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Mortality risk among frail neonates might be associated with maternal BCG scar status: Observational study from Guinea-Bissau

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Running title: Effects of maternal BCG on offspring health

Brief 40-word summary: Mortality by 6 weeks of age tended to be reduced among neonates born to mothers that had a BCG scar versus mothers with no BCG scar.

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ABSTRACT (194 words)

Background: Maternal priming with Bacille Calmette-Guérin (BCG) has been associated with reduced offspring mortality. We investigated this association in a cohort of frail neonates.

Methods: We performed an observational study within a randomized BCG trial conducted at the Neonatal Intensive Care Unit (NICU) in Guinea-Bissau from 2015-2017. At NICU admission and following informed consent, the maternal scar status was evaluated by visual inspection before neonates were randomized 1:1 to receive BCG+Oral Polio Vaccine (OPV) immediately versus BCG+OPV at hospital discharge. Stratified by maternal scar status, we assessed overall in-hospital and post-discharge mortality up to 42 days of age in Cox Proportional Hazards models providing adjusted Mortality Rate Ratios (aMRRs).

Results: 62% (903/1451) of mothers had a BCG scar. During NICU admission, the mortality risk was 1.7% (15/903) for neonates born to mothers with a scar vs 3.3% (18/548) for those born to mothers with no scar, the maternal scar/no scar aMRR=0.53 (0.26-1.05); the aMRR was 0.39 (0.13-1.05) for unvaccinated neonates and 0.70 (0.26-1.87) for vaccinated neonates.

Conclusion: This small study indicates that maternal BCG might be associated with reduced all-cause NICU mortality. If confirmed elsewhere, this finding would have substantial ramifications for global health.

Keywords: Bacille Calmette-Guérin (BCG) vaccine; maternal BCG scar; NICU mortality; non-specific effects of vaccines; vertical priming; maternal BCG priming; perinatal mortality.

BACKGROUND

A series of observational studies and randomized controlled trials (RCTs) have associated Bacille Calmette-Guérin (BCG) vaccination with beneficial *non-specific effects* (NSE), substantially reducing all-cause mortality in low-income countries with high infectious disease prevalence.[1–8] In 2014, a WHO-commissioned review of NSE concluded that BCG halves child mortality and encouraged further research.[9] Even in a high-income country with lower prevalence of infectious diseases, contrasting BCG vs no-BCG at birth in a large-scale RCT revealed that if the mother had also been BCG-vaccinated, the benefit of being randomized to neonatal BCG was substantial.[10]

To further explore the novel finding of maternal- or "vertical" BCG priming effects, the Bandim Health Project (BHP, www.bandim.org) has initiated a series of maternal scar studies in the low-income and high-infection prevalent country Guinea-Bissau.[11–14] We found that for children born to mothers with a scar, childhood BCG scars were associated with a 66% (33-83%) lower mortality risk when compared to children with no scar; there was no effect of child BCG scar if the mother had no scar.[11] Similarly, presence of a maternal scar was associated with a 60% (4-83%) reduction in all-cause mortality by 6 weeks of age in a recent retrospective analysis.[12] Finally, in a large cohort of healthy BCG-vaccinated newborns, presence of a maternal scar was associated with fewer fatal infections such as sepsis by 6 weeks of age.[13] Most of the available datasets to examine the effect of maternal BCG involves BCG-vaccinated newborns, since the policy is to provide BCG at birth. In a recent analysis from the rural areas of Guinea-Bissau, however, the maternal scar status was evaluated for pregnant women, and having a maternal scar was associated with a trend towards fewer adverse pregnancy outcomes, the maternal scar vs no scar hazard ratio being 0.78 (0.59-1.01).[14] With the a priori hypothesis that maternal BCG would reduce the overall mortality risk especially for BCG-vaccinated neonates, we here report the first prospective study evaluating all-cause mortality effects of maternal priming with BCG. Our analysis was conducted within a unique

cohort of frail neonates admitted to the Neonatal Intensive Care Unit (NICU) that were randomly allocated to receive BCG at NICU admission or delayed to discharge (usual practice).[15]

METHODS

Setting

The main trial was conducted at the NICU of Hospital Nacional Simão Mendes (HNSM)'s maternity ward, which is Guinea-Bissau's principal birthplace with ~7,000 deliveries/year, located in the capital Bissau. BHP maintains a routine data collection system to register births, vaccinations, admissions and admission outcomes at the HNSM maternity and pediatric wards.[4,15–19]

Study Design

This prospective study was based on data collected from 2015-17 within an RCT initiated in 2013 that compared the overall health effects of providing neonatal vaccines immediately at NICU admission vs at discharge, as described in detail.[15] Briefly, the main outcome was all-cause mortality during NICU admission. Known risk factors for NICU admission are birthweight <1500g, Apgar score $\leq$3, single motherhood and delivery by C-section.[17] Exclusion criteria were weight on admission <1250g and Apgar score <2 at birth.[15]

The recommended vaccination schedule at birth in Guinea-Bissau is BCG and Oral Polio Vaccine (OPV), and the usual practice is co-administration at discharge from HNSM. Thus, NICU-admitted neonates were randomized 1:1 to BCG+Oral Polio Vaccine (OPV) immediately versus BCG+OPV at hospital discharge as per standard of care. The BCG strains used in the trial were BCG-Denmark during 2013-16 and BCG-Japan in 2016-17, due to a BCG-Denmark production halt.[15]

Ethical approval

The study protocol was approved by the Guinea-Bissau Health Ministry's Research Coordination Committee (Reference number: CNES-2013-0054) and given consultative approval by the Central

Danish Ethical Committee (Case No: 1303771-1). A subsequent protocol revision was equally approved by both ethical committees (CNES-2014-001, 1303771-2). The trial was conducted in accordance with the Helsinki Declaration ethical standards, and a Data and Safety Monitoring Board (DSMB) oversaw the trial. Free healthcare consultations and essential drugs were provided to all infants invited to participate in the study, which was registered at **ClinicalTrials.gov** with registration number NCT01989026 on November 20, 2013.

Enrollment

Among all neonates admitted to the NICU during the trial, 86% were eligible for participation.[15] Enrollment procedures occurred the morning following admission to the NICU, where mothers or guardians were provided written study information in Portuguese and a verbal explanation of the study in the local language Creole and invited to ask questions. If the mother or guardian of the newborn gave written informed consent to participate, the newborn was enrolled in the main trial. At enrollment, we collected socio-economic data and recorded the maternal mid-upper-arm circumference along with newborn weight and twinning status.

The study group became aware of the possible importance of maternal BCG priming for offspring outcomes in July 2015 and conducted a training course for the study assistants. As part of inclusion procedures, we initiated assessments of the maternal BCG scar status by visual inspection of both upper arms on July 10, 2015, when 1750 neonates had been enrolled in the trial.

Follow-up

The weight and vital status for enrolled neonates was monitored daily and follow-up continued at the adjacent pediatric ward for neonates transferred there. At hospital discharge, we ensured that control neonates received the recommended BCG+OPV. After discharge, the neonate and family was provided home transport by BHP and a map was drawn to facilitate follow-up home-visits, which

were conducted at 3 days post-discharge and at 2-, 6- and 12-months of age, applying the same sequence of visits as in previous trials.[1–3]

Statistical analyses

Mortality Rate Ratios (MRRs) by maternal BCG scar status (scar/no scar) were estimated in Cox Proportional Hazards Models with age as the underlying time variable. Age was thus inherently controlled for. Tests of proportionality of hazards were computed using Schoenfeld's residuals.

We tested associations between baseline inclusion characteristics and maternal BCG scar status using Kruskal-Wallis and Pearson chi-squared tests. The maternal level of schooling (no education, primary school, secondary school, high school, or university) differed significantly between the maternal scar and no maternal scar cohorts and was therefore included in a multivariate Cox-model containing maternal BCG scar, sex and the outcome variable providing adjusted MRRs (aMRRs). For nine mothers (0.6% of the cohort) with missing information, the level of schooling was imputed as no education. We present mortality risk estimates by maternal scar status during NICU admission, overall (main outcome) and by offspring BCG randomization allocation (secondary outcome). A senior pediatrician blinded to both randomization allocation and maternal scar status evaluated NICU deaths and assigned a probable cause of death, which was used to differentiate between causes of death in a supplementary analysis. Furthermore, estimates including follow-up time after NICU discharge (when all neonates had been BCG-vaccinated) and up to 42 days of age are presented (secondary outcome). This approach was chosen to evaluate maternal BCG effects in both BCG-vaccinated, unvaccinated and the combined cohort and further to eliminate interference from other childhood vaccines administered from 42 days of age, in accordance with previous studies.[7,12,16,20]

For the NICU analysis, neonates contributed risk-time until they died, were lost to follow-up, or were discharged, whichever came first. In the analysis including post-discharge follow-up time, neonates

contributed risk-time until they died, migrated, or reached 42 days of age, whichever came first. Since neonatal BCG has previously been shown to be beneficial in low birth weight (LBW, birth weight <2500g) neonates[3,7] and since there might be differences between BCG strains[21], we conducted supplementary analyses assessing effects of maternal BCG scars by LBW status (yes/no) and by BCG strain. All analyses were intention-to-treat, performed overall and by sex using StataIC 16 (Stata Corp, College Station, Texas) and all estimates are reported with 95% Confidence Intervals (CIs). The sample size for the present study was pragmatic, based on the number of neonates enrolled in the main trial from when the potential importance of maternal BCG priming was recognized and the collection of maternal scar data initiated, and until the main trial was halted.[15]

RESULTS

Between October 2013 and August 2017, 3353 neonates were enrolled in the main trial; 1750 of these were enrolled before we initiated maternal scar assessments. For the remaining 1603 neonates, the maternal scar status was captured for 90% (1451/1603) of the mothers; 62% (903/1451) had a BCG scar. Mothers with a BCG scar had received more schooling, a higher proportion was literate (p<0.001 for both comparisons) and the proportion of males recruited in the study was 50% (452/903) for mothers with a BCG scar vs 57% (311/548) for mothers with no scar (p=0.01) (**Table 1**). Six infants (4 maternal scar, 2 no maternal scar) corresponding to 0.4% were lost to follow-up during the NICU admission.

All-cause mortality risk during NICU admission by maternal BCG scar status

For the entire cohort of both vaccinated and unvaccinated neonates, the overall mortality risk during NICU admission was 1.7% (15/903) if the mother had a scar and 3.3% (18/548) if not, the scar/no scar aMRR being 0.53 (0.26-1.05) (**Table 2**). By cause of death, the aMRR was 0.92 (0.08-10.8) for

birth complications, 0.80 (0.21-3.02) for infectious diseases, 0.38 (0.12-1.13) for prematurity/respiratory insufficiency and 0.39 (0.09-1.80) for unknown causes (**Table 2**).

All-cause mortality risk during NICU admission by maternal BCG scar status, stratified by offspring vaccination status

Among neonates randomized to vaccination with BCG+OPV at NICU admission, the overall mortality risk during NICU admission was 1.8% (8/441) if the mother had a scar and 2.8% (8/287) if not, the scar/no scar aMRR being 0.70 (0.26-1.87) (**Table 2**).

For the neonates randomized to control (vaccination at NICU discharge), the overall mortality risk during NICU admission was 1.5% (7/462) if the mother had a scar and 3.8% (10/261) if not, the scar/no scar aMRR being 0.39 (0.15-1.05) (**Table 2**). There was no difference in the effect of maternal BCG within randomization strata (p for same effect=0.42).

All-cause mortality risk during NICU admission and post-NICU discharge and up to 6 weeks of age (includes only vaccinated follow-up time)

The unvaccinated control group of the main trial was vaccinated at discharge from the NICU and for the combined vaccinated cohort, the mortality risk up to 6 weeks of age was 1.9% (17/896) for neonates born to mothers with a BCG scar and 3.0% (16/538) for those born to mothers with no scar, the corresponding overall aMRR being 0.74 (0.37-1.48) (**Table 3**).

Overall mortality risk from enrolment to 42 days of age (includes both vaccinated and unvaccinated follow-up time)

The overall mortality risk during NICU admission and post-discharge up to 42 days of age was 2.7% (24/903) if the mother had a scar and 4.7% (26/548) if the mother had no scar, the scar/no scar aMRR being 0.59 (0.34-1.04) (**Table 3**).

By birth weight, among 451 LBW neonates, the aMRR was 0.60 (0.30-1.18). For 1000 normal birth weight (NBW) neonates, the aMRR was 0.48 (0.18-1.39).

By strain of BCG administered, there were 834 neonates that received BCG-Denmark, 60% (498/834) were born to mothers with a BCG scar, and the overall aMRR was 0.57 (0.22-1.51). Among 600 neonates that received BCG-Japan, 66% (398/600) were born to mothers with a BCG scar, and the overall aMRR was 0.94 (0.34-2.59) (see **Supplementary Table 1**).

All-cause NICU mortality risk by main trial randomization allocation (BCG vs control)

The overall BCG/control MRR in the subgroup with maternal scar assessments was 0.90 (0.45-1.78). For neonates born to mothers with a BCG scar, the immediate BCG vs. no-BCG MRR was 1.21 (0.44-3.34) vs 0.65 (0.25-1.64) for neonates born to mothers with no BCG scar (p for same effect=0.37, **Supplementary Table 2**).

DISCUSSION

Main findings

Maternal BCG scars appeared to mainly affect the risk of death from prematurity/respiratory insufficiency during NICU admission and while maternal scars were associated with a trend for protection from death in the unvaccinated neonates, effects were comparable within randomization strata and not statistically significant overall.

The unvaccinated control group received BCG+OPV at discharge from the NICU, after which the beneficial effects of maternal priming were less pronounced.

Strengths and weaknesses

To our knowledge, this is the first prospective study to assess all-cause survival data by maternal BCG scar status within a frail cohort enrolled in an RCT with a strict protocol for the provision of neonatal vaccines. The control group was thus not vaccinated without our knowledge.

We initiated the assessments of maternal BCG scars in 2015 when we became aware of the possible importance. Enrolment into the trial was more than halfway completed at this time point and the NICU mortality risk had declined during the course of the trial and was substantially lower (<3%) than expected a priori (12%)[15], factors that reduced study power. We furthermore conducted several subgroup analyses of outcomes, which were assessed both pre- and post-discharge, but did not adjust estimates for multiple testing. Results from this explorative observational study should therefore be interpreted with caution.

In our data, maternal BCG scars were associated with the maternal education level, consistent with two BHP studies from the same period.[13,14] Similarly, a HNSM maternal scar study also reported a greater age spread for mothers with no scar, and that their babies had a slightly lower inclusion weight.[13] For the inclusion weight, the difference (21 g) in the present study was not significant, but even small differences in weight can be vital in LBW (<2500 g) and very-LBW (<1500 g) neonates. In our supplementary analysis, however, effects of maternal scar tended to be more pronounced in NBW rather than LBW neonates and mothers with no scar were not more likely to deliver LBW babies. Residual confounding cannot be ruled out. Speaking against this possibility, for BCG-vaccinated newborns, we note that both at the NICU and after discharge when the control group had been BCG-vaccinated, there was a consistent trend for a beneficial effect of maternal scars in males only, an observation consistent with recently published data.[13] If the effects had been caused by an undetected confounder associated with the maternal scar status and mortality risk, it would likely have affected mortality risk equally in both sexes.

Our team conducted daily follow-up at the NICU and the adjacent pediatric ward. Causes of death during admission were assessed by a senior pediatrician blinded to the neonatal and maternal vaccination status. However, our data regarding causes of deaths was limited by the availability of diagnostic tools, e.g., no laboratory results or blood-cultures, and sometimes insufficient clinical data. Hence, we encourage caution also in regard to interpreting the cause of death data.

Two separate BCG strains were used in the main trial, but the overall mortality risk was also declining during the study and substantial improvements at the NICU occurred in the last part of the study, which employed BCG-Japan.[15] Any deductions regarding differential effects of maternal BCG by offspring BCG strain is therefore likely confounded by chronology and there were no differences in maternal scar effects across three BCG strains used in our larger maternal scar sister-study.[13]

BCG vaccination does not always result in the development of a scar and the determinants for scar development is the BCG strain used and the vaccination technique, not host factors.[6,22] In a study from rural Guinea-Bissau involving >15,000 BCG-vaccinated children, only 52% developed a BCG scar[23], which is the lowest scar prevalence reported in the literature.[5]

Mothers with no scar in the present cohort will represent a proportion that was BCG-vaccinated but did not develop a scar, and a proportion that never received BCG. The relative proportions are unknown since maternal BCG vaccination data for the cohort is not available. Likewise, the coverage of other childhood vaccines in the cohort's mothers is unknown. In case maternal BCG vaccination not leading to a scar is also protective of NICU mortality, then the effects we report might have been reduced by misclassification bias. Alternatively, maternal BCG scars might be associated with other characteristics that influence early-life survival without being the protective factor itself. We reviewed available covariates and did not detect other factors associated with having a BCG scar aside from the maternal educational status. Control for maternal educational status did not change

estimates much. The fact that the same pattern of sex-differential effects of maternal BCG among the BCG-vaccinated offspring was found both in the present study and a recent study[13] speaks against an overarching unmeasured confounder being the explanation that best fits the available data. It is necessary for the hypotheses generated by the present study to be rigorously tested, from the epidemiological and immunological angle and preferably also within other settings to further pinpoint the relative importance for offspring outcomes.

Consistency with previous findings

The hypothesis that maternal BCG influences offspring outcomes came from studies finding a particularly beneficial effect of measles vaccination when given in the presence of maternal measles antibody.[24,25] In a concurrent Danish trial, we therefore investigated whether neonatal BCG was particularly beneficial when given to BCG-vaccinated rather than BCG-unvaccinated mothers, and that turned out to be the case.[10] Based on these observations, we initiated the present data collection with the hypothesis that maternal BCG would enhance the beneficial effect of BCG provided to the offspring. There are two additional observational studies from Guinea-Bissau focusing on maternal scars from the same period; the sister-study from the HNSM Maternity Ward[13] and a study from rural Bissau.[14] Both studies report maternal scar prevalences of 60-64% in accordance with the prevalence reported in the present study.

The study from rural Guinea-Bissau involves 1320 pregnant women that had their scar status assessed during pregnancy which allowed for an assessment of perinatal outcomes. The study reported a trend for fewer adverse pregnancy outcomes associated with maternal BCG scars; this trend was evident for stillbirths and early neonatal deaths, but not miscarriages.[14] Enrollment in the present study occurred later – after the child had been born – precluding any assessment of impacts on adverse pregnancy outcomes. But we identified indications of protection against perinatal deaths due to prematurity.

Our analysis is the first to present prospective data on maternal priming effects strictly controlled for offspring vaccination status. During the NICU admission, neonates were monitored daily with minimal loss-to-follow-up, and the unvaccinated control group was only vaccinated at discharge, after which we continued to monitor effects. This "controlled cross-over" RCT design enabled us to report maternal scar effects by offspring vaccination status, providing a unique 2x2 possibility to study the effects of maternal priming among both BCG-vaccinated and unvaccinated neonates.

In a recent retrospective analysis of a cohort of LBW neonates that had been randomized to BCG vs no-BCG at discharge from the hospital, the maternal scar effect estimate was slightly larger than in the present study.[12] This previous retrospective cohort featured a mix of BCG-vaccinated and unvaccinated neonates, where cross-over would have occurred naturally during follow-up. Effects of maternal priming appeared more enhanced in the BCG-vaccinated group, but the study included only a total of 23 deaths and was underpowered to thoroughly investigate subgroup effects by vaccination status and sex.

Outcomes by maternal BCG status have now been reported for six BHP cohorts with different study designs and thus differences in underlying confounding structures, yet maternal BCG scars have consistently been associated with beneficial overall effects on perinatal and infant health.[10–14]

While possible immunological pathways for NSEs of neonatal administration of BCG are "*trained innate immunity*", induction of emergency granulopoiesis and/or heterologous T-cell immunity.[26–29], the immunological pathways behind maternal priming effects in offspring remain to be identified. One possibility was suggested in a Ugandan study, where the presence of maternal BCG scars in BCG-vaccinated offspring was strongly associated with an enhanced offspring proinflammatory immune profile at 1- and 6-weeks post neonatal BCG.[30] In a study from the UK, it was reported that neonates born to BCG-vaccinated parents have a 4-fold higher Th17 (CD4+ IL-17+)

cell population as percentage of the total CD4+ T cell population.[31] The importance of the induction of Th17 cells by parental BCG priming for clinical outcomes is unknown, however.

Implications

BCG is among the world's most widely used vaccines with >120 million infants vaccinated per year and total vaccinations exceeding 4 billion.[32] Vaccines are often delayed for various logistic reasons[33] and vaccination often does not result in a scar due to inadequate vaccination technique and less effective strains.[5,6,16] A series of RCTs has demonstrated substantial beneficial effects on neonatal mortality and morbidity after BCG vaccination[1–4,7] and observational studies have demonstrated marked beneficial effects associated with BCG scars.[5,6,9] An emerging series of studies, including the present one, indicate that the presence of a maternal BCG scar is associated with marked beneficial health effects in the offspring. This emphasizes that there is a strong rationale to ensure provision of immunogenic BCG vaccines with adequate vaccination technique as early as possible after birth to produce high BCG scar rates at the population level.

CONCLUSION

In a cohort of frail neonates admitted to the NICU, presence of a maternal BCG scar tended to be associated with protection from death. This extends the findings of recent studies that point to marked beneficial overall effects for neonatal outcomes associated with maternal priming with BCG, warranting further studies.

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Footnote page

Conflict of interest statement

None of the authors have any conflict of interest to declare.

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Previous presentations of this information

The data presented in this article has not been presented at any meetings or conferences.

Data sharing statement

Deidentified 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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Authors' contributions

CSB and MBA were the principal investigators and guarantors of the main trial. CSB, PAA, MBA, FSB and TRK designed the study. FSB, IM, IS, MBA and NA supervised the data collection and data entry. FSB conducted the statistical analyses and wrote the first draft of the paper, and all authors approved the final manuscript.

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Supplementary Table 1. *Mortality risk by maternal BCG scar status and offspring BCG vaccination status, overall and by sex.*

Group	Maternal BCG scar % (deaths/enrolled)	No scar % (deaths/enrolled)	**MRR** (95% CI)[a]	**Adjusted MRR** (95% CI)[b]
Overall mortality risk by 42 days of age during NICU admission and after discharge (includes only BCG-vaccinated follow-up time)				
BCG-Denmark				
Overall (n=834)	1.4% (7/498)	3.0% (10/336)	0.46 (0.18-1.21)	0.57 (0.22-1.51)[c]
Males (n=436)	1.3% (3/235)	4.0% (8/201)	0.31 (0.08-1.16)	0.35 (0.09-1.34)
Females (n=398)	1.5% (4/263)	1.5% (2/135)	1.07 (0.20-5.88)	1.20 (0.22-6.67)
BCG-Japan				
Overall (n=600)	2.5% (10/398)	3.0% (6/202)	0.85 (0.31-2.35)	0.94 (0.34-2.59)[c]
Males (n=317)	3.8% (8/213)	4.8% (5/104)	0.77 (0.25-2.36)	0.83 (0.27-2.54)
Females (n=283)	1.1% (2/185)	1.0% (1/98)	1.12 (0.10-12.4)	1.26 (0.11-14.1)

[a] Cox Proportional Hazards model (crude).

[b] Cox Proportional Hazards model adjusted for level of maternal schooling and sex.

[c] p for same overall effect=0.38.

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval ; MRR, Mortality Rate Ratio.

Supplementary Table 2. *Mortality risk while admitted at the NICU by maternal BCG scar status and offspring BCG vaccination status.*

	Maternal scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)	**No maternal scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)**	**Maternal scar vs. no scar adjusted MRR[a]** (95% CI)	**p for same effect of maternal scar by randomization allocation**
Neonatal BCG	1.8% (8/441) [1.2] (6.7)	2.8% (8/287) [1.7] (4.7)	0.70 (0.26-1.87)	0.42
Control	1.5% (7/462) [1.0] (6.9)	3.8% (10/261) [2.8] (3.6)	0.39 (0.15-1.05)	
BCG/Control MRR[b]	1.21 (0.44-3.34)	0.65 (0.25-1.64)		
p for same effect of immediate vs delayed BCG by maternal scar status	0.37			

[a] Cox Proportional Hazards model adjusted for level of maternal schooling and sex.

[b] Cox Proportional Hazards model (crude). The overall BCG/Control MRR was 0.90 (0.45-1.78).

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio; NICU, Neonatal Intensive Care Unit; PYRS, Person-years.

Table 1. *Baseline Inclusion Characteristics at admission to the NICU by Maternal BCG scar status*

	Maternal scar	**No maternal scar**	
Included % (n/N)	62% (903/1451)[a]	38% (548/1451)	
Maternal Characteristics	**Maternal scar**	**No maternal scar**	**P-value**
Median maternal age in years (IQR)[b]	27 (23-30)	27 (20-33)	0.45
Median arm-circumference (MUAC) in mm (IQR)[b]	274 (252-298)	274 (250-298)	0.67
Reside in BHP HDSS % (n/N)[c]	13% (115/903)	14% (77/548)	0.47
Reside in rural areas % (n/N)[c]	29% (261/903)	31% (168/548)	0.48
No maternal schooling % (n/N)[c]	24% (214/897)	36% (198/545)	<0.001
Mother is literate % (n/N)[c]	75% (676/896)	63% (342/542)	<0.001
Infant Characteristics	**Maternal scar**	**No maternal scar**	**P-value**
Delivered by Caesarean Section, n/N %[c]	72% (652/901)	69% (374/545)	0.13
Mean Apgar score (1 minute after birth) [SD][b]	6.7 [2.1]	6.5 [2.0]	0.12
Low birth weight (<2500 g), n/N %[c]	31% (279/903)	31% (172/548)	0.85
Median birthweight in grams (IQR)[b]	2854 (2330-3360)	2836 (2250-3365)	0.94
Median inclusion weight in grams (IQR)[b]	2753 (2240-3250)	2732 (2150-3235)	0.82
Recruited on day of birth % (n/N)[c]	27% (247/903)	24% (131/548)	0.15
Male sex % (n/N)[c]	50% (452/903)	57% (311/548)	0.01
Twinning percentage % (n/N)[c]	21% (185/901)	17% (90/545)	0.10

[a] The same inclusion supervisor was responsible for all inclusions, including the assessment of the maternal BCG scar status.

[b] Kruskal-Wallis test.

[c] Pearson chi-squared test.

Abbreviations: BCG, Bacille Calmette-Guérin; BHP, Bandim Health Project; HDSS, Health and Demographic Surveillance System; IQR, Interquartile Range (25%-quartile – 75%-quartile); MUAC, Mid Upper Arm Circumference; NICU, Neonatal Intensive Care Unit.

Table 2. *Mortality risk during NICU-admission by maternal scar status, overall and by randomization allocation, stratified by sex.*

All neonates admitted to the NICU

Group	Maternal BCG scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)	No scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)	MRR (95% CI)[a]	Adjusted MRR (95% CI)[b]
Overall	1.7% (15/903) [1.1] (13.6)	3.3% (18/548) [2.2] (8.4)	0.50 (0.25-0.99)	0.53 (0.26-1.05)
Male	2.0% (9/452) [1.2] (7.2)	3.9% (12/311) [2.6] (4.6)	0.49 (0.20-1.16)	0.51 (0.21-1.21)
Female	1.3% (6/451) [0.9] (6.4)	2.5% (6/237) [1.6] (3.7)	0.55 (0.18-1.71)	0.56 (0.18-1.77)
By cause of death				
Birth complications	0.2% (2/903)	0.2% (1/548)	1.10 (0.10-12.2)	0.92 (0.08-10.8)
Male	0.2% (1/452)	0.3% (1/311)	0.58 (0.04-9.28)	0.54 (0.03-8.88)
Female	0.2% (1/451)	0.0% (0/237)	NA	NA
Prematurity/respiratory insufficiency	0.6% (5/903)	1.6% (9/548)	0.34 (0.11-1.02)	0.38 (0.12-1.13)
Male	0.9% (4/452)	1.6% (5/311)	0.55 (0.15-2.05)	0.57 (0.15-2.14)
Female	0.2% (1/451)	1.7% (4/237)	0.13 (0.01-1.20)	0.15 (0.02-1.39)
Infectious diseases	0.6% (5/903)	0.7% (4/548)	0.75 (0.20-2.81)	0.80 (0.21-3.02)
Male	0.4% (2/452)	1.0% (3/311)	0.38 (0.06-2.41)	0.39 (0.06-2.50)
Female	0.7% (3/451)	0.4% (1/237)	1.89 (0.19-18.7)	2.12 (0.21-21.4)
Unknown cause	0.3% (3/903)	0.7% (4/548)	0.45 (0.10-2.00)	0.39 (0.09-1.80)
Male	0.4% (2/452)	1.0% (3/311)	0.45 (0.08-2.70)	0.39 (0.06-2.35)
Female	0.2% (1/451)	0.4% (1/237)	0.52 (0.03-8.26)	0.41 (0.02-6.64)

NICU-admitted and allocated to immediate BCG+OPV				
Group	**Maternal scar**	**No scar**	**MRR** (95% CI)[a]	**aMRR** (95% CI)[b]
Overall	1.8% (8/441) [1.2] (6.7)	2.8% (8/287) [1.7] (4.7)	0.69 (0.26-1.83)[c]	0.70 (0.26-1.87)
Male	2.2% (5/231) [1.3] (3.7)	3.8% (6/157) [2.5] (2.4)	0.60 (0.18-1.96)	0.62 (0.19-2.04)
Female	1.4% (3/210) [1.0] (3.0)	1.5% (2/130) [0.9] (2.3)	1.02 (0.17-6.13)	1.19 (0.20-7.23)

NICU-admitted and allocated to control				
Group	**Maternal scar**	**No scar**	**MRR** (95% CI)[a]	**aMRR** (95% CI)[b]
Overall	1.5% (7/462) [1.0] (6.9)	3.8% (10/261) [2.8] (3.6)	0.37 (0.14-0.96)	0.39 (0.15-1.05)
Male	1.8% (4/221) [1.1] (3.5)	3.9% (6/154) [2.7] (2.2)	0.41 (0.11-1.45)	0.41 (0.11-1.48)
Female	1.2% (3/241) [0.9] (3.4)	3.7% (4/107) [2.8] (1.4)	0.32 (0.07-1.43)	0.31 (0.07-1.39)

[a] Cox Proportional Hazards model (crude).

[b] Cox Proportional Hazards model adjusted for level of maternal schooling and sex (overall estimate).

[c] Test of the Proportional Hazards assumption using Schoenfeld's residuals: p = 0.02

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio; NICU, Neonatal Intensive Care Unit.

Table 3. *Mortality risk up to 42 days of age by maternal BCG scar status and offspring BCG vaccination status, overall and by sex*

Group	Maternal BCG scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)	No scar % (deaths/enrolled) [Mortality rate per PYRS] (PYRS)	**MRR** (95% CI)[a]	**Adjusted MRR** (95% CI)[b]
Deaths among BCG-vaccinated neonates during NICU admission and after NICU discharge and up to 42 days of age[c]				
Overall	1.9% (17/896) [0.2] (80)	3.0% (16/538) [0.4] (46)	0.63 (0.32-1.25)	0.74 (0.37-1.48)
Male	2.5% (11/448) [0.3] (40)	4.3% (13/305) [0.5] (26)	0.56 (0.25-1.24)	0.61 (0.27-1.38)
Female	1.3% (6/448) [0.2] (40)	1.3% (3/233) [0.2] (20)	1.06 (0.27-4.45)	1.28 (0.32-5.15)
Overall mortality risk by 42 days of age during NICU admission and after discharge (includes BCG-vaccinated and unvaccinated neonates)				
Overall	2.7% (24/903) [0.3] (87)	4.7% (26/548) [0.5] (49)	0.53 (0.31-0.93)	0.59 (0.34-1.04)
Male	3.3% (15/452) [0.3] (43)	6.1% (19/311) [0.7] (28)	0.52 (0.26-1.02)	0.55 (0.28-1.09)
Female	2.0% (9/451) [0.2] (44)	3.0% (7/237) [0.3] (21)	0.64 (0.24-1.73)	0.70 (0.26-1.88)

[a] Cox Proportional Hazards model (crude).

[b] Cox Proportional Hazards model adjusted for level of maternal schooling and sex.

[c] Deaths among neonates allocated to BCG during NICU admission and after discharge plus control neonates that were BCG-vaccinated at discharge from the NICU.

Abbreviations: BCG, Bacillus Calmette-Guérin; CI, Confidence Interval; MRR, Mortality Rate Ratio; NICU, Neonatal Intensive Care Unit.