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Published in:
Vaccine

DOI:
10.1016/j.vaccine.2021.06.077

Publication date:
2021

Document version:
Final published version

Vaccine 39 (2021) 7286–7294

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Neonatal Bacille Calmette-Guérin vaccination and tuberculin skin test reactions at 2- and 6-months: Effects on mortality up to 1 year of age

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ARTICLE INFO

Article history:
Available online 3 July 2021

Keywords:
Bacille Calmette-Guérin (BCG)
Purified Protein Derivative (PPD)
Tuberculin Skin Test (TST)
Mantoux reaction
Infant mortality
Non-specific effects of vaccines
Heterologous effects of vaccines

ABSTRACT

Background: In randomized trials, Bacille Calmette-Guérin (BCG) vaccine has been associated with reduced all-cause mortality. BCG-induced Tuberculin Skin Test (TST) reactions have also been associated with reduced all-cause mortality. We aimed to assess the association between TST responses and subsequent mortality in three birth cohorts and conducted a meta-analysis of existing studies.
Methods: Observational study within three Guinea-Bissau BCG trial birth cohorts (conducted 2002–04, 2009–2013 and 2014–18) that encompassed children who were BCG-vaccinated within 28 days with TSTs performed at 2- (n = 1389) and 6-months (n = 2635) of age. We evaluated TST reaction determinants by binomial regression and assessed the association between TSTs > 1 mm (reactors) vs. ≤ 1 mm (non-reactors) and subsequent mortality risk up to age 12 months in Cox-models providing Mortality Rate Ratios (MRRs). We searched PubMed for studies to calculate meta-estimates of the association between TST reactivity by age 2- and 6-months and all-cause mortality.
Results: Large post-vaccination wheal size was associated with 6-month TST positivity and so was receiving BCG-Denmark or BCG-Japan, compared with BCG-Russia. By age 2 months, 22% (302/1389) of infants were TST reactors with a 2–12-month mortality risk of 1.7% (5/302) vs. 3.3% (36/1087) for non-reactors, the corresponding reactor/non-reactor MRR = 0.49 (0.19–1.26). By age 6 months, 44% (1149/2635) of infants were reactors and the 6–12-month mortality risk was 0.4% (4/1149) vs. 0.6% (9/1486) for non-reactors, the MRR = 0.87 (0.27–2.86). The literature search provided 3 studies. The meta-analysis revealed a uniform pattern of reduced mortality associated with TST reactivity, a TST response by 2 months being associated with an MRR of 0.59 (0.39–0.90); for 6-month TST responses the MRR was 0.65 (0.43–1.00).
Conclusion: Among BCG-vaccinated infants, TST reactions were associated with markedly reduced mortality. Improved vaccination technique and using certain BCG strains could lead to a higher TST reaction prevalence, which would enhance BCG's beneficial non-specific effects.

1. Introduction

Vaccination with Bacille Calmette-Guérin (BCG) against tuberculosis (TB) has been associated with reduced all-cause mortality [1,2]. In three randomized controlled trials (RCTs) conducted in Guinea-Bissau, receiving early BCG-Denmark vs no-BCG (usual practice) was associated with a 38% (95% Confidence Interval (CI): 17%–54%) reduction in neonatal mortality [3] and a 54% (2%–78%) reduction in the risk of fatal sepsis [4]. Similarly, a recent trial from Uganda reported that receiving BCG-Denmark at birth was associated with a 29% (5%–47%) reduction in the risk of non-TB infections in the first 6 weeks of life, when compared to no-BCG [5]. In contrast to these findings, two RCTs conducted in India reported no effect of BCG-Russia [6]. Observational studies have

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https://doi.org/10.1016/j.vaccine.2021.06.077
0264-410X/

consistently indicated that among BCG-vaccinated infants, those that develop a BCG scar at the local injection site have a 30–50% lower mortality risk compared to those that do not develop a scar [7–14].

Aside from assessing BCG scars, the delayed type hypersensitivity response to BCG can be evaluated using the tuberculin skin test (TST). The association between having a TST reaction and all-cause mortality has been evaluated in three studies that included four birth cohorts from Guinea-Bissau; having a TST reaction was associated with 30–50% reductions in subsequent all-cause mortality [7–9].

There are indications that BCG strains vary with regards to their ability to induce BCG scars and TST reactions [15]. More than 120 million infants receive BCG yearly [16] and if immunogenic BCG strains induce more scars and TST conversions, then providing such strains early could markedly benefit the world's most vulnerable populations.

We therefore analysed additional data for infants that had been vaccinated in the neonatal period with various BCG strains in Guinea-Bissau, reviewing determinants of TST reactions and testing whether having a TST reaction versus no reaction affects subsequent all-cause mortality risk. We conducted a meta-analysis of the available studies to provide estimates of the effect of having a TST at 2- and 6-months of age on subsequent all-cause mortality risk up to 1 year of age.

2. Methods

2.1. Setting

The 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, a developing country with high child mortality. The country's main hospital facility and principal birthplace with 6000–7000 births/year, Hospital Nacional Simão Mendes (HNSM), is located within a few kilometres. Approximately 40% of HDSS births occur at HNSM, where BHP staff documents all deliveries, and two experienced BHP vaccinators provide neonatal vaccines (BCG and Oral Polio Vaccine (OPV)) at discharge from the maternity ward. Neonates delivered at home are typically brought for vaccination at the local health centre, where the subsequent infant vaccines are also provided. Since 2002, BHP has conducted a series of trials at HNSM evaluating the overall health effects of neonatal BCG vaccination [1,3,15,17,18].

2.2. Participants

The eligibility criteria for infants to be included in the present study were: 1) being enrolled in one of three BCG RCTs (Table 1) and having been BCG-vaccinated by our team during the neonatal period (first 28 days of life), 2) to have received standardised routine RCT home-visits providing mortality data up to age 1 year, and 3) having had a TST performed at age 2 and/or 6 months.

2.3. Study design

This observational study includes data collected within two RCTs of BCG-Denmark versus no-BCG that were conducted between 2002 and 2004 (RCT I) [18] and 2009–2014 (RCT II) [3], and an RCT of BCG strains conducted between 2014 and 2018 (RCT III) [15]. For the present study, we included only neonates randomized to receive early-BCG by our study team within the neonatal period to allow time for a TST reaction to have developed by the time of the home visit. We also conducted a trial of BCG-Denmark versus no-BCG between 2004 and 2009 and have published the data comparing all-cause mortality for TST reactors versus non-reactors [9].

The three trials provided BCG shortly after birth with the following study designs: RCT I and RCT II randomized healthy low-weight (LW) neonates at HNSM and HDSS health centres to early BCG-Denmark (Copenhagen strain 1331, Statens Serum Institut (SSI), Denmark) versus no-BCG; infants were offered TSTs at 2- and 6-months of age irrespective of where they resided. RCT III compared different BCG strains provided to LW and normal-weight neonates. The trial was divided in two phases: from 2014 to 2016, neonates were randomized to BCG-Denmark (strain 1331, SSI) versus BCG-Russia (Moscow strain 361-I, Serum Institute of India (SII), India) in Phase I and BCG-Japan (Tokyo strain 172–1, Japan BCG Laboratory, Japan) versus BCG-Russia (strain 361-I, SII) in Phase II from 2016 to 2018. Infants from RCT III that resided within the HDSS were offered TSTs at 6 months of age and were therefore eligible for the present study.

BHP staff vaccinated neonates included in the present study by intradermal injection of 0.05 ml BCG in the left deltoid region, followed by vaccination with OPV. Information on maternal age and mid-upper-arm-circumference (MUAC) and the infant birth- and inclusion weight was registered during inclusion procedures. Children were excluded if they were severely malformed or moribund.

2.4. Study hypotheses and sample size

The hypotheses of the study was that 1) having a TST reaction at 2- or 6-months of age is associated with reduced subsequent all-cause mortality and 2) that the main determinants for developing a TST reaction are vaccination technique and the strain of BCG used.

The sample size for the present study was based on the available cohorts where TSTs had been applied within the three respective trials that had recruited neonates for the purpose of assessing effects of BCG vs no-BCG or different strains on all-cause mortality risk [3,15,18].

2.5. Data collection procedures

The HDSS provided address information to locate HDSS infants while non-HDSS infants were transported home after enrolment (RCT I-II); our assistant drew a map to relocate the house. Field assistants conducted standardised home-visits at age 2, 6 and 12 months (RCT I-II) or at age 2 and 6 months (RCT III). For RCT III, the HDSS provided 6–12-month mortality data. Families are sometimes absent/travelling when our team arrives. We therefore returned three times to each house and if all visits were unsuccessful, information on survival was collected from those present. Infants whose family had moved were censored in the survival analysis from the date they had moved.

At the 2- and 6-month home-visits, growth and BCG scar data was collected, and a trained nurse informed the family about the possibility of having a TST performed and explained the purpose. Provided that informed consent was given, a 0.1 ml TST (PPD RT23, SSI, Copenhagen, Denmark) was applied intradermally in the ventral aspect of the forearm. Most of the infants enrolled in Phase I of RCT III received TSTs of 10 Tuberculosis Units (TU) due to unavailability of 2 TU TSTs, while TST 2 TU was used exclusively in RCTs I-II and Phase II of RCT III.

The TST response was read by measuring the reaction height and width using the ballpoint pen technique [19] 48–72 h after application, as recommended by the manufacturer. The TST reaction size was calculated as the height plus width divided by two. If the TST was > 10 mm, the child was considered a TB suspect and referred for medical consultation.

Table 1
Overview of the RCTs which formed the basis for the present study.

	Time Period	Study design	Place of inclusion (%)	Eligibility criteria	Strain(s) of BCG given	Infants enrolled	Infants that received neonatal BCG	Present at 2-month home-visit	TST at 2-month home visit[a]	Present at 6-month home-visit	TST at 6-month home visit[a]
RCT (#)											
LBW I[b] [16]	2002–2004	Early-BCG vs. no-BCG, follow-up at 2, 6 and 12 months	Health Centers (100%)	LBW, healthy, no severe malformations	Denmark	104	47	35	33	29	27
LBW II[b] [2]	2009–2013	Early-BCG vs. no-BCG, follow-up at 2, 6 and 12 months	HNSM (90%) Health Centers (10%)	LBW, healthy, no severe malformations	Denmark	4158	2058	1657	1356	1451	1208
BCGSTRAIN III[c] [14]	2014–2018	BCG-Denmark vs BCG-Russia, BCG-Japan vs BCG-Russia, follow-up at 2 and 6 months, 6–12 months HDSS follow-up	HNSM (100%)	Healthy LBW and NBW neonates born at HNSM with no severe malformations	Denmark, Japan, Russia	2529 from HDSS area	2528	1992	0	1729	1398
RCTs I-III combined	2002–2004, 2009–2018	–	HNSM (92%) Health Centers (8%)	–	Denmark, Japan, Russia	12 465	14 093	3684	1389	3209	2635

Abbreviations: BCG, Bacille Calmette-Guérin; HDSS, Health Demographic Surveillance System; HNSM, Hospital Nacional Simão Mendes; LBW, Low Birthweight; NBW, Normal Birthweight; RCT, Randomized Controlled Trial.
[a]These numbers include only infants that had the TST applied and where the test result was evaluated within 48–72 h. [b]RCTs of early BCG-Denmark versus no-BCG to neonates with low weight (<2500 g) on the day of inclusion. Within RCTs I-II, a total of 2105 infants received neonatal BCG and were visited at home at 2 months of age where a TST could be applied. Of the 2105 infants, 1692 (80%) were found at home by 2 months of age (40 had moved, 94 had died, 39 were absent, 232 had travelled, 3 refused the visit and 5 could not be located). Of these, 94% (1588/1692) had a TST applied and of these, 87% (1389/1588) had the TST reaction evaluated within 48–72 h (3 had moved, 163 were absent, 17 had travelled and 16 were not evaluated for other reasons). Equally by 6 months and within RCTs I-III, a total of 4618 infants received neonatal BCG and were visited at home at 6 months of age where a TST could be applied. Of the 4618 infants, 3209 (69%) were present at the home-visit (300 had moved, 154 had died, 146 were absent, 778 had travelled, 2 refused the visit, 2 were hospitalized and 27 could not be located). Of the 3209 infants found at home, 95% (3038/3209) had a TST applied and of these, 87% (2635/3038) were found at home between 48 and 72 h later and had the TST reaction evaluated (5 infants had moved, 329 were away, 33 had travelled, 1 refused the visit and 35 were not evaluated for other reasons).
[c]RCT of BCG-Denmark versus BCG-Russia (Phase I, 5677 neonates) and BCG-Japan versus BCG-Russia (Phase II, 6344 neonates); approx. 20% of the cohort were from residents in the Health and Demographic Surveillance System (HDSS) and could thus be included for the present study provided that a TST test was applied and evaluated within 48–72 h.

2.6. Statistical Methods

Since our focus was on the more modest effect of BCG on TST conversions rather than TB exposure, we classified TSTs > 1 mm as positive (reactors) and ≤ 1 mm readings as negative (non-reactors), as done in previous studies [7–9]. We tested the following determinants of 2-month TST reactions to calculate risk ratios (RRs) using binomial regression: maternal MUAC, age, place of inclusion (hospital or health centre), weight at vaccination, vaccinator, and age at vaccination. Missing data was addressed by mean substitution. For 6-month TST reactions, we furthermore tested whether maternal BCG scarring, post-vaccination wheal size and BCG strain were TST reaction determinants. We tested whether the weight at vaccination, maternal MUAC, 2-month infant MUAC, age at BCG vaccination, place of vaccination, sex, age at 2-month visit and the assistant evaluating the 2-month reaction influenced mortality estimates by >5%. Only the 2-month infant MUAC affected the estimate by >5%. We did not adjust for infant MUAC as we considered the infant MUAC a mediator of the TST effect, as in our recent analysis of early BCG reactions [14].

The 2–12-month and 6–12-month mortality risk by 2- and 6-month TST reaction status was assessed in crude Cox Proportional Hazards Models providing Mortality Rate Ratios (MRRs) with age as the underlying time variable; age was thus inherently controlled for in all analyses; the 6-month analysis was stratified with equal coefficients across RCT strata to account for differences between RCTs I-II and RCT III, such as the underlying mortality risk and the prevalence of positive TST reactions.

Infants entered the survival analysis on the day that their TST reaction status had been assessed. We computed cumulative mortality curves using the Kaplan-Meier estimate based on the date of death. Person-years-at-risk (Pyrs) were calculated from the date of TST assessment and infants contributed risk-time until they died, migrated, or reached 12 months of age, whichever came first. Tests of proportionality of hazards were computed using Schoenfeld's residuals. Since having an early BCG skin reaction (BCG scar) and the scar size has been associated with enhanced survival [13,14], we evaluated whether there was a correlation between the 2-month TST reaction status (yes/no) and BCG scars (scar vs no scar, scar size tertile) at 2 months of age by binomial regression providing RRs.

We conducted a meta-analysis based on a PubMed literature search in April 2021 (search terms: PPD reaction AND mortality/death, TST reaction AND mortality/death, TST AND mortality/death, Tuberculin reaction AND mortality/death). The meta-analysis includes the present study and three additional studies comprising four BCG-vaccinated cohorts that had evaluated the association between TST reactions and all-cause mortality with prospective follow-up. If both a crude and adjusted MRR (aMRR) had been reported, we used the aMRR for the meta-analysis. Since a previous combined analysis of studies evaluating the association between BCG scars and enhanced survival reported stronger effects closer to birth, we calculated meta-estimates of subsequent all-cause mortality risk by TST reaction status at 2- and 6-months of age. The first-authors of two of the previously published studies [8,9] conducted a re-analysis for the meta-analysis to be conducted using aMRRs by 2 and 6 months of age, respectively, and the meta-analysis was performed using effect estimates and confidence intervals for each cohort using the Stata *metan* command. Analyses were conducted using StataIC 16 (Stata Corp, College Station, Texas) and all estimates are reported with 95% CIs.

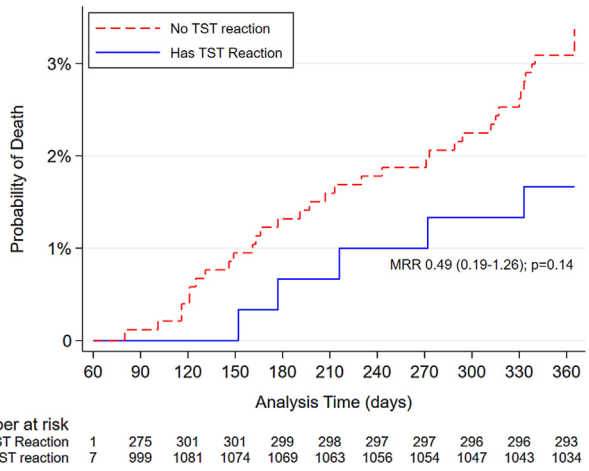

Fig. 1. Kaplan-Meier curve of cumulative deaths up to 1 year of age among infants with a 2-month TST reaction versus no reaction.

3. Results

3.1. TST reactions by 2 months of age

Within RCTs I-II, a total of 2105 infants received neonatal BCG, 1750 (83%) of which were recruited at HNSM. Of the 2105 infants, 1692 (80%) were found at home by 2 months of age, while 40 had moved, 94 had died, 39 were absent, 232 had travelled, 3 refused the visit and 5 could not be located. Of the 1,692 infants found at home, 94% (1588/1692) had a TST applied and of these, 87% (1389/1588) had a valid TST reading (evaluated within 48–72 h), while 3 had moved, 163 were absent, 17 had travelled and 16 were not evaluated for other reasons.

Among the infants with a valid TST reading at age 2 months, 22% (302/1389) had a TST reaction. Between 2 and 12-months there were 1074 person-years (PYRS) of follow-up and the mortality risk was 1.7% (5/302) for reactors and 3.3% (36/1087) for non-reactors, the corresponding crude reactor/non-reactor MRR being 0.49 (0.19–1.26) (Table 2, Fig. 1).

Among the 1389 infants, the 2-month BCG scar status was evaluated for 1386. For infants with a TST reaction, 99% (297/300) had a visible BCG scar versus 95% (1028/1086) of infants with no TST reaction, the non-reactor/reactor RR for no scar being 4.56 (1.51–13.8).

Table 2
Mortality risk between 2 and 12-months by 2-month TST skin reaction status and RCT (RCTs I and II).

	RCT	2–12-month mortality % (n/N)	2–12-month mortality rate per 100 Pyrs (Deaths/Pyrs)	Crude[a] MRR (95% CI)
No TST reaction	I	7.4% (2/27)	9.7 (2/21)	Ref.
	II	3.2% (34/1060)	4.1 (34/828)	Ref.
	Combined	3.3% (36/1087)	4.2 (36/848)	Ref.
Has TST reaction	I	0% (0/6)	0 (0/5)	–
	II	1.7% (5/296)	2.2 (5/232)	0.52 (0.20–1.33)
	Combined	1.7% (5/302)	2.1 (5/237)	0.49 (0.19–1.26)

Abbreviations: MRR, Mortality Rate Ratio; CI, Confidence Interval; Pyrs, person-years; TST, Tuberculin Skin Test (2 TU).
[a] Cox Proportional Hazards survival analysis, comparing mortality by reaction status (yes/no).

Table 3
Studies reporting prospective mortality by 2- or 6-month TST reaction status.

Study	Infants TST evaluated (n)	TST dose applied	Prevalence of TST reactions	Period of follow-up	Reactor/nonreactor aMRR (95% CI)
TST reactions by 2 months of age					
Epidemiology 2006 [6]	1906	2 TU	26% (500/1906)	2–12 months	0.50 (0.21–1.20)[1]
TMIH 2015 LBW [7]	803	2 TU	16% (132/803)	2–12 months	0.47 (0.14–1.54)
TMIH 2015 NBW [7]	1651	2 TU	36% (595/1651)	2–12 months	0.75 (0.40–1.42)
Present study	1389	2 TU	22% (302/1389)	2–12 months	0.49 (0.19–1.26)[2]
Combined estimate (fixed effects)	5749	2 TU	27% (1529/5749)	2–12 months	0.59 (0.39–0.90)
TST reactions by 6 months of age					
Vaccine 2003 [5]	813	Undeclared	59% (479/813)	7.5–19.5 months	0.48 (0.25–0.90)
Epidemiology 2006 [6]	1716	2 TU	25% (430/1716)	6–12 months	0.66 (0.22–1.98)[1]
TMIH 2015 LBW [7][3]	619	2 TU	27% (171/619)	6–12 months	0.52 (0.12–2.33)
TMIH 2015 NBW [7][3]	1340	2 TU	35% (474/1340)	6–12 months	1.13 (0.45–2.86)
Present study	2635	2 and 10 TU	44% (1149/2635)	6–12 months	0.87 (0.27–2.86)[2]
Combined estimate (fixed effects)	7123	2 and 10 TU	38% (2703/7123)	–	0.65 (0.43–1.00)

Abbreviations: aMRR, adjusted Mortality Rate Ratio; CI, Confidence Interval; NBW, normal birthweight; LBW, low birthweight; TST, Tuberculin Skin Test; TU, Tuberculosis Units.
[1] Adjusted as in Epidemiology 2006 (BCG strain, age at BCG vaccination, electricity in the house, maternal schooling, reader of TST) and provided by the first author of the original paper [6].
[2] MRR (unadjusted).
[3] The TST reaction vs. no TST reaction estimates between 6 and 12 months of age used in this meta-analysis was provided by the first author of the original paper [7].

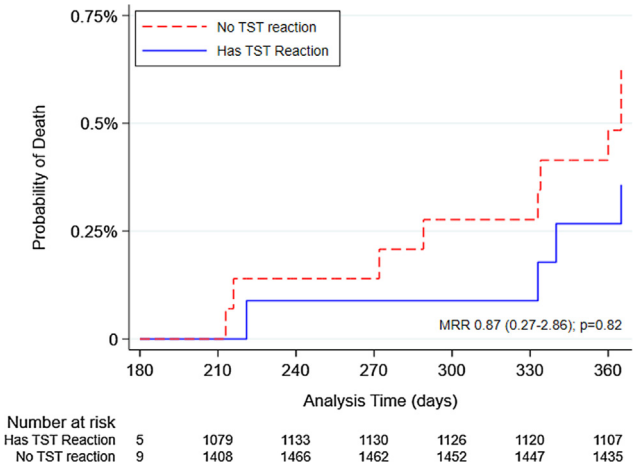

Fig. 2. Kaplan-Meier curve of cumulative deaths up to 1 year of age among infants with a 6-month TST reaction versus no reaction.

Among infants with no visible BCG scar at 2 months of age, 5% (3/61) had a TST reaction. For infants in the small BCG scar size tertile, 18% (505/616) were TST positive, the no scar vs small scar RR for developing a TST reaction thus being 0.27 (0.09–0.83). The prevalence of TST reactions was 26% (85/330) for the medium scar size tertile and 27% (101/379) in the large scar size tertile, the corresponding medium/small TST reaction RR being 1.43 (1.11–1.83) and the large/small RR being 1.48 (1.17–1.87), respectively.

3.2. TST reactions by 6 months of age

Within RCTs I-III, a total of 4618 infants received neonatal BCG, 4263 (92%) of which were recruited at HNSM. Of the 4618 infants, 3209 (69%) were found at home by 6 months of age while 300 had moved, 154 had died, 146 were absent, 778 had travelled, 2 refused the visit, 2 were hospitalized and 27 could not be located. Of the 3,209 infants found at home, 95% (3038/3209) had a TST applied and 87% (2635/3038) had the TST evaluated at home within 48 to 72 h, while 5 infants had moved, 329 were away, 33 had travelled, 1 refused the visit and 35 were not evaluated for other reasons. Among infants with a valid TST reading at age 6 months, 44% (1149/2635) had a reaction (Table 3). Between 6 and 12-months there were 1209 person-years (PYRS) of follow-up and the mortality risk was 0.4% (4/1149) for reactors and 0.6% (9/1486) for non-reactors, the corresponding crude MRR (stratified by RCT) being 0.87 (0.27–2.86) (Fig. 2).

3.3. Meta-analysis of the association between TST reactions and all-cause mortality

We identified three observational studies [7–9], all from Guinea-Bissau and conducted by our research group, that reported prospective mortality data for four cohorts of BCG-vaccinated infants with TST assessments performed at 2 or 6 months of age. Including the present study, the overall TST reaction prevalence by 2 months of age was 27% (1529/5749) and the combined meta-estimate an MRR of 0.59 (0.39–0.90) (Table 3, Fig. 3). By 6 months of age, the overall prevalence of TST reactions was 38% (2703/7123) and the combined meta-analysis MRR = 0.65 (0.43–1.00) (Table 3, Fig. 3).

3.4. Determinants for developing a TST reaction

The maternal MUAC, maternal age, maternal BCG scar status (RCT III) and the infant age at BCG vaccination were not determinants for developing a TST reaction, nor was inclusion site or vaccinator (Table 4). Reactors at 2 months tended to have a slightly higher inclusion weight in RCTs I-II (Table 4).

The post-vaccination wheal size (measured only in RCT III) was associated with more subsequent TST reactions by 6 months of age. Compared with having a large (≥5 mm) wheal, the RR for having a TST reaction was 0.73 (0.62–0.87) for wheals smaller than 5 mm. The BCG-Denmark and BCG-Japan strains were markedly associated with more reactions to TST 2 TU than BCG-Russia, the BCG-Denmark/BCG-Russia TST reaction RR being 2.09 (1.29–3.39) and the BCG-Japan/BCG-Russia RR being 1.57 (1.29–1.90) (Table 4).

4. Discussion

Among infants that were BCG-vaccinated in the neonatal period, having a TST reaction by 2- or 6 months of age tended to be associated with reduced mortality. The meta-analysis indicate that the mortality reduction associated with TST reactions at 2 and

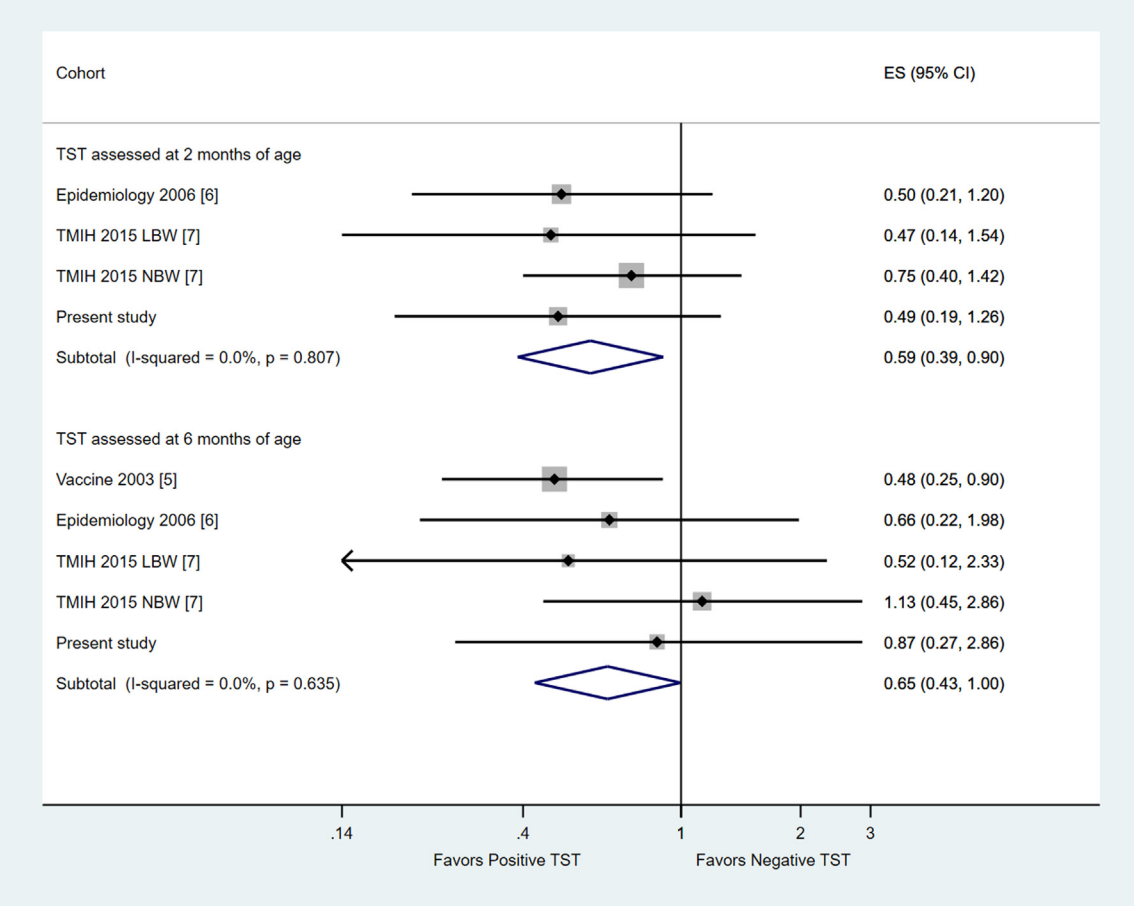

Fig. 3. Forest plot of studies evaluating the association between having a TST reaction versus no reaction at 2 and 6 months of age.

6 months of age is in the magnitude of 30%-40%. Vaccination technique and the BCG strain provided were the main determinants for inducing TST reactions; the formation of TST reactions was independent of the assessed host factors. Having a BCG scar and the BCG scar size at 2 months of age was positively associated with TST reactivity.

4.1. Strengths and weaknesses

While there were differences between the three RCTs included in the present study regarding inclusion criteria, BCG strains used and the underlying mortality risk, all neonates included were provided neonatal BCG by our team. Our cohort is thus representative of the recommended vaccination schedule of providing BCG and OPV shortly after birth, and any interference on the effects of BCG from pre-vaccination exposure to environmental mycobacteria should be minimal. Since determinants for developing a TST reaction were related to vaccination technique and the strain of BCG used and not the maternal MUAC nor the weight of the neonate at inclusion, we considered the 2-month MUAC as a mediator of the beneficial effect on overall health of having a TST response. Given that the cohorts only included infants that were present at the 2- or 6-month home-visit and also had the TST assessed at an additional visit 48–72 h later, our analysis did not include infants that had travelled or died prior to the visits.

For the RCT I-II analysis of determinants of TST responses at 2 months of age, data on the post-vaccination wheal size was not available and all infants received BCG-Denmark. It is a weakness that RCT III only included TSTs at 6 months of age and that both TST 2 TU and TST 10 TU were utilized, since the latter inherently induces more TST reactions. Furthermore, the mortality was low in RCT III, reducing the power of the 6–12-month mortality comparison. The standardized home-visits conducted across the RCTs applied the same methodology, but they were conducted by several field assistants and nurses, as was the TST injections and reaction readings. There could be assessment disparities but adjusting for assessor did not affect the mortality analyses.

We did not analyse our data by TB exposure and the observed effects on all-cause mortality might thus represent both specific protective effects against TB and beneficial NSEs, but it should be noted that TB is rare in infancy [20]. In a recent analysis of 39 431 children in the Bandim urban HDSS in Bissau, only 8% had observation time as TB-exposed between 28 days and 3 years of age [21]. Likewise, excluding children exposed to TB did not affect estimates in a previous analysis assessing overall effects of TST reactions on mortality [8].

We chose a cut-off for the TSTs classifying reactions > 1 mm as positive reactions and < 1 mm readings as non-reactors in accordance with the previously published TST literature, ensuring that the studies could be included in the meta-analysis. Arguments can be made as to why a 5 mm cut-off would be better; fewer would then have a positive reaction. In a previous analysis [8], the survival benefit was larger for infants with a TST reaction of > 5 mm indicating that our approach likely produced conservative estimates. Since our main interest was to assess whether TST is an important indicator for assessing “beneficial immune training”, we were more interested in the 1 mm cut-off which affect far more children.

It is problematic that all studies evaluating the association between TSTs and mortality originate from Guinea-Bissau. Given

Table 4
Determinants for developing a TST reaction at 2- and 6-months of age.

Determinants for developing a TST reaction at 2 months of age				
Determinant		TST reaction % (n/N) or median (IQR)		Risk ratio for presenting a reaction[a] (95% CI) or p-value[b]
Maternal MUAC in mm	Median (IQR)	Reactors	244 (230–262)	0.23
		Non-reactors	246 (230–266)	
Maternal age in years[c]	Median (IQR)	Reactors	23 (19–27)	0.36
		Non-reactors	23 (19–28)	
Place of inclusion	HDSS Health Center	25% (58/234)	1.17 (0.91–1.51)	
	HNSM	21% (244/1155)	Ref.	
Weight at inclusion	Median (IQR)	Reactors	2.230 (2.060–2.360)	0.06
		Non-reactors	2.210 (1.960–2.370)	
HNSM vaccinator	1	19% (85/443)	Ref.	
	2	22% (156/701)	1.16 (0.92–1.47)	
Age at BCG vaccination	≤3 days	22% (197/910)	Ref.	
	>3 days and < 29 days	22% (105/479)	1.01 (0.82–1.25)	
Determinants for developing a TST reaction at 6 months of age				
Determinant			TST reaction % (n/N) or median (IQR)	Risk ratio for presenting a reaction[a] (95% CI) or p-value[b]
Maternal BCG scar status (RCT III)	Mother has scar	54% (424/786)	1.03 (0.92–1.15)	
	Mother has no scar	52% (232/443)	Ref.	
Maternal MUAC	RCT I-II	Reactors	250 (226–268)	P = 0.27
		Non-reactors	244 (230–264)	
	RCT III	Reactors	270 (250–300)	P = 0.49
		Non-reactors	274 (252–300)	
Maternal age in years[d]	Reactors	26 (21–30)	P = 0.97	
	Non-reactors	26 (21–30)		
Place of inclusion (RCTs I-II)	HDSS Health Center	27% (50/183)	Ref.	
	HNSM	32% (341/1054)	1.18 (0.92–1.52)	
Weight at inclusion[e]	RCT I-II	Reactors	2220 (2010–2370)	P = 0.07
		Non-reactors	2210 (1950–2350)	
	RCT III	Reactors	3040 (2730–3320)	P = 0.88
		Non-reactors	3020 (2710–3340)	
Vaccinator (HNSM inclusions)	1	47% (432/915)	1.09 (0.99–1.19)	
	2	43% (660/1523)	Ref.	
Age at BCG vaccination[e]	≤3 days	36% (577/1601)	1.17 (1.00–1.36)	
	>3 days and < 29 days	31% (132/427)	Ref.	
Size of post-vaccination wheal in millimeters (RCT III)[e]	Small (<4 mm)	37% (26/70)	0.78 (0.57–1.08)	
	Medium (≥4mm, <5mm)	34% (128/374)	0.72 (0.60–0.86)	
	Large (≥5mm)	47% (163/344)	Ref.	
Strain of BCG (RCT III, Phase I)[e]	BCG-Russia	25% (16/64)	Ref.	
	BCG-Denmark	52% (34/65)	2.09 (1.29–3.39)	
Strain of BCG (RCT III, Phase II)[e]	BCG-Russia	32% (106/335)	Ref.	
	BCG-Japan	50% (162/327)	1.57 (1.29–1.90)	

Abbreviations: BCG, Bacille Calmette-Guérin; CI, Confidence Interval; HNSM, Hospital National Simão Mendes; IQR, Interquartile Range; mm, millimeters; MUAC, Mid Upper-Arm Circumference; RCT, Randomized Controlled Trial; TST, Tuberculin Skin Test.
[a]Binomial regression. [b]Kruskal-Wallis chi-squared test with ties. [c]57% (787/1389) missing datapoints evenly distributed between reactors (57% (171/302) missing) vs non-reactors (57% (616/1087) missing). [d]29% (753/2635) missing datapoints; 21% (238/1149) for reactors and 35% (515/1486) for non-reactors. [e]Analysis conducted among infants that were tested using TST 2 TU.

the public health implications, it is important that data from other settings is presented to test whether the strong association between TST reactivity and enhanced survival found in Guinea-Bissau can be reproduced in other settings.

4.2. Interpretation

The previous studies that evaluated effects of TST reactions on survival – all from Guinea-Bissau – mostly included infants that were BCG-vaccinated later in infancy, after the neonatal period [7–9]. Similar conclusions were reached, however; having a TST reaction reduced all-cause mortality by 30–50%. While BCG-Denmark has been associated with a substantial reduction in deaths from infections in randomized trials conducted in Guinea-Bissau [3,17], two recent RCTs from India reported no beneficial effect of BCG-Russia [6]. BCG-Denmark and BCG-Japan both contain substantially more viable mycobacteria than BCG-Russia [22]. We bring additional data forward emphasizing the association between immunogenic BCG strains, large post-vaccination wheals, and the TST reaction prevalence, in alignment with two previous reports from Guinea-Bissau [15,23]. These factors influences both the prevalence of early BCG skin reactions, their size, the formation of the final BCG scar and the TST reaction prevalence, all of which in a series of studies from Guinea-Bissau positively affects survival [7–14]. A clear link between the use of specific BCG strains and enhanced all-cause survival rather than surrogate outcomes have not been made. There are two RCTs conducted by BHP in Guinea-Bissau that will provide data to this end [24,25].

Addressing the vaccination program efficacy by surveying the TST reaction prevalence could both improve TB protection and reduce all-cause mortality.

BCG is a strong immunostimulant [26] and a study in mice has demonstrated that access of BCG to the bone marrow changes the transcriptional landscape of hematopoietic stem cells and multipotent progenitors, inducing immune training [27]. Similarly, a trial in humans demonstrated that BCG induces transcriptomic myeloid

priming of the hematopoietic stem and progenitor cell compartment, upregulating myeloid and granulocytic pathways and inducing transcription factors connected to myeloid cell function [28]. This resulted in elevated granulocyte numbers in BCG-vaccinated infants and induced lasting innate immune system memory. Similarly, two recent studies evaluating the effects of intradermal application of BCG to human newborns indicate that human newborn BCG vaccination increases peripheral blood neutrophil counts through a process termed emergency granulopoiesis [29,30]. It should be stated that it is unknown whether having a TST reaction is indicative of enhanced innate immune training in infants. Biological effects depending on BCG's access to the bone marrow are likely influenced by the absolute number of viable mycobacteria deposited within the host; the BCG strain and the dose injected would thus be important factors [31]. The manufacturer of BCG-Japan has specifically prioritized to ensure a high content of viable mycobacteria [32], but other manufacturers might be inclined to allow lower amounts to reduce production costs and the risk of adverse events [15,33].

A large observational study with low risk of bias indicated that BCG-Japan provides better protection against TB than BCG-Russia [34] and BCG is most protective against the disseminated, bloodborne forms of TB such as miliary and cerebral TB [35]. Hospital data from the three BCG-Denmark versus no-BCG RCTs indicate that BCG-Denmark protects against fatal neonatal sepsis [4] and recent RCTs have demonstrated that prior BCG provides protection against viremia following vaccination with yellow-fewer [36] and against malarial parasitemia [37]. Furthermore, a recent large observational study reported that BCG is associated with reduced malaria prevalence in children under 5 years of age in Sub Saharan Africa [38].

The current evidence thus indicates that adequate BCG vaccination can provide ample non-specific protection against bloodborne bacteria, viruses, and parasites, which can explain the substantially lowered all-cause mortality associated with BCG vaccination in the high mortality setting that is Guinea-Bissau. BCG scars and TST reactions thus likely represents a well-trained infant immune system induced by adequate BCG vaccination. To ensure high prevalences of BCG scars and TST reactions at the population level, proper vaccination technique is essential. Providing BCG to newborns is technically difficult and improvements in vaccination programs could be done by surveying the prevalence of BCG scars among vaccinated infants and, if logistically possible, TST reactions. If it is then identified that a vaccinator produces low rates of scars and/or TST reactions, these individuals could be retrained by the most qualified vaccinators in the program; those that produce the highest scar and/or TST prevalence rates.

5. Conclusion

Our analysis indicates a low degree of heterogeneity between studies of BCG-vaccinated infants which demonstrate that having a TST reaction is associated with markedly reduced all-cause mortality, when compared to no reaction. Correct administration of BCG induces more TST reactions, as does immunogenic strains such as BCG-Denmark and BCG-Japan, when compared to BCG-Russia. This indicates a long-term association between early adequate vaccination with specific BCG strains, TST reaction kinetics, and survival. To enhance BCG's beneficial NSEs and protection against TB, vaccination at birth with immunogenic BCG strains is thus to be encouraged and novel TB vaccines in phase-III testing must be tested against an efficient BCG strain. Vaccination programs should prioritize the provision of BCG-at-birth and monitor the prevalence of early BCG skin reactions and TST conversions thoroughly, to detect immunization failures and weak strains. It is paramount to introduce measures that eliminate known logistic barriers to vaccination so that a well-administered BCG can be provided as early as possible after birth [39,40]. An increased focus on timely vaccination, immunogenic BCG strains and injection quality would save many infant lives globally at low cost.

Notes

Author contributions

CSB and PA were the principal investigators and guarantors of the three RCTs. IM was the data collection supervisor for all three RCTs. AR supervised RCT I and reanalysed previously reported TST data. SBS supervised RCT II. CAGT reanalysed previously reported TST data. FSB unified and prepared the TST reaction data from the three RCTs, supervised RCTs II-III, conducted a systematic PubMed search of studies evaluating TST reactions, conducted the data analysis and wrote the first draft of the paper. All authors contributed to and approved the final paper.

Funding

This work was supported by The European Research Council (starting grant ERC-2009-StG-243149, which also funded SBS, IM, and CSB); the Novo Nordisk Foundation (research professorship grant to PA); the Danish National Research Foundation (grant DNRF108 to the Research Center for Vitamins & Vaccines); and the Danish International Development Agency (DANIDA), European Union FP7, and OPTIMUNISE [grant number Health-F3-2011–261375 to the Bandim Health Project] (RCT I-II). RCT III was supported by The Danish National Research Foundation (DNRF) [grant number DNRF108]. DNRF and the University of Southern Denmark [grant ref. HNP] funded the PhD scholarship to FSB.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors wish to thank the mothers and infants who participated in the study.

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.

Ethics approval

The study is based on already-collected data from three RCTs that were approved by the ethical committees in Guinea-Bissau (Comité Nacional de Ética na Saúde, approval number 19/2014 (RCT III)) and Denmark (National Committee on Biomedical Research Ethics in Denmark, approval number: 2007-7041121 (RCT II), 2015-1407397 (RCT III)). Informed consent was obtained for all participants, both for participation in the BCG trial and for applying the TST, and the trials were conducted in accordance with the Helsinki declaration standards and the reporting of the results are in accordance with the STROBE statement. BHP offered free medical consultations and essential medicine to all infants accessed for eligibility. Participants could request to leave a trial at any given time.

Data availability statement

Deidentified participant data with a data dictionary will be shared on reasonable request by sending a data-sharing proposal to Professor Christine Stabell Benn (cbenn@health.sdu.dk).

Ethical approval: The present article is based on already-collected data from studies conducted in Guinea-Bissau. Original study protocols were approved by the Guinean Ministry of Health's Research Coordination Committee and the Central Danish Ethical Committee gave its consultative approval and the trials were conducted in accordance with the Helsinki Declaration Ethical Standards.

ClinicalTrials.gov registration numbers: NCT00146302, NCT00625482 & NCT02447536.

Data sharing statement

Raw data used to conduct the analyses and accompanying STATA 16 dofiles can be shared after approval of a data-sharing proposal sent to Dr. Frederik Schaltz-Buchholzer (fschaltz-buchholzer@health.sdu.dk).

Appendix A. Supplementary material

Mortality Risk Between 6-12-Months By 6-Month TST Skin Reaction Status to 2 TU and 10 TU and RCT (RCTs I-III). Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.06.077.

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