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Commentary

Maternal BCG primes for enhanced health benefits in the newborn

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SUMMARY

Objectives: Bacille Calmette-Guérin (BCG) vaccination lowers the risk of severe infection; we tested whether effects are modulated by maternal BCG in a large cohort of BCG-vaccinated newborns from Guinea-Bissau.

Methods: Maternal BCG scar status were inspected at enrolment in a BCG trial conducted from 2014 to 17 in Bissau, Guinea-Bissau. We tested associations with background factors for potential confounding; maternal age affected effect estimates >5% and accordingly, all analyses were adjusted for maternal age. Hospitalization data was collected prospectively and assessed in Cox-models providing adjusted Incidence Rate Ratios (aIRRs). In-hospital risk of death (case-fatality) risk was assessed using binomial regression providing adjusted Risk Ratios (aRRs).

Results: 60% (6,309/10,598) of mothers had a scar. The maternal-scar/no-scar admission aIRR was 0.96 (0.81–1.14) from 0 to 6 weeks and 1.12 (0.97–1.28) for 6 weeks-3 years. The 6-week in-hospital case-fatality infection aRR was 0.59 (0.34–1.05); 0.40 (0.17–0.91) for males and 0.86 (0.38–1.94) for females. Protection was especially evident against sepsis, the overall 6-week aRR=0.49 (0.26–0.91); no effect was observed for non-infectious deaths or after 6 weeks of age. Effects were similar across BCG strains and multivariate models adjusted for socioeconomic status did not affect estimates.

Conclusion: Among BCG-vaccinated newborns, there was a trend for fewer in-hospital deaths from infection associated with maternal BCG priming, especially for males. Providing BCG to adults without a vaccination scar might enhance their offspring's capacity to handle severe infections.

Brief 40-word summary: Within a trial comparing BCG strains for their overall effects on morbidity and mortality in Guinea-Bissau, vertical priming with BCG (represented by the maternal BCG scar) was associated with beneficial sex-differential effects on offspring survival.

Introduction

The early-life mortality remains high in developing countries and nearly half of under-5 deaths occur in the neonatal period.[1] Neonatal sepsis caused by a range of different bacterial agents, against which specific vaccines are not available, is a leading cause of death.[2] A strategy to reduce early-life infection severity is the at-birth provision of live vaccines such as Oral Polio Vaccine (OPV) and Bacille Calmette-Guérin (BCG).[3] In a series of randomized controlled trials (RCTs) conducted by the Bandim Health Project (BHP, www.bandim.org) in Guinea-Bissau, BCG provided at hospital discharge a few days after birth reduced infectious disease mortality risk by 40% (95% Confidence Interval (CI): 11–60%)[4], particularly due to protection against neonatal sepsis.[5,6] A recent RCT from Uganda similarly reported protective non-specific effects (NSE) of at-birth BCG against non-tuberculosis infectious diseases.[7]

The interaction between maternal and offspring immune systems across the placenta and during lactation are crucial for offspring immune system development.[8,9] Interestingly, there are indications that previous maternal BCG enhances the beneficial

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NSE of offspring BCG. A Danish RCT of neonatal BCG versus no-BCG found no overall effect on admission risk between 0 and 15 months, except within the subgroup born to BCG-vaccinated mothers, where BCG was associated with a 35% (6–55%) reduced risk of infectious disease admissions, compared to unvaccinated controls.[10] Similarly, a study from Guinea-Bissau reported that, compared to children with no scar, having a BCG scar was associated with a 66% (33–83%) reduction in subsequent all-cause mortality if the mother had a scar and no effect if not.[11] Furthermore, a retrospective study has indicated that maternal BCG is associated with a 60% (4–83%) reduction in all-cause mortality by 6 weeks of age.[12]

Within a large cohort of BCG-vaccinated neonates enrolled in a BCG trial with prospective follow-up[13], we tested the hypothesis that maternal BCG influences early-life morbidity and mortality.

Materials and methods

Setting

BHP maintains a Health and Demographic Surveillance System (HDSS) covering approximately 100,000 inhabitants in Bissau, Guinea-Bissau's capital. The data for the present study stems from an RCT conducted from 2014 to 2017 at the Hospital Nacional Simão Mendes (HNSM), which is the country's principal birthplace (6–7000 deliveries/year). BHP staff registers births and vaccinations at the maternity ward, where all neonates receive BCG+OPV at discharge. HNSM also hosts Guinea-Bissau's only specialized pediatric ward, where a dedicated BHP team registers admission data, including diagnoses assigned by the treating physicians.[6,13]

Study design

The main trial has been described elsewhere.[13] Briefly, BHP conducted a parallel-group RCT from December 2014 to October 2017 that enrolled 12,000 neonates for randomization 1:1 to BCG-Denmark or BCG-Russia. By July 2016, BCG-Denmark was substituted with BCG-Japan due to a manufacturing halt. Healthy neonates were vaccinated shortly after birth with the allocated BCG strain and OPV.

Enrollment and informed consent

Healthy neonates with no severe malformations were invited to participate at hospital discharge. We collected maternal socioeconomic data such as ethnicity, age, residential area and telephone contacts and recorded the neonatal weight and assessed the maternal BCG scar status and mid-upper-arm circumference (MUAC).

Assessment of parental BCG scars

We became aware of the possible importance of maternal priming in 2015[14] and conducted a 1-day BCG scar recognition training course for all study personnel, after which scar assessments by visual inspection of both arms was initiated on July 11, 2015.

Follow-up

Information on outcomes was obtained from HDSS home-visits, through telephone interviews and the HNSM pediatric ward.

Hospital registration: The BHP pediatric ward team collects data all days of the year in the triage room and on two daily rounds to all department beds. The registration system has functioned continuously during the trial and the following 3 years, providing long-term admission data. Admission diagnoses were registered from the charts of the treating physicians and divided into infectious and non-infectious diseases (**Appendix**). Infants were considered at risk of admission if they had not been registered to have died at follow-up, as done in the main trial analysis.[13] While the BHP pediatric ward is the main department that treats ill infants in the country and the cohort had been recruited and vaccinated at the adjacent maternity ward of HNSM, some infants in the cohort will have been treated and/or died at other hospitals or at home.

Telephone follow-up: We conducted telephone interviews at 6 weeks of age collecting data on admissions and deaths from families with a telephone contact registered.[13]

HDSS follow-up: HDSS infants received home-visits at 2- and 6 months of age where data on admissions and deaths were collected.[13]

Outcomes

The primary outcome of the main trial was all-cause admission at HNSM by 6 weeks of age and secondary outcomes were mortality (hospital and community deaths), also by 6 weeks of age. Recent BCG trials have focused on outcomes from 0 to 6 weeks of age to avoid interactions with subsequent vaccines administered from 6 weeks of age.[7,13,15]

For the present study, we have therefore examined the effect up to 6 weeks of age of maternal BCG priming on all-cause admission risk and in-hospital case-fatality risk. We assessed effects overall and on infectious and non-infectious admissions, since the previous studies indicate that BCG mainly affects the severity of infectious diseases.[5–7,10] As a secondary exploratory outcome, we examined effects between 6 weeks-3 years of age using the HNSM admission data.

Statistical analyses

Incidence Rate Ratios (IRRs) of hospital admission events by maternal BCG status (yes/no) were estimated in a recurrent-event Andersen-Gill Cox Proportional Hazards Model with age as the underlying time variable. Age was thus inherently controlled for. Person-years-of-risk (Pyrs) were calculated from the date of main trial enrollment. Infants were not considered at risk of admission while admitted; days admitted thus did not contribute Pyrs. Proportionality of hazards tests were computed using Schoenfeld's residuals. We tested associations between baseline inclusion characteristics and maternal BCG scar status; parameters that deviated between groups ($p<0.05$) and which altered the IRR estimate by >5%, when included in a model containing maternal BCG scar and the outcome variable, were included in a multivariate Cox-model. The same approach was done for the analysis of in-hospital case-fatality Risk Ratios (RRs), which are reported as Cohort Study RRs assessed using a generalized linear model with a log link function (binomial regression) providing approximate CIs. We conducted an analysis with censoring for two national child OPV vaccination campaigns, which provided OPV to all children between 0 and 59 months of age regardless of OPV vaccination status and occurred during the trial (I: October 2015, affecting both the 6-week and 3-year outcome; II: November 2017 when all cohort infants were >42 days old, affecting only the 3-year outcome), since OPV campaigns have been shown to influence all-cause mortality.[16,17] We conducted a complete case sensitivity analysis with adjustment for all parameters that were significantly different between the two groups. All analyses were performed overall, by sex and BCG strain using StataIC 16 (Stata Corp, College Station, Texas) and all estimates are reported with 95% CIs.

Results

The main trial analysis included 12,021 neonates of which 10,777 were recruited after initiation of maternal BCG scar assessments, which were performed for 98% (10,598/10,777); 60%

Table 1
Baseline inclusion characteristics by maternal BCG scar status.

	Maternal scar	No maternal scar	
Included % (n/N)	60% (6309/10,598)[1]	40% (4289/10,598)	
Maternal Characteristics			**P-value**
Median maternal age in years (IQR)[2],[4]	25 (22–29)	25 (20–30)	0.01
Median arm-circumference (MUAC) in mm (IQR)[2]	270 (250–296)	266 (246–290)	<0.001
Supplied at least one telephone contact % (n/N)[3]	93% (5841/6309)	89% (3822/4289)	<0.001
Reside in BHP Study Area % (n/N)[3]	19% (1192/6309)	18% (767/4289)	0.19
Reside in rural areas % (n/N)[3]	11% (690/6309)	13% (575/4289)	<0.001
Median maternal years of schooling, (IQR)[2, 4] [missing]	9 (6; 11) [712]	7 (4; 10) [760]	<0.001
Infant Characteristics			**P-value**
Delivered by Caesarean Section, % n/N[3, 5]	5.9% (368/6254)	6.0% (254/4240)	0.82
Admitted to pediatric department before inclusion % (n/N)[3]	1.6% (102/6309)	1.8% (76/4289)	0.54
Median birthweight in grams (IQR)[2]	3130 (2820–3420)	3090 (2790–3400)	<0.001
Median inclusion weight in grams (IQR)[2]	3000 (2710–3280)	2970 (2680–3250)	<0.001
Recruited on day of birth % (n/N)[3]	23% (1447/6309)	21% (915/4289)	0.05
Male sex % (n/N)[3]	51% (3216/6309)	53% (2259/4289)	0.09
Twinning percentage % (n/N)[3]	3.2% (203/6309)	3.6% (156/4289)	0.24

[1] Six inclusion assistants assessed maternal scars, with the prevalence ranging from lowest 57% (423/739) to highest 61% (709/1160) ($p = 0.10$) between assistants. There was no significant differences between the assessments of the different assistants (binomial regression).
[2] Kruskal-Wallis test.
[3] Pearson chi-squared test.
[4] 1,472 (14%) missing data points; maternal scar: 11% (712/6309), no maternal scar: 18% (760/4289).
[5] 143 (1.4%) missing data points.Abbreviations: BCG, Bacille Calmette-Guérin; BHP, Bandim Health Project; IQR, Interquartile Range (25%-quartile – 75%-quartile); MUAC, Mid Upper Arm Circumference.

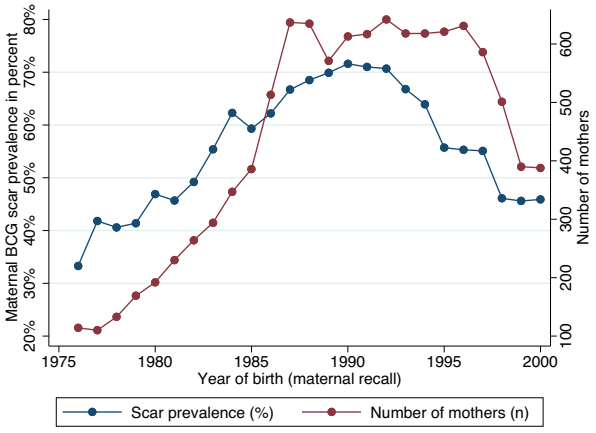

Fig. 1. Prevalence of maternal scars by self-reported year of birth. Mothers born before 1980 or after 2000 were grouped in 1980 and 2000, respectively. Note: In 1986–87, UNICEF initiated universal childhood immunization in Guinea-Bissau. In 1998–99, a civil war severely affected Guinea-Bissau.

(6309/10,598) of mothers had a scar (Table 1). There were no assessor differences in scar prevalence, which increased slightly from 58% (1352/2343) in 2015 to 60% (2144/3566) in 2017 and was associated with maternal age in a hill-shaped pattern (Fig. 1). There were fewer scars for older mothers born before 1983 (when regular childhood immunization programs began) and for younger mothers born in 1998 or later, where a civil war struck Bissau.

Associations between maternal BCG scarring and mother-child baseline characteristics

The age span of mothers with a scar was smaller (closer to 25 years of age) and their MUAC slightly larger (scar: 270 mm vs no scar: 266 mm), more supplied ≥1 telephone number (93% vs 89%), fewer were from rural areas (11% vs 13%), and mothers with a scar had more years of schooling (9 vs 7 years, Table 1). There was no difference in C-section (5.9% vs 6.0%) or twinning rates (3.2% vs 3.6%, Table 1). Neonates born to mothers with a scar tended to be recruited on the day of birth more often (23% vs 21%) and fewer tended to be male (51% vs 53%), and their inclusion weight was slightly higher (3000 g vs 2970 g, Table 1).

Morbidity and in-hospital deaths by 6 weeks of age

There were 550 admissions and 53 in-hospital deaths (46 from infection, 7 from non-infectious causes) by 6 weeks of age, the overall admission incidence being 5% (550/10,598) and the in-hospital Case-Fatality Rate (CFR) being 10% (53/550). The rate of successful follow-up for vital status during hospital admission was 99.8% (549/550).

The crude maternal scar/no maternal scar all-cause IRR was 1.00 (0.84–1.19) and the crude all-cause maternal scar/no maternal scar in-hospital mortality RR=0.60 (0.36–1.01).

In the multivariate analysis, only maternal age affected hospital admission and in-hospital case-fatality estimates, the overall aIRR being 0.96 (0.81–1.14) and the aRR being 0.62 (0.37–1.05) (Table 2). By sex, the 6-week male aIRR was 0.89 (0.70–1.11) vs 1.08 (0.82–1.41) for females (Table 2), and the all-cause case-fatality aRR for males was 0.48 (0.23–0.98) vs 0.83 (0.38–1.81) for females (Fig. 2, p for same effect=0.30).

For infectious disease deaths, the CFR was 7% (21/300) for maternal scar vs 12% (25/209) for no scar, the aRR being 0.59 (0.34–1.05) (Table 2). For males, the aRR was 0.40 (0.17–0.91) vs 0.86 (0.38–1.94) for females (Fig. 2, p for same effect=0.20). Censoring for the OPV campaign did not affect 6-week infectious disease estimates.

Maternal scars appeared especially protective against early-life sepsis, the aRR being 0.44 (0.21–0.92) in the first week of life; 0.39 (0.14–1.09) for males and 0.49 (0.16–1.44) for females (**S**Table 1). By 6 weeks of age, the same aRRs were 0.49 (0.26–0.91); 0.35 (0.14–0.89) for males and 0.62 (0.26–1.47) for females (Fig. 3, **S**Table 1).

Sensitivity analysis

When adjusting for all background factors that differed significantly between the 2 groups among neonates with no missing data ($n = 9096$), the all-cause aIRR was 0.92 (0.76–1.12) and the all-

Table 2
Admission incidence and case-fatality rate within 6 weeks of age by maternal BCG scar status and sex.

	Maternal BCG scar (N = 6309)		**No maternal scar** (N = 4289)		Maternal scar/no scar Risk of admission aIRR (95% CI) [a]	Maternal scar/no scar Case-Fatality aRR (95% CI) [b]
	Admissions [Fatal]	Admission rate per Pyrs (total Pyrs)	Admissions [Fatal]	Admission rate per Pyrs (total Pyrs)		
All admissions						
Male (n = 5475)	176 [11]	0.50 (355)	133 [18]	0.54 (249)	0.89 (0.70–1.11)	0.48 (0.23–0.98) [c]
Female (n = 5123)	152 [14]	0.44 (343)	89 [10]	0.39 (225)	1.08 (0.82–1.41)	0.83 (0.38–1.81)
Total (n = 10,598)	328 [25]	0.47 (697)	222 [28]	0.47 (474)	0.96 (0.81–1.14)	0.62 (0.37–1.05) [d]
Infectious diseases [e]						
Male (n = 5475)	160 [8]	0.45 (355)	127 [16]	0.51 (249)	0.84 (0.67–1.07)	0.40 (0.17–0.91) [f]
Female (n = 5123)	140 [13]	0.41 (343)	82 [9]	0.37 (225)	1.08 (0.82–1.42)	0.86 (0.38–1.94)
Total (n = 10,598)	300 [21]	0.43 (697)	209 [25]	0.44 (474)	0.94 (0.78–1.12)	0.59 (0.34–1.05)

Abbreviations: aIRR, adjusted Incidence Rate Ratio; aRR, adjusted Risk Ratio; Pyrs, person-years.
[a] Recurrent-event Andersen-Gill Cox Proportional Hazards Model adjusted for maternal age, tests of the assumption of proportionality of hazards using Schoenfeld's residuals: $p>0.05$.
[b] Cohort Study Risk Ratio assessed by binomial regression adjusted for maternal age.
[c] p for same effect (male vs female)=0.30.
[d] The overall effect of the maternal scar status on in-hospital mortality risk was comparable for city dwellers, RR=0.62 (0.35–1.09) and infants from rural areas, RR=0.64 (0.17–2.47).
[e] There were 46 in-hospital deaths (maternal scar: 21, no maternal scar: 25) due to infectious causes by 42 days of age: abscess, 1 vs 0; bronchitis, 2 vs 1; malaria, 2 vs 1; sepsis, 16 vs 23). Non-infectious causes of death (maternal scar: 4, no maternal scar: 3) were: severe anemia, 3 vs 0; dehydration, 1 vs 2; malnutrition, 0 vs 1).
[f] p for same effect (male vs female)=0.20.

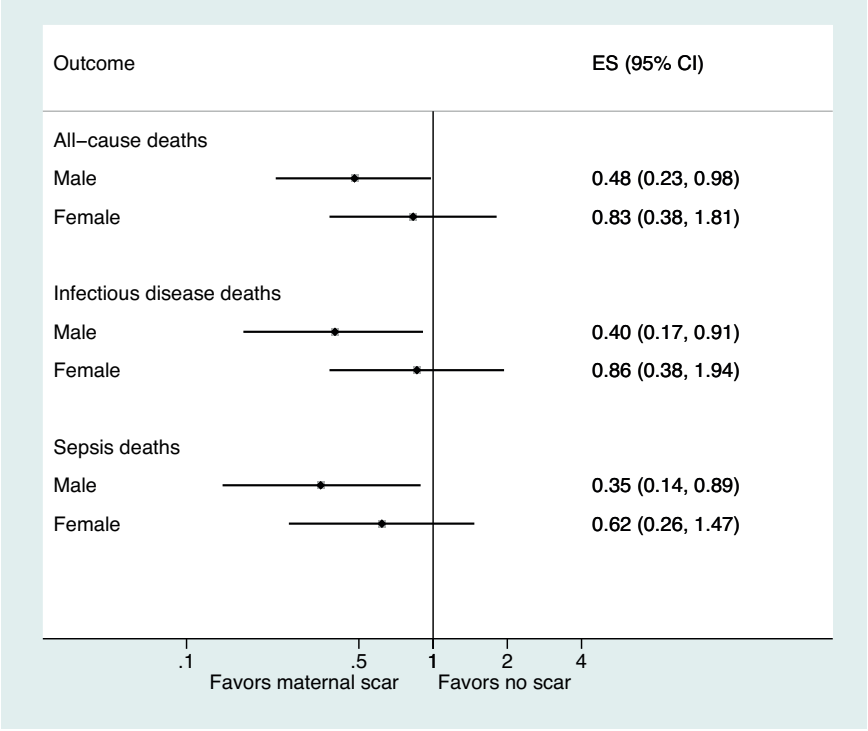

Fig. 2. Maternal scar versus no maternal scar in-hospital mortality risk up to 6 weeks of age by admission cause.
Abbreviations: CI; Confidence Interval; ES, Effect Size.

cause in-hospital mortality aRR 0.70 (0.40–1.24). By sex, the male aIRR was 0.82 (0.64–1.05) vs 1.11 (0.82–1.51) for females and the all-cause case-fatality aRR was 0.51 (0.24–1.12) for males vs 0.98 (0.42–2.27) for females. For infectious disease deaths, the fully adjusted aRR was 0.68 (0.37–1.28); 0.43 (0.17–1.06) for males vs 1.03 (0.42–2.53) for females.

Effects by BCG strain and after 6 weeks of age

There were no observed differences in maternal priming effects between the three BCG strains administered during the main trial (**STable 2**) and we did not detect effects of maternal priming between 6 weeks to 3 years of age (**STable 3**).

Discussion

To our knowledge, this study is the first to prospectively compare the early-life and long-term health effects of maternal priming with BCG. We have previously reported that neither BCG vs no BCG[6] nor BCG strains[13] affect hospital admission risk in Guinea-Bissau, but rather the neonatal mortality risk, indicating that BCG reduces the *severity* of infection rather than the *risk* of infection. The same pattern was evident for maternal BCG priming, which

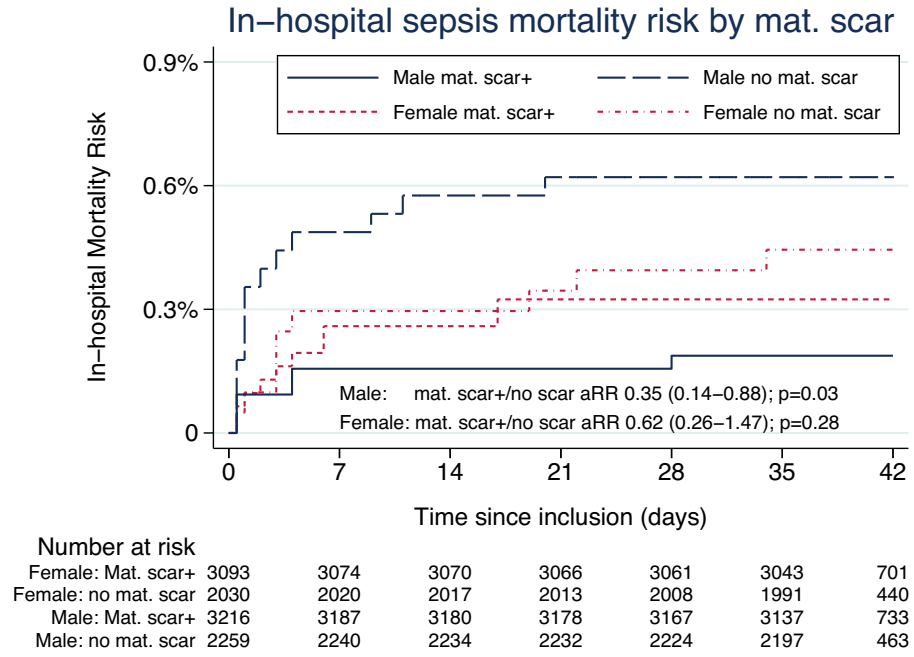

Fig. 3. In-hospital sepsis mortality risk by maternal scar status up to 42 days after birth.
Abbreviations: aRR; adjusted Risk Ratio. The statistical analysis for the in-hospital case-fatality risk ratios is a generalized linear model with a log link function (binomial regression) providing approximate confidence intervals.

was associated with a trend for reduced case-fatality risk for admitted newborns.

Based on an analysis of three RCTs, we previously reported strong protective effects of neonatal BCG vs. no neonatal BCG on the in-hospital neonatal mortality risk; the protective effect against neonatal sepsis was especially pronounced.[6] In the present study, maternal BCG priming was associated with an in-hospital mortality risk reduction of similar magnitude, and protective effects were likewise more pronounced against neonatal sepsis. Maternal priming appeared to be mainly beneficial for males during the first 6 weeks of life. Effects were less pronounced from 6 weeks and up to 3 years of age.

Strengths and weaknesses

Practically all neonates were vaccinated at birth making our analysis representative of the recommended vaccination schedule; interference on BCG's effects from pre-vaccination exposure to environmental mycobacteria should therefore be minimal.

Mothers with a BCG scar were socio-economically better positioned. They had an age distribution that might enhance offspring survival, more years of schooling, and the inclusion weight of their offspring was slightly higher. These differences could affect offspring outcomes and the cohort born to mothers with no scar could thus have been weaker than the maternal scar cohort, but statistical adjustments accounting for these imbalances did not alter effect estimates.

We present a large cohort of maternal scar assessments with prospectively collected follow-up data focusing on the first 6 weeks of life to exclude interactions from subsequent infant vaccinations such as diphtheria-tetanus-pertussis vaccination, which could affect outcomes.[18] We could indeed only detect an effect in the first 6 weeks of life, when both the overall mortality risk and the burden of infectious diseases are also higher.

At the HNSM pediatric ward, diagnoses are mainly based on clinical presentation due to scarce resources. We conducted a BCG scar assessment course for all trial personnel prior to initiation of data collection, and there were no inter-observer discrepancies in scar prevalence.

Interpretation and consistency with previous findings

The present data corroborates previous studies indicating that BCG's beneficial NSE in offspring are enhanced when administered in the presence of maternal immunity.[10–12] Consistent with previous reports regarding offspring BCG effects[5–7,19], the maternal priming effects we report were mainly against deaths from infectious diseases and especially sepsis, corroborating the plausibility of these novel findings.

RCTs from Guinea-Bissau[20] and Uganda[7] have reported sex-differential effects of neonatal BCG vaccination with effects being more marked for males. Previous data[11,12] and the present study indicates that the prevalence of maternal scars was likely high when the Guinea-Bissau RCTs were conducted, which could help explain the marked effect of BCG on male mortality risk. A recent retrospective study from Guinea-Bissau did not report sex-differential effects of maternal priming, however, but the study also involved a cohort of both BCG-vaccinated and unvaccinated newborns.[12]

There has been substantial effect heterogeneity in historical trials that evaluated the protective effect of BCG against TB, efficacy estimates ranging from 0%−80% across different trial designs, settings and time.[21] The variations in geographical settings and time periods makes it likely that there has been a substantial variation in the underlying maternal scar prevalence. If maternal priming substantially enhances immune responses to BCG, it might also affect protection against TB. Maternal priming might therefore have been an important unmeasured confounder.

While the mechanism of action behind maternal priming is unknown, maternal BCG has been associated with an enhanced offspring proinflammatory immune profile.[22] Placental colonization after maternal blood-to-decidua transfer early in gestation is a possible mechanism of vertical transmission to the fetus of mycobacterial L-forms of BCG.[23] Since BCG induces trained immunity by

epigenetic modifications in the bone marrow[24], another route of transmission could be the transfer of a more adept maternal epigenetic immune system setup to the developing fetal immune system in utero or shortly after birth through lactation and/or the microbiota. A recent study also provides evidence for inheritance of trained immunity in mammals, enhancing offspring protection against infections.[25] The rapid BCG-induced protection from severe sepsis might be mediated by Granulocyte Colony-Stimulating Factor (G-CSF) induction of an emergency granulopoiesis response, but it is unknown whether maternal priming affects this response.[26] Interestingly, a recent study in UK neonates also reported a BCG-induced emergency granulopoiesis response resulting in elevated neutrophil counts, but only for males.[27] Also of possible importance, another UK study focusing on neonatal immune responses reported a 4-fold expansion of Th17 (CD4+ IL-17+) cell prevalence associated with parental priming with BCG.[28]

BCG has been shown to enhance antibody responses to subsequent vaccines[29–31] and it has been established that infant immunity against early-life respiratory infections is strengthened by vertical transfer of protective antibodies.[32] If prior BCG vaccination had equipped the mother with a more well-functioning immune system producing enhanced antibody responses after subsequent vaccines and infectious challenges, then vertical transfer of an enhanced array of protective antibodies to the child provides another feasible mechanism.

A series of studies conducted in Guinea-Bissau, where a substantial proportion of mothers would be BCG-vaccinated, have demonstrated that randomization to BCG versus no-BCG, developing an early BCG skin reaction+reaction size[19], having a BCG scar[33] or a positive tuberculin skin test (TST)[34] are all associated with substantial 30%–50% reductions in subsequent infant mortality. Both the formation of scars and TST responses are unaffected by maternal BCG status[19,34], but a previous study indicated that among infants whose mother had no scar, having a scar vs. no scar had no effect.[11] Perhaps vaccination in the presence of maternal immunity should thus be considered a booster dose of BCG, which has previously been shown to be associated with additional beneficial NSE.[35]

Perspectives

Enhancing the maternal-offspring immune system dyad towards a more favorable profile is an important strategy to reduce newborn mortality. It would be useful if additional studies further evaluate the effects of maternal BCG, preferably in other settings with different underlying mortality risk and maternal BCG coverage. Furthermore, a randomized prospective design studying the effects of maternal vaccination on the offspring's immune system and general health would be ideal to further pinpoint effects and the immunological mechanisms. One such study, *Measles and BCG Vaccines for Mother and Child (MATVAC)*, is underway.[36] The possibility that maternal BCG enhances BCG's beneficial NSE in offspring could have far-reaching consequences. If BCG vaccination not only markedly benefits the recipient, but also future offspring, then ensuring a high neonatal BCG vaccination coverage is even more crucial. To increase the impact of immunization programs, barriers to vaccination should be reduced or avoided completely.[37,38] Furthermore, vaccinating with efficacious strains using adequate technique would increase scar prevalence, scar sizes and TST response rates.[13,34,39,40]

It should be considered to revaccinate scar-negative infants and adults, and the effects of increasing the BCG dose from 0.05 ml to the previously used 0.1 ml is worth evaluating, perhaps especially for less immunogenic strains such as BCG-Russia, since the main determinants of scar development and TST responses are the BCG strain[13,39,40] and the dose administered.[41,42]

Since 1978, we have monitored the Guinea-Bissau all-cause child mortality rate, which has declined significantly between 2000 and 15, even though standards of living and hospital standards are largely unchanged. A large proportion of this reduction can be attributed to fewer infectious disease deaths and our data indicates that a higher neonatal BCG and OPV coverage[5,6,43], a high frequency of OPV campaigns[16] and an increasing BCG scar prevalence among women in the fertile age[11,12] have likely contributed importantly to this decline.

Conclusion

There was a trend of reduced risk of in-hospital death associated with maternal BCG priming, a protection that was strongest against neonatal sepsis, and for males. Children of mothers with a scar are not more likely to develop a scar after vaccination.[11,19] Since careful assessment of possible confounders did not identify major confounding of this sex-differential effect, it appears unlikely that genetic or confounding factors would explain why maternal BCG scars appears to have marked beneficial effects on infant survival. The available data thus associates offspring survival with the mother-child dyad of successful BCG vaccination and suggests that the BCG scar is an important marker of well-trained immune systems both in mother and child. Providing BCG to infants and women in the fertile age that have no scar should be considered as a public health strategy to reduce infant mortality.

Ethical approval

The study protocol was approved by the Guinea-Bissau Health Ministry's Research Coordination Committee (Reference number: 0020 CNES/INASA/2014) and consultative approval was given by the Central Danish Ethical Committee (Case No: 1,407,397). The trial was conducted in accordance with the Helsinki Declaration ethical standards and a Data Safety Monitoring Board oversaw the main trial.

ClinicalTrials.gov registration number: NCT02447536.

Notes

Author contributions. CSB and PA were the principal investigators and guarantors. CSB, FSB, MBA and PA designed and initiated the RCT. CNG, EBS, FSB and IM were responsible for the recruitment and follow-up of participants and FSB wrote the first draft of the paper. CBØ and FSB were responsible for the statistical analyses assisted by MB, who conceptualized the paper with FSB. All authors contributed to and approved the final paper.

Disclaimer

The funding agencies had no influence on the study design, data collection, analysis, interpretation or writing of the manuscript, nor the decision to submit the paper for publication. The authors had full access to all study data and bears the responsibility for their analysis and the decision to submit for publication.

Declaration of Competing Interest

At the time the data collection was conducted, several of the authors were affiliated with the Statens Serum Institute (SSI) in Copenhagen which administered, but did not finance, their grants. SSI was a producer of BCG. However, SSI did not fund the vaccines, the study, or the researchers and neither the SSI or funders had any influence on the study design, data collection, analysis, interpretation or writing of the report, nor the decision to submit the paper for publication. None of the authors have any commercial,

financial, or personal interests, relationships or other associations that might pose a conflict of interest in relation to this study.

Data sharing agreement. 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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