strains in use today [2]. In addition, there are substantial differences between BCG strain preparations in regard to absolute mycobacterial contents and the live versus dead mycobacterial ratio [3]. Despite not being bioequivalent, BCG strains are currently considered equipotent and administered in the same dosage to >120 million infants per year [4]. The BCG strains currently prequalified by the World Health Organization (WHO) for procurement by United Nations agencies (eg, the United Nations Children's Fund) include BCG-Denmark, BCG-Japan, BCG-Russia, and BCG-Bulgaria [5].

It has been discovered that, aside from providing protection against the target disease, vaccines can have nonspecific effects (NSEs), which in low-income countries manifest as a modulated susceptibility to infections other than the target disease [6]. The current understanding of the immunological pathway(s) behind BCG's marked epidemiological effects are that BCG induces emergency granulopoiesis responses [7] and innate trained immunity effects [8]. For BCG, observational studies and randomized controlled trials (RCTs) have demonstrated

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that vaccination with the Danish BCG strain is associated with marked beneficial NSEs in the form of reduced mortality and morbidity from infections [9–18]. Compared to no-BCG, receiving BCG-Denmark at birth was associated with a 38% (95% Confidence Interval: 17%–54%) reduction in all-cause neonatal mortality risk across 3 RCTs in Guinea-Bissau [11]. An RCT from Uganda likewise found a reduced risk of physician-diagnosed nontuberculous infectious disease incidence [12]. The beneficial NSEs of BCG may be more pronounced in males [12, 19, 20] and less pronounced when provided to frail, hospitalized neonates [14, 21]. The BCG strain may also be an important factor since, in an Indian RCT comparing BCG-Russia versus no-BCG provided to neonates weighing <2000 g, BCG-Russia was not associated with reduced mortality [21]. Importantly, among BCG-vaccinated children, those who develop a BCG scar and/or a positive tuberculin skin test (TST) following vaccination have a 40%–50% reduced all-cause mortality risk, compared with BCG-vaccinated children having no scar or a negative TST [15, 16]. Furthermore, the size of the BCG skin reaction at 2 months of age is associated with both induction of the TST reaction and reduced subsequent infant mortality risk [16, 17]. In 2014–2017, we conducted an RCT testing NSEs of BCG strains [22] (BCGSTRAIN I); while the trial had low power to compare all-cause morbidity and mortality by strain, it was clear that BCG-Denmark and BCG-Japan induced both more and larger BCG skin reactions and TSTs, when compared with BCG-Russia [22]. Given the implications for global health, we conducted an additional large-scale RCT contrasting BCG-Japan and BCG-Russia, to assess whether BCG strains have differential effects on all-cause morbidity, all-cause mortality, and the induction of skin reactions.

METHODS

Setting

Bandim Health Project (BHP; www.bandim.org) maintains a Health and Demographic Surveillance System (HDSS) covering approximately 100 000 inhabitants in Bissau, the capital of Guinea-Bissau. The trial was conducted at the Hospital Nacional Simão Mendes (HNSM) maternity ward, which is Guinea-Bissau's principal birthplace with approximately 7000 deliveries per year, located in Bissau. BHP maintains a routine data collection system to register births, vaccinations, admissions, and outcomes at HNSM's maternity and pediatric wards [13, 14, 22–26]. In Guinea-Bissau, neonates are recommended BCG and oral polio vaccine (OPV) at birth; other infant vaccines are provided from 6 weeks of age.

Trial Design

We initiated this hospital-based, open-label, parallel-group RCT (BCGSTRAIN II) in October 2017 aiming to individually randomize 15 600 newborns to BCG-Japan (intervention) or BCG-Russia (control). There is no routine vaccination service at the HNSM maternity ward, so our team has for many years provided BCG and OPV on the day of discharge. This has formed the basis for conducting several BCG trials, including BCGSTRAIN II. Instead of using the BCG strain available in the country (when available), the team randomized children whose parents/guardians consented to take part in the trial to 1 of 2 types of WHO-preapproved BCG vaccines. While BCGSTRAIN II was ongoing, it was decided to conduct an additional trial of BCG strains [27]. Our team maintained enrollment of newborns while awaiting the ethics approval for the subsequent trial. Approval was given in March 2020. At that point, BCGSTRAIN II enrolled 17 506 children. Recruitment ceased on 25 March 2020.

Patient Consent Statement

Eligible for enrollment into the trial were healthy unvaccinated neonates at the maternity ward with no severe malformations. They were invited on the day of discharge. Exclusion criteria were age >42 days, gross malformation, and acute illness. Noneligible children and those refusing to participate were offered vaccine from the BCG vial that had most doses left, if there were no contraindications for vaccination, along with OPV.

BHP recruitment assistants assessed neonates for enrollment eligibility, ensuring informed oral and written consent from the mother/guardian. Mothers/guardians were provided written study information in Portuguese and an oral explanation of the study in Portuguese Creole, as well as the opportunity to ask questions. At the inclusion interview, we registered birth weight, weight at randomization, twin status, and delivery mode. From the mother (if present), we registered years of schooling, area of residence, age, BCG scar status, and the mid-upper arm circumference (MUAC).

Randomization and Blinding

The study team individually randomized participants 1:1 by letting the mother/guardian select, from a stack of sealed opaque envelopes, 1 envelope containing the treatment allocation. Envelopes were prepared in blocks of 40 for each sex by a senior study supervisor. Same-sex twins were allocated to the same treatment to avoid confusion. Parents and the enrollment team were not blinded to the treatment allocation, but providers of care and follow-up staff were blinded. On the vaccination card, as per normal procedure, it is only noted that the child was vaccinated with BCG; strain/batch is not recorded.

Intervention

The trial vaccines were BCG-Japan (Tokyo strain 172, Japan BCG Laboratory) and BCG-Russia (Russia BCG-I strain, Serum Institute of India). The vaccine bottles have distinguishable

appearances. Two experienced vaccinators performed all vaccinations by injecting 0.05 mL reconstituted BCG intradermally in the left deltoid region (Supplementary Figure 1), followed by administration of OPV. During a short period between 24 June and 6 August 2018, BCG-Japan was out of stock at the study site, for which reason enrollment was paused.

Outcomes

The primary outcome was all-cause hospital admissions at HNSM within 6 weeks of age. Secondary outcomes were admissions by 6 months of age as well as in-hospital case-fatality risk and all-cause mortality at the same time points. It was prespecified to analyze outcomes both overall, by sex, by maternal BCG scar status, and with censoring at the time of national OPV campaigns.

Registration of Pediatric Admissions at HNSM

HNSM hosts the only specialized pediatric ward in Guinea-Bissau, where a BHP team is present every day, collecting follow-up information for in-hospital outcomes. A triage room assistant maintains a daily list of outpatient consultations and admissions to the ward, documenting the child's name, date of birth, parents' names, telephone contacts, weight, socioeconomic information, unique BHP identification number (ID), and study ID (where applicable). Using the list of admissions updated on the day before and the triage list, the data entry supervisor performs a morning and afternoon round to all pediatric ward beds, sequentially updating the location and vital status (alive, died, discharged) for each child. To identify study participants listed in the pediatric ward database, we applied a standardized data-linkage protocol using the reclink2 Stata module [28] with individual confirmation of each event.

Follow-up

Blinded telephone interviews with the mother (or, if not available, other family or close contacts of the infant), conducted at approximately 6 weeks and 6 months of age, were the main source of all-cause mortality data. Available contacts were telephoned up to 3 times on 3 separate days. We also collected BCG skin reaction data (yes/no, type) by telephone. The telephone follow-up assistant first requested that the interviewee described the location and appearance of the skin reaction with his/her own words, and if necessary asked follow-up questions to determine the likely type of skin reaction (scar, papule, or pustule). To minimize loss to follow-up, home transport from the maternity ward was offered to participants with no available telephone contact, to obtain telephone numbers from family members.

Home Visit Follow-up

Participants residing in the BHP HDSS study area (16% of the cohort) received standardized home visits at 2 and 6 months of age to collect anthropometric data, BCG skin reaction characteristics, history of ipsilateral lymphadenitis, and mortality data.

Sample Size

The sample size was calculated based on the BCGSTRAIN I trial [22] with an expected HNSM admission rate of 4.5% by 6 weeks of age. To detect if 1 strain is associated with a 20% lower admission rate with 80% power, an α of .05, and 10% loss to follow-up, a total of 631 admission events would be necessary, corresponding to a sample size of 15 600.

Statistical Analyses

Incidence rate ratios (IRRs) of admission events comparing randomization groups were estimated in recurrent-event Andersen-Gill Cox proportional hazards models with age as the underlying time variable; age was thus inherently controlled for. Person-years of risk were calculated from enrollment (day of randomization), with no contribution of risk-time during admission at HNSM. In-hospital case-fatality risk ratios (RRs) were assessed using a generalized linear model with a log link function (binomial regression) providing approximate confidence intervals (CIs). All-cause mortality risk was assessed in Cox models providing mortality rate ratios (MRRs). Infants contributed risk-time until they died, were lost to follow-up, or reached 6 weeks of age (primary outcome) or 6 months of age (secondary outcomes), whichever came first. Tests of proportionality of hazards were computed using Schoenfeld residuals and reported where $P < .05$. BCG skin reaction and lymphadenitis prevalence was assessed by binomial regression providing prevalence ratios. We assessed whether the distribution of BCG skin reaction sizes was Gaussian using Q-Q plots; Student *t* test provided estimates of the mean difference between BCG strains with approximate CIs.

All morbidity and mortality outcomes were also analyzed with predefined censoring on the first day of national OPV campaigns, since OPV campaigns can affect the overall health status [29–31]. To estimate the effect of the BCG strains in the absence of OPV campaigns, we censored all subsequent follow-up on the first day of the first campaign occurring after enrollment of the infant. We also conducted a sensitivity analysis censoring events that occurred on the day of inclusion. All analyses were performed as intention-to-treat (ITT), overall (stratified by sex), by sex, and by maternal BCG scar status using StataIC 16 (StataCorp, College Station, Texas). Estimates are reported with 95% CIs and level of significance (α) <.05.

RESULTS

Our team assessed 17 656 newborns for inclusion eligibility and of these, 150 infants were excluded (Figure 1). A total of 17 485 newborns, recruited before 6 weeks of age, were included in the 6-week ITT analysis and 17 506 in the 6-month ITT analysis (Figure 1).

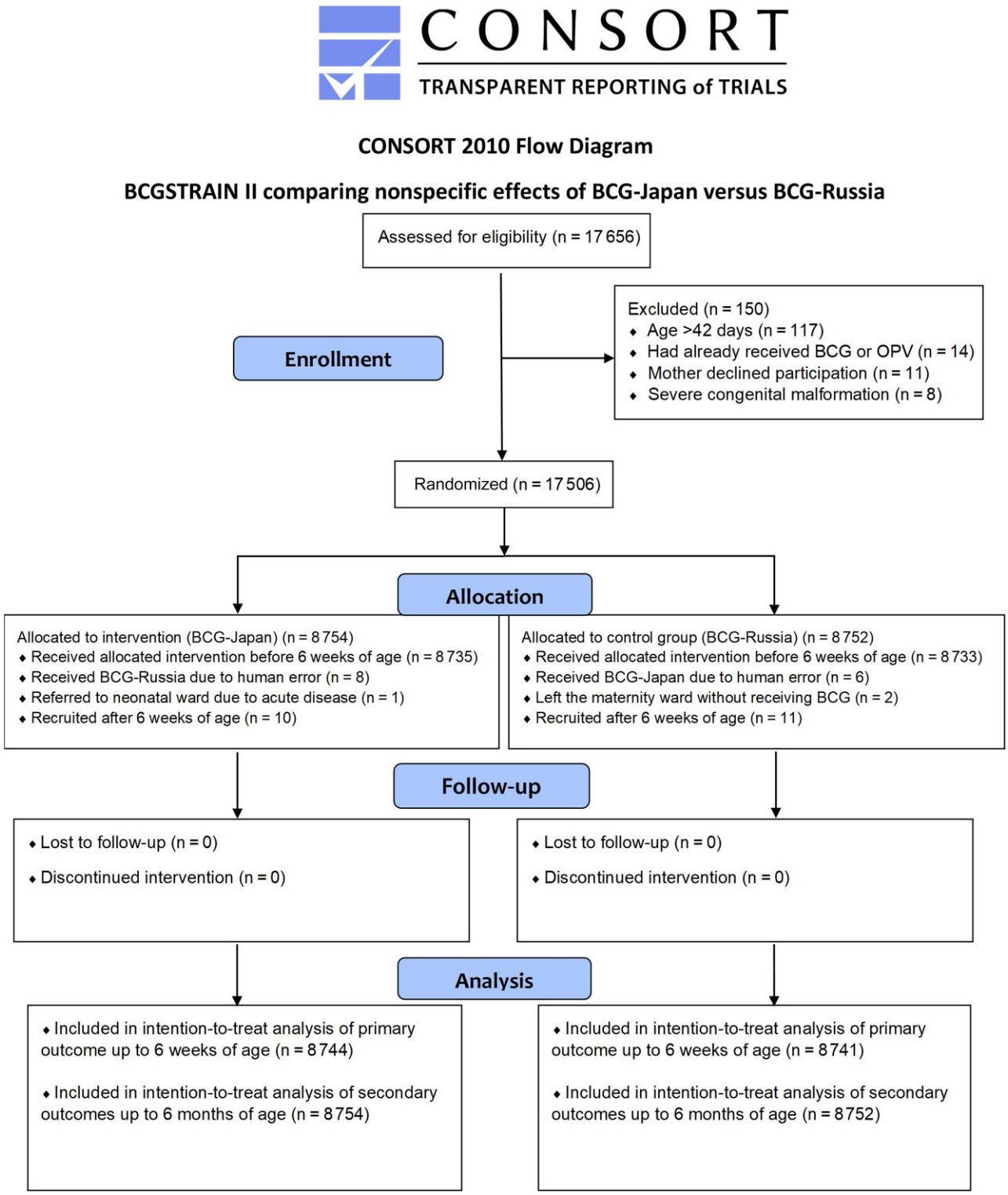

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) study flowchart. Abbreviations: BCG, bacille Calmette-Guérin; OPV, oral polio vaccine.

Baseline Inclusion Characteristics

Baseline characteristics were evenly distributed between the 2 randomization arms (Table 1). Our team enrolled 80% (13 932/17 506) of participants on day 0–1 after birth, and the median inclusion weight was 2980 g. For 99.2% (17 366/17 506) of the newborns, 1 or more family telephone numbers were provided. The mean size of the postvaccination wheal (measured just after intradermal BCG injection) was 4.5 mm.

All-Cause Admission Incidence and In-Hospital Case-Fatality Risk

There were 934 admissions (446 BCG-Japan, 488 BCG-Russia) by 6 weeks of age, resulting in a BCG-Japan/BCG-Russia admission IRR of 0.92 (95% confidence interval [CI], .81–1.05) (Figure 2). By sex, the BCG-Japan/BCG-Russia IRR was 0.84 (95% CI, .71–1.00) for males and 1.03 (95% CI, .85–1.25) for females (P for same effect = .13; Table 2, Figure 3). Eighty infants (8.6%; 42 BCG-Japan, 38 BCG-Russia) died during admission. The corresponding 6-week BCG-Japan/BCG-Russia case-fatality RR was 1.22 (95% CI, .80–1.87); 0.97 (95% CI, .52–1.78) for males and 1.52 (95% CI, .82–2.81) for females (P for same effect = .30; Table 2).

By 6 months of age, 1297 admissions (618 BCG-Japan, 679 BCG-Russia) had occurred, resulting in a BCG-Japan/BCG-Russia admission IRR of 0.92 (95% CI, .82–1.02); 0.86 (95% CI, .74–.99) for males and 0.99 (95% CI, .84–1.17) for females (P for same effect = .21; Table 3, Figure 3). By 6 months, there were 118 in-hospital deaths (9.1%; 58 BCG-Japan, 60 BCG-Russia), the 6-month BCG-Japan/BCG-Russia case-fatality RR being 1.06 (95% CI, .75–1.51); 0.78 (95% CI, .48–1.27) for males and 1.52 (95% CI, .90–2.59) for females (P for same effect = .07; Table 3).

By both 6 weeks and 6 months of age, BCG strain effects on admission risk and case-fatality risk were comparable when stratified by maternal scar status (Tables 2 and 3).

Table 1. Baseline Inclusion Characteristics by Randomization Allocation

Characteristic	BCG-Japan	BCG-Russia
Included	50% (8754/17 506)	50% (8752/17 506)
Maternal characteristics		
Mother has a BCG scar	65% (5584/8621)	63% (5486/8654)
Maternal age, y, median (IQR)	26 (21–30)	26 (21–30)
MUAC, mm, median (IQR)	274 (252–300)	272 (252–300)
Supplied at least 1 telephone contact	99.1% (8676/8754)	99.3% (8690/8752)
Family resides in BHP HDSS study area	16% (1369/8754)	16% (1361/8752)
Maternal years of schooling, median (IQR)	8 (4–11)	7 (3–11)
Neonate characteristics		
Cesarean delivery	14% (1213/8742)	13% (1169/8743)
Admitted at hospital before inclusion[a]	7.4% (639/8649)	6.7% (582/8636)
Low birth weight (<2500 g)	17% (1452/8754)	16% (1403/8752)
Inclusion weight, g, median (IQR)	2975 (2660–3295)	2980 (2660–3300)
Recruited on day of birth	18% (1615/8754)	19% (1673/8752)
Age at inclusion, d, median (IQR)	1 (1–1)	1 (1–1)
Male sex	52% (4543/8754)	52% (4521/8752)
Twinning percentage	5.0% (441/8754)	4.6% (406/8752)
Size of postvaccination wheal, mm, mean (SD)	4.5 (0.5)	4.5 (0.5)

Data are presented as % (no./No.) unless otherwise indicated.

Abbreviations: BCG, bacille Calmette-Guérin; BHP, Bandim Health Project; HDSS, Health and Demographic Surveillance System; IQR, interquartile range; MUAC, mid-upper arm circumference; SD, standard deviation.

[a]Newborns who were transferred to neonatal intensive care or the pediatric ward before they were discharged from the hospital and included in the trial.

Follow-up for Mortality Outcomes

The 6-week telephone interview was successfully completed for 91.3% (15 967/17 485) of participants at a median participant age of 52 days. The success rate for the subsequent 6-month interview was 87.0% (15 223/17 506; median participant age, 187 days).

All-Cause Mortality Risk by BCG Strain

By 6 weeks of age, 199 deaths (98 BCG-Japan, 101 BCG-Russia) had occurred, the corresponding BCG-Japan/BCG-Russia

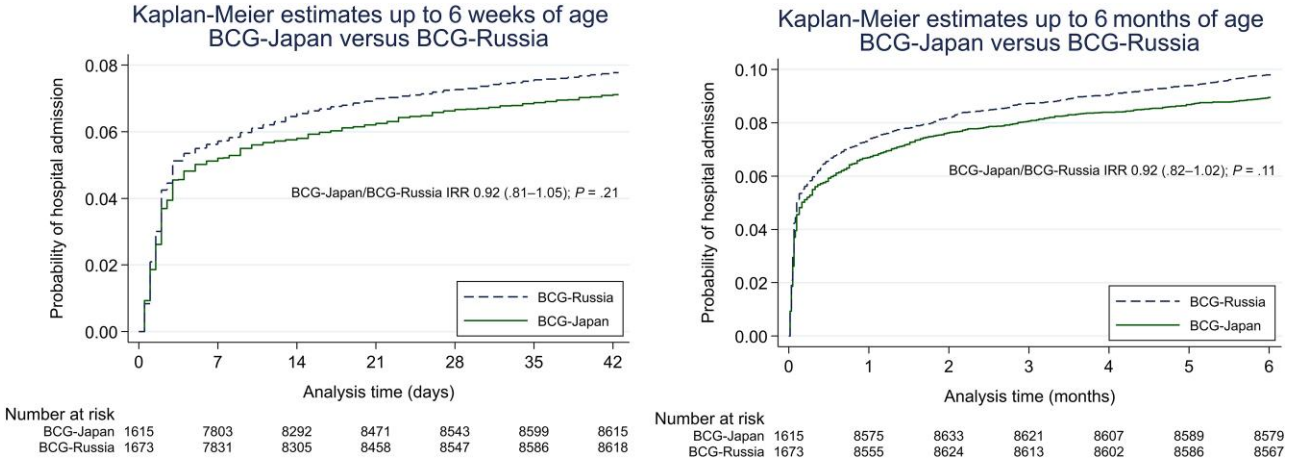

Figure 2. Kaplan-Meier cumulative admission incidence up to 6 weeks and 6 months of age. Data in parentheses indicate the 95% confidence interval. Abbreviations: BCG, bacille Calmette-Guérin; IRR, incidence rate ratio.

Table 2. All-Cause Admission Incidence and Case-Fatality Risk Within 6 Weeks[a], by BCG Strain and Sex

All-Cause Hospital Admissions by 6 Weeks of Age

Sex	Admission Rate per PY (Admissions/PY)		Japan/Russia 6-Week Admission IRR (95% CI)[b]	*P* Value for Interaction With Sex	Case-Fatality Risk, % (Deaths/Admissions)		Japan/Russia 6-Week Case-Fatality RR (95% CI)[c]	*P* Value for Interaction With Sex
	BCG-Japan	BCG-Russia			BCG-Japan	BCG-Russia		
	n = 8744	n = 8741			n = 8744	n = 8741		
Male	0.50 (241/485)	0.59 (286/482)	0.84 (.71–1.00)[d]		7.5% (18/241)	7.5% (22/286)	0.97 (.52–1.78)	
Female	0.45 (205/453)	0.44 (202/455)	1.03 (.85–1.25)	.13	12% (24/205)	7.9% (16/202)	1.52 (.82–2.81)	.30
Total	0.48 (446/937)	0.52 (488/938)	0.92 (.81–1.05)		9.4% (42/446)	7.8% (38/488)	1.22 (.80–1.87)	
Mother has a BCG scar[e]								
	n = 5576	n = 5478			n = 5576	n = 5478		
Male	0.50 (154/308)	0.59 (178/304)	0.86 (.69–1.06)		5.8% (9/154)	5.1% (9/178)	1.17 (.47–2.92)	
Female	0.48 (139/291)	0.46 (132/285)	1.04 (.82–1.32)	.24	12% (16/139)	7.6% (10/132)	1.60 (.74–3.46)	.61
Total	0.49 (293/599)	0.53 (310/589)	0.93 (.80–1.10)		8.5% (25/293)	6.1% (19/310)	1.44 (.80–2.58)	
Mother has no BCG scar								
	n = 3035	n = 3165			n = 3035	n = 3165		
Male	0.48 (81/169)	0.59 (103/174)	0.82 (.61–1.09)		11% (9/81)	12% (12/103)	0.93 (.40–2.14)	
Female	0.39 (61/155)	0.39 (64/166)	1.03 (.72–1.46)	.32	11% (7/61)	6.3% (4/64)	1.80 (.54–5.97)	.37
Total	0.44 (142/325)	0.49 (167/340)	0.90 (.72–1.12)		11% (16/142)	9.6% (16/167)	1.15 (.58–2.25)	

A total of 89% (834/934) of admissions (436 BCG-Russia, 398 BCG-Japan) were due to infectious diseases and 11% (100/934) (52 BCG-Russia, 48 BCG-Japan) were due to noninfectious causes. The Japan/Russia IRR for admission due to infectious diseases was 0.92 (95% CI, .80–1.05) overall, 0.86 (95% CI, .72–1.03) for males, and 1.00 (95% CI, .82–1.23) for females. Corresponding case-fatality RRs were 1.21 (95% CI, .75–1.96) overall, 0.78 (95% CI, .38–1.58) for males, and 1.84 (95% CI, .91–3.73) for females.

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; IRR, incidence rate ratio; PY, person-years; RR, risk ratio.

[a]Twenty-one infants (11 BCG-Russia, 10 BCG-Japan) were enrolled when >42 days old. These were included in the 6-month estimates.

[b]Andersen-Gill recurrent-event Cox proportional hazards model providing IRRs. For infants admitted within 6 weeks of age, 3 newborns (2 BCG-Russia, 1 BCG-Japan) were lost to follow-up.

[c]Binomial regression providing RRs.

[d]*P* = .052.

[e]The maternal BCG scar status was unknown for 1.3% (231/17 506) for the cohort in which there was 22 admissions and 4 in-hospital deaths (11 [1 fatal] BCG-Japan, 11 [3 fatal] BCG-Russia).

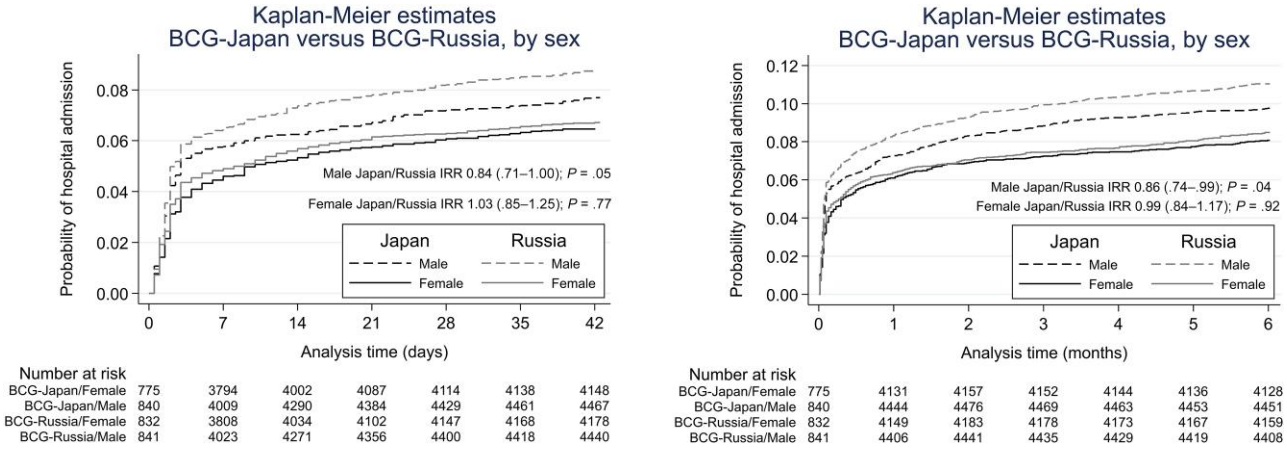

Figure 3. Kaplan-Meier cumulative admission incidence up to 6 weeks and 6 months of age, by sex. Data in parentheses indicate the 95% confidence interval. Abbreviations: BCG, bacille Calmette-Guérin; IRR, incidence rate ratio.

MRR being 0.99 (95% CI, .75–1.30) (Table 4). By 6 months of age, the BCG-Japan/BCG-Russia MRR was 0.96 (95% CI, .78–1.19). By sex, the male 6-month BCG-Japan/BCG-Russia MRR was 0.82 (95% CI, .62–1.09) and the female MRR was 1.19 (95% CI, .86–1.65) (*P* for same effect = .09; Table 4).

Mortality effects of BCG strains were comparable when stratified by maternal scar status (Table 4).

BCG Skin Reaction Prevalence and Risk of Lymphadenitis at 2 and 6 Months of Age

In the HDSS subgroup of 2730 children who received home visits, the BCG strain did not affect the prevalence of left axillary lymphadenitis (Table 5). A significantly higher proportion of infants vaccinated with BCG-Japan presented a BCG skin reaction by 2 (99% [531/534]) and 6 (99% [467/473]) months of age

Table 3. All-Cause Admission Incidence and Case-Fatality Risk Within 6 Months, by BCG Strain and Sex

All-Cause Hospital Admissions by 6 Months of Age								
	Admission Rate per PY (Admissions/PY)		Japan/Russia 6-Month Admission IRR (95% CI)[a]	*P* Value for Interaction With Sex	Case-Fatality Risk, % (Deaths/Admissions)		Japan/Russia 6-Month Case-Fatality RR (95% CI)[b]	*P* Value for Interaction With Sex
Sex	BCG-Japan	BCG-Russia			BCG-Japan	BCG-Russia		
	n = 8754	n = 8752			n = 8754	n = 8752		
Male	0.15 (342/2209)	0.18 (397/2194)	0.86 (.74–.99)[c]		7.6% (26/342)	9.6% (38/397)	0.78 (.48–1.27)	
Female	0.13 (276/2054)	0.14 (282/2068)	0.99 (.84–1.17)	.21	12% (32/276)	7.8% (22/282)	1.52 (.90–2.59)	.07
Total	0.14 (618/4263)	0.16 (679/4261)	0.92 (.82–1.02)		9.4% (58/618)	8.8% (60/679)	1.06 (.75–1.51)	
Mother has a BCG scar[d]								
	n = 5584	n = 5486			n = 5584	n = 5486		
Male	0.16 (224/1404)	0.18 (249/1384)	0.89 (.74–1.07)		5.4% (12/224)	8.0% (20/249)	0.66 (.33–1.34)	
Female	0.14 (183/1321)	0.13 (174/1291)	1.03 (.84–1.27)	.28	12% (22/183)	7.5% (13/174)	1.69 (.87–3.30)	.06
Total	0.15 (407/2725)	0.16 (423/2675)	0.95 (.83–1.09)		8.4% (34/407)	7.8% (33/423)	1.09 (.68–1.75)	
Mother has no BCG scar								
	n = 3037	n = 3168			n = 3037	n = 3168		
Male	0.14 (111/771)	0.17 (137/790)	0.84 (.65–1.07)		13% (14/111)	11% (15/137)	1.12 (.55–2.26)	
Female	0.12 (86/703)	0.14 (102/751)	0.91 (.68–1.21)[e]	.21	10% (9/86)	6.9% (7/102)	1.50 (.57–3.94)	.63
Total	0.13 (197/1474)	0.16 (239/1541)	0.87 (.72–1.05)		12% (23/197)	9.2% (22/239)	1.24 (.70–2.18)	

A total of 86% (1112/1297) of admissions were due to infection and 14% (185/1297) were due to noninfectious causes. The Japan/Russia IRR for admission due to infectious diseases was 0.92 (95% CI, .82–1.04) overall, 0.87 (95% CI, .75–1.02) for males, and 1.00 (95% CI, .84–1.20) for females. Corresponding case-fatality RRs were 1.09 (95% CI, .72–1.64) overall, 0.74 (95% CI, .42–1.30) for males, and 1.74 (95% CI, .92–3.27) for females.

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; IRR, incidence rate ratio; PY, person-years; RR, risk ratio.

[a]Andersen-Gill recurrent-event Cox proportional hazards model providing IRRs. Three newborns (2 BCG-Russia, 1 BCG-Japan) were lost to follow-up during hospital admission.

[b]Binomial regression providing RRs.

[c]*P* = .049.

[d]The maternal BCG scar status was unknown for 1.3% (231/17 506) for the cohort in which there was 22 admissions and 4 in-hospital deaths (11 [1 fatal] BCG-Japan, 11 [3 fatal] BCG-Russia).

[e]Test of the proportional hazards assumption using Schoenfeld residuals: *P* = .041.

Table 4. All-Cause Mortality Within 6 Weeks and 6 Months of Age[a] by Strain of BCG, Sex, and Maternal BCG Scar Status

All-Cause Mortality by 6 Weeks of Age					All-Cause Mortality by 6 Months of Age				
	Mortality Rate by 6 Weeks of Age per 100 PY (No. of Deaths/Total PY)		Japan/Russia 6-Week MRR[b] (95% CI)	*P* Value for Interaction With Sex		Mortality Rate by 6 Months of Age per 100 PY (No. of Deaths/Total PY)		Japan/Russia 6-Month MRR[b] (95% CI)	*P* Value for Interaction With Sex
Sex	BCG-Japan	BCG-Russia			Sex	BCG-Japan	BCG-Russia		
	n = 8744	n = 8741				n = 8754	n = 8752		
Male	11 (51/463)	13 (61/469)	0.85 (.59–1.24)		Male	4.6 (90/1968)	5.6 (111/1986)	0.82 (.62–1.09)	
Female	11 (47/437)	9.1 (40/440)	1.19 (.78–1.81)	.25	Female	4.3 (80/1846)	3.6 (68/1877)	1.19 (.86–1.65)	.09
Total	11 (98/900)	11 (101/909)	0.99 (.75–1.30)		Total	4.5 (170/3815)	4.6 (179/3862)	0.96 (.78–1.19)	
Mother has a BCG scar[c]					Mother has a BCG scar				
	n = 5576	n = 5478				n = 5584	n = 5486		
Male	9.1 (27/296)	12 (34/297)	0.80 (.48–1.32)		Male	3.8 (48/1266)	4.8 (61/1264)	0.79 (.54–1.15)	
Female	9.9 (28/283)	9.4 (26/276)	1.06 (.62–1.80)	.45	Female	4.0 (48/1204)	3.6 (43/1184)	1.10 (.73–1.66)	.25
Total	9.5 (55/579)	11 (60/573)	0.91 (.63–1.31)		Total	3.9 (96/2471)	4.2 (104/2448)	0.92 (.69–1.21)	
Mother does not have BCG scar					Mother does not have BCG scar				
	n = 3035	n = 3165				n = 3037	n = 3168		
Male	15 (24/160)	15 (26/168)	0.98 (.56–1.70)		Male	6.2 (42/672)	6.4 (45/703)	0.98 (.65–1.50)	
Female	12 (17/148)	7.6 (12/158)	1.52 (.72–3.17)	.35	Female	4.7 (29/616)	3.4 (23/670)	1.36 (.79–2.35)	.36
Total	13 (41/308)	12 (38/327)	1.15 (.74–1.79)		Total	5.5 (71/1288)	4.9 (68/1373)	1.11 (.80–1.55)	

Abbreviations: BCG, bacillus Calmette-Guérin; CI, confidence interval; MRR, mortality rate ratio; PY, person-years.

[a]Twenty-one (11 BCG-Russia, 10 BCG-Japan) infants were enrolled when >42 days old. These were included in the 6-month estimates. Follow-up was conducted by 2 telephone calls at 6 weeks and 6 months of age, which provided 6-week outcome data for 95.1% (16 624/17 485) of the cohort. Home visits within the HDSS yielded follow-up data for an additional 0.3% (54/17 485), and there were 34 six-week admissions among newborns not reached by telephone and home visits, 6 of which were fatal. The combined 6-week follow-up rate was 95.6% (16 712/17 485); 95.2% (8324/8744) for BCG-Japan and 96.0% (8388/8741) for BCG-Russia.

[b]Cox proportional hazards model providing MRRs.

[c]The maternal BCG scar status was unknown for 1.3% (231/17 506) of the cohort neonates, and their 6-month mortality risk was 4.3% (10/231); 2.3% (3/133) for BCG-Japan and 7.1% (7/98) for BCG-Russia.

Table 5. BCG Skin Reaction Prevalence and Risk of Lymphadenitis by BCG Strain in the Subgroup of Infants Reached by Home Visits

Sex	Infants With No Visible BCG Skin Reaction			Mean Size of BCG Skin Reaction			History of Left Axillary Lymphadenitis[a]		
	% (no./No.)		Japan/Russia No Reaction PR (95% CI)[b]	Size, mm (SD)		Reaction Size, MD (95% CI)[c]	% (no./No.)		Japan/ Russia RR (95% CI)[b]
	BCG-Japan	BCG-Russia		BCG-Japan	BCG-Russia		BCG-Japan	BCG-Russia	
Home visits conducted by 2 months of age									
Male	0.6% (3/534)	2.0% (11/559)	0.29 (.08–1.02)	5.3 (1.4)	4.8 (1.2)	0.51 (.35–.67)[d]	0.0% (0/530)	0.4% (2/556)	…
Female	1.0% (5/518)	1.9% (10/526)	0.51 (.17–1.48)	5.2 (1.3)	4.7 (1.1)	0.52 (.36–.67)[d]	0.2% (1/514)	0.4% (2/526)	0.51 (.05–5.63)
Total	0.8% (8/1052)	1.9% (21/1085)	0.39 (.17–.88)	5.2 (1.3)	4.7 (1.2)	0.51 (.40–.62)[d]	0.1% (1/1044)	0.4% (4/1082)	0.26 (.03–2.31)
Home visits conducted by 6 months of age									
Male	1.3% (6/473)	2.5% (12/489)	0.52 (.20–1.37)	5.1 (1.6)	4.5 (1.3)	0.54 (.33–.76)[d]	0.0% (0/471)	0.2% (1/485)	…
Female	0.4% (2/486)	2.1% (10/471)	0.19 (.04–.88)	5.1 (1.3)	4.4 (1.3)	0.66 (.46–.85)[d]	0.2% (1/485)	0.0% (0/468)	…
Total	0.8% (8/959)	2.3% (22/960)	0.36 (.16–.81)	5.1 (1.5)	4.5 (1.3)	0.61 (.46–.74)[d]	0.1% (1/956)	0.1% (1/953)	1.00 (.06–15.9)

Abbreviations: CI, confidence interval; MD, mean difference; PR, prevalence ratio; RR, risk ratio; SD, standard deviation.

[a]History of left axillary lymphadenitis reported by the mother/guardian.

[b]Binomial regression.

[c]Student *t* test providing estimates of MD in BCG reaction size (height + width divided by 2) between intervention and control groups.

[d]$P < .001$.

and the skin reactions were significantly larger, when compared with BCG-Russia (2 months of age: 98% [548/559]; 6 months of age: 98% [477/489]) (Table 5).

For the BCG reaction data collected by telephone follow-up, BCG-Japan was also associated with a higher skin reaction prevalence at both 6 weeks and 6 months of age, when compared with BCG-Russia (Table 6, Figure 4). In addition, BCG-Japan resulted in significantly more BCG pustules and fewer papules at 6 weeks of age (Table 6, Figure 4).

Sensitivity Analyses

Censoring events that occurred after 2 OPV campaigns during the trial or events occurring at the day of inclusion did not modify estimates (data not shown).

DISCUSSION

Main Findings

We conducted a large RCT to test the hypothesis that BCG-Japan would reduce the 6-week admission rate and the mortality risk by 20%, when compared with BCG-Russia. Overall, there were no differences between the strains regarding admission risk, case-fatality risk, or all-cause mortality risk. For males, receiving BCG-Japan might be associated with a reduction in admission risk; however, the test for interaction by sex was not significant. The maternal BCG scar status did not affect BCG strain effects.

Strengths and Weaknesses

Vaccinators, Development of the BCG Scar, and OPV Coadministration

Our study was conducted by a core team that has been responsible for providing vaccines at the HNSM maternity ward since 2002 and that have conducted 6 RCTs [10, 11, 14, 22, 32, 33] with a total of >46 000 newborns enrolled. The same 2 vaccinators have administered all BCG and OPV vaccines through these years, and their vaccinations result in large post-vaccination wheals, large BCG skin reactions, and high BCG scar prevalences compared with other trial sites [17, 34–36]. As an example, the proportion of infants vaccinated with BCG-Russia that develop a BCG scar—98% in the present trial—has been reported to be as low as 52% in a study from rural Guinea-Bissau involving children vaccinated at smaller health centers [37]. In a recent large multicountry RCT providing BCG-Denmark to adults, 76% of the participants developed a BCG scar—84% in Brazil and only 47% in Spain [38].

The very high scar success rate in our trial may have hampered our ability to show differences between strains, as it would likely be in favor of a weak BCG strain. Differences in BCG strains such as the content of live culturable mycobacteria might be more important when BCG is administered by a less experienced vaccinator, where successful administration of the complete vaccine dose occurs less frequently. In addition, there might also be other factors at play that are not reflected by the induction of BCG skin reactions, such as the vaccine content of dead mycobacteria, which might still be important for the immediate overall health effects of neonatal vaccination with different BCG strains. Most neonates received the allocated BCG strain coadministered with OPV. OPV provided at birth has nonspecific effects of its own [33], which might have made BCG strain differences harder to detect.

Power Considerations

The trial included 12% more newborns than originally planned and the follow-up success rate and the admission rates were higher than anticipated a priori, while mortality was lower. Therefore, the trial had adequate power to detect a 20% difference in the rate of hospital admission, but not to detect a 20% difference in overall mortality by 6 weeks of age.

Table 6. BCG Skin Reaction Prevalence and Characteristics Reported at Telephone Interviews at 6 Weeks and 6 Months of Age

Infants With No Visible BCG Skin Reaction at the Injection Side (Left Upper Deltoid)						
	6 Weeks of Age			6 Months of Age		
	% (no./No.)			% (no./No.)		
Sex	BCG-Japan	BCG-Russia	Japan/Russia RR for No Reaction (95% CI)[a]	BCG-Japan	BCG-Russia	Japan/Russia RR for No Reaction (95% CI)[a]
Male	1.8% (58/3294)	5.8% (187/3215)	0.30 (.23–.40)[b]	1.1% (37/3505)	2.2% (76/3480)	0.48 (.33–.71)[b]
Female	1.8% (57/3119)	4.3% (131/3071)	0.43 (.32–.58)[b]	1.1% (35/3306)	2.1% (71/3345)	0.50 (.33–.75)[b]
Total	1.8% (115/6413)	5.1% (318/6286)	0.35 (.29–.44)[b]	1.1% (72/6811)	2.2% (147/6825)	0.49 (.37–.65)[b]

Infants With BCG Skin Reaction Reported at the Injection Side (Left Upper Deltoid)						
	6 Weeks of Age			6 Months of Age		
	% (No.)			% (No.)		
Reaction type	BCG-Japan (n = 6413)	BCG-Russia (n = 6286)	Japan/Russia RR for Reaction Type (95% CI)[a]	BCG-Japan (n = 6811)	BCG-Russia (n = 6825)	Japan/Russia RR for Reaction Type (95% CI)[a]
Scar	51% (3199)	57% (3388)	0.92 (.89–.96)[b]	97% (6539)	96% (6406)	1.02 (1.01–1.03)[b]
Pustule	41% (2602)	32% (1903)	1.34 (1.28–1.40)[b]	1.3% (85)	1.4% (93)	0.91 (.68–1.23)
Papule	6.5% (412)	9.8% (587)	0.69 (.61–.78)[b]	0.8% (51)	1.6% (108)	0.47 (.34–.66)[b]
Unknown	1.4% (85)	1.5% (90)	0.93 (.69–1.24)	1.0% (64)	1.1% (71)	0.90 (.65–1.26)

Abbreviations: BCG, bacille Calmette-Guérin; CI, confidence interval; RR, risk ratio.

[a]Binomial regression.

[b]$P < .001$.

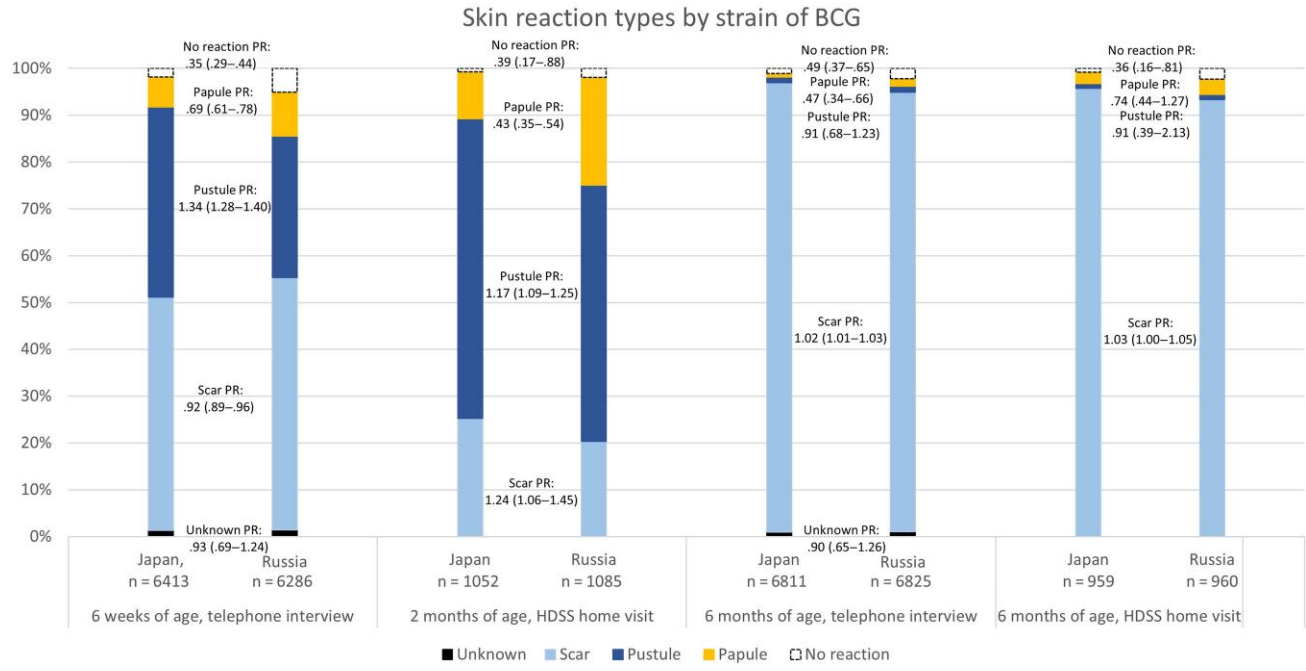

Figure 4. Skin reaction types by BCG strain. Data in parentheses indicate the 95% confidence interval. Abbreviations: BCG, bacille Calmette-Guérin; HDSS, Health and Demographic Surveillance System; PR, prevalence ratio.

As in previous BCG studies conducted at BHP, the mother/guardian was not blinded to the randomization allocation. We have no reason to believe that the knowledge about BCG strain affected maternal behavior. Follow-up staff and outcome assessors were blinded.

Consistency With Previous Findings

The present trial follows the BCGSTRAIN I trial that, for a period, had compared BCG-Japan versus BCG-Russia, with no significant differences detected in terms of mortality or morbidity, but with limited power [22]. There was, however, a trend

for more admissions among males who had received BCG-Japan in the previous trial, where the male Japan/Russia IRR was 1.28 (95% CI, .96–1.71); such an effect was not seen in the present trial, rather the contrary, as BCG-Japan was associated with a modest but significant reduction in admission risk in males.

Our finding of a higher BCG scar prevalence associated with BCG-Japan, when compared to BCG-Russia, corroborates data from our previous trial [22] and an Australian trial [34], which further reported that BCG-Japan induces higher proportions of polyfunctional $CD4^+$ T cells and higher concentrations of secreted T-helper 1 cytokines, compared to BCG-Russia. Furthermore, all 3 trials report that vaccination with BCG-Japan produces larger BCG skin reactions than BCG-Russia [34]. The difference in BCG reaction characteristics is consistent with the substantially lower number of colony-forming units per neonatal dose of BCG-Russia ($1–\times 10^5$), compared with BCG-Japan ($2.2–2.8 \times 10^6$) [3]. Recent data have associated larger BCG reaction size with decreased infant mortality risk [17]. A post hoc analysis of the aforementioned Australian trial revealed that larger reaction size correlates positively with in vitro immune responses likely associated with protection from tuberculosis [35].

CONCLUSIONS

In this large trial, contrasting BCG-Japan versus BCG-Russia did not reveal overall differences in morbidity or mortality. We found that BCG-Japan induced more BCG skin reactions that were larger than for BCG-Russia. This induction of BCG skin reactions, which later forms the final scar, is important because having a BCG scar and the scar size are associated with substantially reduced all-cause mortality during childhood for the vaccinee [15, 17], and likely also improved health outcomes in future offspring [26, 39–42]. When compared to other cohorts, the overall BCG skin reaction prevalence was very high in both groups. There may be greater potential differences between strains in settings with less experienced vaccinators.

Given these results, the substantial number of infants vaccinated with BCG per year, and the large discrepancies in viable mycobacterial content in licensed BCG strain formulations [3], further randomized studies of BCG strains and of the dose of BCG are warranted.

Supplementary Data

Supplementary materials are available at *Open Forum 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

Author contributions. C. S. B. and P. A. were the principal investigators and guarantors of the main trial. C. S. B., P. A., and F. S.-B. designed the study. E. J. C., E. B. S., F. S.-B., I. M., M. K. S., and R. A. F. supervised the data collection and data entry. F. S.-B. and S. N. conducted the statistical analyses. F. S.-B. wrote the first draft of the manuscript, and all authors approved the final manuscript.

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Data availability. De-identified participant data with a data dictionary can be shared after approval of a data-sharing proposal sent to Professor Christine Stabell Benn (cbenn@health.sdu.dk).

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Potential conflicts of interest. All authors: No reported conflicts.

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