BMJ Open

Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children: a prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035595
Article Type:	Original research
Date Submitted by the Author:	07-Nov-2019
Complete List of Authors:	Thysen, Sanne; University of Southern Denmark, Bandim Health Project, OPEN; Bandim Health Project, Bandim Health Project Benn, Christine; Statens Serum Institut, Bandim Health Project; University of Southern Denmark, OPEN Gomes, Victor; Bandim Health Project, ; Serum Statens Institute, Epidemiology Rudolf, Frauke; Bandim Health Project, Bandim Health Project; Aarhus University Hospital, Department of Infectious Diseases Wejse, Christian; Aarhus University, Center for Global Health (GloHAU), Department of Public Health Roth, Adam; Public Health Agency of Sweden, Dept. of Communicable Disease Control and Health Protection; Lund University, Dept. of Translational Medicine Kallestrup, Per; Aarhus University, Center for Global Health, Department of Public Health Aaby, Peter; Bandim Health Project, Fisker, Ane; Bandim Health Project,
Keywords:	EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES, Tuberculosis < INFECTIOUS DISEASES, Paediatric infectious disease & immunisation < PAEDIATRICS, PUBLIC HEALTH

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children: a prospective cohort study

Sanne M. Thysen[a,b,c,d] PhD, Christine S. Benn[a,b,c] DMSc, Victor F. Gomes[b] PhD, Frauke Rudolf[b,d] PhD, Christian Wejse[b,d,e] PhD, Adam Roth[f,g] PhD, Per Kallestrup[d] PhD, Peter Aaby[b,c] DMSc, Ane B. Fisker[a,b,c] PhD

[a] OPEN, Institute of Clinical Research, Odense University Hospital and University of Southern Denmark, J.B. Winsløwsvej 9, 5000 Odense, Denmark

[b] Bandim Health Project, Apartado 861, 1004 Bissau Codex, Guinea-Bissau

[c] Research Centre for Vitamins and Vaccines, Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark

[d] Center for Global Health (GloHAU), Aarhus University, Bartholins Allé 2, 8000 Aarhus, Denmark

[e] Dept of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

[f] Dept. of Communicable Disease Control and Health Protection, Public Health Agency of Sweden, SE-171 81 Solna, Sweden

[g] Dept. of Translational Medicine, Lund University, SE-205 02 Malmö, Sweden

Corresponding author: Sanne Marie Thysen, Bandim Health Project, INDEPTH Network; Apartado 861, 1004 Bissau Codex, Guinea-Bissau; s.thysen@bandim.org, Telephone: +45 31127825/ +245 955859948

Word count: Abstract: 249, Manuscript: 3544

Abstract

Objectives To assess the association between neonatal BCG vaccination and mortality between 28 days and 3 years of age among TB-exposed and TB-unexposed children.

Design Prospective cohort study.

Setting Bandim Health Project runs an urban Health and Demographic Surveillance site in Guinea-Bissau with registration of mortality, vaccination status and TB cases.

Participants Children entered the analysis when their vaccination card was inspected after 28 days of age, and remained under surveillance to 3 years of age. Children residing in the same house as a TB case were classified as TB-exposed from 3 months prior to case registration to the end of follow-up.

Methods Using Cox-proportional hazards models with age as underlying time scale, we compared mortality of children with and without neonatal BCG between October 2003 and September 2017.

Main outcome measure Hazard ratio (HR) for neonatal BCG compared with no neonatal BCG by TB-exposure status.

Results Among the 39,421 children who entered the analyses, 3,022 (8%) had observation time as TB-exposed. In total, 84% of children received neonatal BCG. Children with neonatal BCG had lower mortality both in TB-exposed (adjusted Hazard Ratio: 0.57 (0.26-1.27)) and in TB-unexposed children (HR: 0.57 (95% CI: 0.47-0.69)) than children without neonatal BCG. Children exposed to TB had higher mortality than TB-unexposed children if they had not received neonatal BCG.

Conclusion Neonatal BCG vaccination was associated with lower mortality among both TB-exposed and TB-unexposed children, consistent with neonatal BCG vaccination having beneficial non-specific effects. Interventions to increase timely BCG vaccination are urgently warranted.

Strengths and limitations of this study

- The study was conducted in Bandim Health Project's urban Health and Demographic Surveillance Site with continuous registration of births, deaths and vaccination status and linked with the TB surveillance database, which allowed classification of households as TB-exposed and TB-unexposed
- More than 39,000 children entered the analysis, all children entering the analysis had their vaccination status assessed after the neonatal period
- The analyses were adjusted for potential confounders, however, residual confounding cannot be excluded
- Some TB cases may be undiagnosed, leading to some TB-exposed children being classified as TB-unexposed. We expect any misclassification to be independent of timing of BCG vaccination

Introduction

Bacillus Calmette-Guérin (BCG) vaccine was developed to protect against tuberculosis (TB), and remains the only approved TB vaccine[1]. The efficacy of the BCG vaccine to protect against TB has varied between different trials[2]. However, trials of neonatal BCG vaccination consistently find that BCG is associated with reduced TB incidence[2].

BCG is recommended at birth in countries with high burden of TB[1]. According to WHO/UNICEF estimates, 88 percent of children are BCG vaccinated in countries where BCG is part of the routine vaccination programme[3]. However, WHO/UNICEF coverage estimates are based on coverage at 12 months of age, and BCG is often delayed in low-income countries[4,5]. If delayed BCG vaccination is associated with lower vaccine efficacy, delays may be critical.

Additionally, there is increasing evidence that BCG has non-specific effects (NSEs) on mortality. That is, BCG may affect mortality by more than can be explained by the protection against TB[6,7]. Three randomised trials in low-weight children in Guinea-Bissau found that BCG-Denmark vaccination at birth was associated with 38% (17-54%) lower neonatal mortality compared with children not BCG vaccinated at birth[8]. Already 3 days after enrolment, BCG was associated with 45% (7-68%) lower mortality compared with unvaccinated children[8], suggesting that even small delays in BCG vaccination may be important for survival.

A potential effect of early BCG on childhood mortality could be due to prevention of TB, NSEs of BCG or a combination. If the effect of BCG was merely specific, we should expect strong effects among children exposed to TB[9] and no effect in children not exposed to TB.

TB is difficult to diagnose, especially in children, and many TB cases are not diagnosed and treated[10,11]. Tuberculin skin test (TST) with purified protein derivatives (PPD) is commonly used to test for latent infection with *M. Tuberculosis*[12]. A response to PPD can be due to either *M. Tuberculosis* infection or BCG vaccination; however, BCG vaccination has been shown to result in lower PPD response compared with latent TB infection[12].

The main objective of this study was to assess the association between neonatal BCG versus later BCG vaccination and mortality by registered exposure to TB. We furthermore assessed the association between neonatal BCG vaccination and positive TST reactions by registered exposure to TB.

Methods

Setting and study population

Bandim Health Project (BHP) runs a Health and Demographic Surveillance Site (HDSS) in six suburban districts in Bissau, the capital of Guinea-Bissau. Children are followed through tri-monthly home visits until 3 years of age. At the home visits, a field assistant collects information on vital status, measures mid-upper-arm circumference (MUAC) and registers vaccination status by transcribing information from the child's vaccination card. Each month, field assistants conduct home visits to follow pregnant women and register new births. Children above 3 years of age and adults are followed through censuses conducted every 2-5 years.

The BCG vaccines used in Guinea-Bissau have been provided by UNICEF and mainly the Russian strain has been used. However, during large periods within the study period, the BHP provided vaccines for the study area; these were the BCG-Denmark strain purchased at the Statens Serum Institut, Denmark.

Since 1996, all diagnosed TB cases living in the study area have been registered and followed[13]. In 2003, a register of information on all TB patients from the study area was established, making it possible to identify houses with exposure to TB. We defined inhabitants as exposed to TB from 3 months prior to diagnosis of a TB case in the house and until 2 weeks after diagnosis. In Guinea-Bissau, more than one household usually share a house, and the houses are constructed with a common roof, no ceiling and the walls separating the rooms do not reach the roof. Much of the everyday life takes place at the veranda of the house. Thus, children living in a house with a TB case were classified as TB-exposed, also if the TB case was from a different household within the same house. Children were classified as TB-exposed from the first registered TB exposure and remained classified as TB-exposed throughout the follow-up period. We expect some TB cases to be undiagnosed. Thus, some children classified as TB-unexposed may have been exposed.

Between September 1, 2005 and October 31, 2007, and between January 18, 2011 and August 20, 2013, studies of the effect of preventive treatment to TB-exposed children were conducted in the BHP study area[14,15]. We excluded these periods for all children in the present study (Figure 1). Thus, we included children who had their vaccination status assessed after 28 days of age between October 28, 2003 (Start of the TB registry) and September 15, 2017 excluding periods with studies of preventive TB treatment. From July 2002 to April 2004, a randomised trial of BCG-revaccination at 19 months of age was conducted in the study area, we therefore censored follow-up at 19 months of age for children eligible for the BCG-revaccination trial[16].

Between start of study and July 1, 2008, a sample of children living in the study area were TST tested using the Mantoux method with an intradermal application of 0.1 ml of PPD (2 tuberculin units RT23, Statens Serum Institut, Denmark) in the forearm at ages 6 and 12 months. The PPD reactions were measured after 48-72 hours using a ruler and ballpoint technique to measure two diameters[17]. Among children with measured PPD reactions, we assessed the effect of neonatal BCG vaccination versus later BCG vaccination on PPD reactions using cut-offs of 10mm and 15mm.

Statistical analyses

We compared baseline characteristics of children with and without neonatal BCG in TB-exposed and TB-unexposed children using chi^2 and paired t-tests. We also compared baseline characteristics of children registered and not registered as TB-exposed.

In Cox-proportional hazards models with age as underlying time scale, we compared mortality rates of children with neonatal BCG with mortality rates of children without neonatal BCG (delayed BCG or no BCG) separately for TB-exposed and TB-unexposed children, allowing for different baseline hazards according to sex and place of birth (maternity ward, health centre or home). Children entered the analysis the first time their vaccination status was assessed at a home visit after the neonatal period. Observation time was split at first registered TB exposure and thus, TB-exposed children could contribute with observation time in the TB-unexposed group until TB exposure. To control for potential confounding, we assessed whether baseline characteristics

changed the estimate by including the factors in the analysis one by one. We adjusted for baseline characteristics that changed the estimate by more than 5%.

In a secondary analysis, we explored whether the effect of neonatal BCG differed by timing of BCG within the neonatal period, thus we divided children with neonatal BCG in two groups, children with early neonatal BCG (vaccinated within 7 days after birth), and children with late neonatal BCG (vaccinated between day 8 and day 28).

In sensitivity analyses, we: A) Excluded all children, who potentially had been exposed to preventive TB treatment as part of the aforementioned studies (Supplementary Figure 1). B) Restricted the analysis to children with a registered BCG vaccine (i.e. allowing a child only to contribute time at risk from the first visit at which a BCG vaccine was registered, therefore, children with no registered BCG vaccine would not enter the analysis). C) Extended follow-up to 5 years of age. D) Excluded twins, as these are more likely to be low-birth-weight (LBW) children and thus receive delayed BCG. E) Stratified the analysis by LBW status for the subset of children for whom we had information on birthweight.

BCG vaccination may cause a transient weak response to PPD[18], but reactions above 15mm would be less likely to be caused by BCG[19]. Using log-binomial regression, we compared the prevalence of positive TST in children with neonatal BCG with children without neonatal BCG among TB-exposed and TB-unexposed children, respectively. We limited comparison to children BCG vaccinated prior to TST assessment and evaluated PPD reaction at age 6 and 12 months using cut-offs of 10mm and 15mm.

Ethical considerations

Most data was obtained from the routine data collection in the urban HDSS of the BHP. The BHP data collection was initiated in 1978 at the request of the Ministry of Health in Guinea-Bissau. Oral consent was obtained from mother/guardian of the children prior to TST.

Patient and public involvement

The communities were involved in locating households, when the HDSS was setup and contributed information allowing tracing of internal migrants between suburbs throughout the study period. No participant was involved in setting the research question or the outcome measure, nor were they involved in developing plans for recruitment, design, or implementation of the study. No participant was asked to advise on interpretation or writing up the results. The results are disseminated to the national public health institute. There are no plans to disseminate the results of the research to study participants or the community.

Results

A total of 39,421 children contributed time at risk, and among these 3,022 had observation time while living in houses of registered TB cases. Among children with a vaccination card seen after 28 days, 33,137 (84%) had received neonatal BCG. The median age of vaccination in the neonatal BCG group was 2 days (interquartile range (IQR): 1-10). Among the 6,284 children not BCG vaccinated in the neonatal period, 5,450 (87%) had a BCG vaccine registered at some point of time during the follow up period (median age of vaccination: 48 days (IQR: 36-69)).

Baseline characteristics

We compared baseline characteristics of children with and without neonatal BCG according to TB-exposure status. In this large dataset, most statistically significant differences were small absolute differences. In both groups, mothers of children with neonatal BCG were better educated, older, and had better socioeconomic status (toilet, electricity, among not TB-exposed children also type of roof). Children with neonatal BCG were more likely to be born at a health facility and less likely to be twins. Ethnic groups also varied between children with and without neonatal BCG. Among TB-unexposed children, the distribution of season of birth, year of birth, number of pregnancies and suburb differed between neonatal BCG vaccinated and not neonatal BCG vaccinated children (Table 1).

Several baseline characteristics were statistically significant different between TB-exposed and TB-unexposed children (birth location, number of pregnancies, maternal age, suburb, ethnicity, and socioeconomic factors) (Table 2).

Effect of neonatal BCG on mortality among children registered as TB-exposed

We included 3,022 TB-exposed children in the analyses, among these 2,605 (86%) were neonatally BCG vaccinated, whereas 417 (14%) were not. TB-exposed children with neonatal BCG had a mortality rate (MR) of 12.2 per 1000 person years (PYRS, 30 deaths during 2,462 PYRS), and children without neonatal BCG (delayed or no BCG) had a MR of 35.2 per 1000 PYRS (13 deaths during 369 PYRS). The hazard ratio (HR) comparing TB-exposed children with and without neonatal BCG was 0.29 (95% CI: 0.14-0.57). Adjusting for the baseline characteristics that changed the estimate by more than 5% resulted in an adjusted hazard ratio (aHR) of 0.57 (0.26-1.27) (Table 3). Neonatal BCG was beneficial for TB-exposed boys (aHR: 0.23 (0.08-0.62)), but not for TB-exposed girls (aHR: 1.61 (0.36-7.08)) (p-value=0.03) (Table 4).

The mortality was lowest among children with early neonatal BCG (aHR compared with children with no neonatal BCG: 0.39 (0.15-0.98) and an aHR of 0.90 (0.36-2.25) for children with late neonatal BCG compared with children with no neonatal BCG) (Supplementary table 1).

Extending follow-up to 5 years of age or excluding children with no registered BCG vaccination, or excluding twins from the analysis did not alter the conclusions (Supplementary table 2). Excluding children who had potentially been eligible for studies of preventive TB treatment (Supplementary figure 1) resulted in an aHR of 0.95 (95% CI: 0.31-2.97) (Supplementary table 2). Stratifying the analysis by LBW status (<2500g), had limited power (Supplementary table 3).

Effect of neonatal BCG on mortality among children registered as TB-unexposed

We included 38,145 children classified as TB-unexposed in this analysis, 32,040 (84%) were neonatally BCG vaccinated, and 6,105 (16%) were not. Children with neonatal BCG vaccination had a MR of 13.5 per 1000 PYRS (533 deaths during 39,608 PYRS) and not neonatal BCG vaccinated children had a MR of 22.8 per 1000 PYRS (157 deaths during 6,880 PYRS). Children with neonatal BCG had 43% lower mortality (HR: 0.57 (95% CI: 0.47-0.69) compared with not neonatal BCG vaccinated children (Table 3). No background factor changed the estimate by more than 5%, and thus adjusted estimates are not presented. The effect of neonatal BCG did not differ by sex (p=0.87) (Table 4).

The effect of BCG within the neonatal period was similar: HR: 0.59 (0.48-0.74) for children with early neonatal BCG compared with no neonatal BCG and HR: 0.55 (0.44-0.68) for children with late neonatal BCG (Supplementary table 1).

Extending follow-up to 5 years of age, excluding children with no registered BCG vaccine, excluding twins from the analysis or excluding children potentially eligible for studies of preventive TB treatment (Supplementary figure 1) did not alter the conclusions (Supplementary table 2). Stratifying the analysis by LBW status, indicated that neonatal BCG was associated with an HR of 0.70 (0.44-1.11) in LBW children, and an HR of 0.76 (0.50-1.16) in NBW children (Supplementary table 3).

Mortality in TB-exposed and TB-unexposed children according to vaccination status

We also examined the effect of being TB-exposed on mortality among children with neonatal BCG and children without neonatal BCG, respectively. Among children with neonatal BCG, TB-exposed children had slightly higher mortality than TB-unexposed children (HR: 1.11 (0.77-1.61)). Among not neonatally BCG-vaccinated children, TB-exposed children had higher mortality (HR: 1.93 (1.10-3.41)) (p=0.11 for interaction between neonatal BCG and TB-exposure, Table 5).

We explored whether the effect differed for boys and girls. TB-exposure was associated with higher mortality for girls both with and without neonatal BCG (HR: 1.50 (0.94-2.39) and 1.90 (0.83-4.35), respectively). In boys, TB-exposure was associated with higher mortality for children without neonatal BCG (HR: 1.98 (0.91-4.28)), whereas this was not the case for children with neonatal BCG (HR: 0.77 (0.42-1.41)) (Table 5).

Tuberculin skin test reactions

Among 53 TB-exposed children with a TST assessed at 6 months, a total of 3 (5.7%) children (all with neonatal BCG) had a PPD reaction above 10mm. Only seven TB-exposed children had a TST assessed at 12 months and no child had a PPD reaction above 15mm (Supplementary Table 4).

Among 1384 TB-unexposed children with assessed TST at 6 months, neonatal BCG did not affect PPD reactions; among the 335 children with TST assessed at 12 months fewer children with neonatal BCG had large TSTs (Supplementary Table 4).

Discussion

Main findings

Neonatal BCG was associated with lower mortality among both TB-exposed (aHR: 0.57 (95%CI: 0.26-1.27)) and TB-unexposed children (HR: 0.57 (95%CI: 0.47-0.69)), resulting in a combined HR of 0.54 (0.45-0.65), not adjusted as no factor changed the estimate by more than 5%. The results were robust to sensitivity analyses of longer follow-up, exclusion of children with no registered BCG vaccine, exclusion of children who were twins and exclusion of children potentially eligible for studies of preventive TB treatment. Children exposed to TB had higher mortality than TB-unexposed children if they had not received neonatal BCG.

Strengths and weaknesses

The study was conducted within the setup of Bandim Health Project's urban HDSS that since 1978 has followed the population in the study area with regular home visits to collect information on health status of children. Information on vaccination status and vital status is collected by experienced field assistants at home visits every third month, and hard endpoints like death are therefore unlikely to be misclassified. Only children with assessed vaccination status after 28 days of life were included in the analysis, and children only entered the analyses when the vaccination status was assessed at a home visit, thus avoiding differential misclassification of vaccination status and survival bias.

Self-selection to BCG vaccination could create healthy vaccinee bias, where early BCG is associated with lower mortality because the healthiest children receive BCG early, rather than early BCG causing healthy children. To remove potential confounding, we controlled for baseline characteristics that changed the estimate by more than 5% when included in the analyses one by one. Adjusting for available potential confounders affected the estimates for TB-exposed children, but did not alter the conclusions, neither did the sensitivity analyses conducted. Thus, self-selection for vaccination is unlikely to explain all of the effect. Since we find marked effects of neonatal BCG on all-cause mortality in both TB-exposed and TB-unexposed children, our results support that the effect of neonatal BCG is not merely due to protection against TB. However, there may be residual confounding not accounted for.

The registration of diagnosed TB cases from the study area allows for classifying children as TB-exposed. However, some TB cases will be undiagnosed, and some TB-exposed children may therefore have been misclassified as TB-unexposed. We expect any misclassification to be independent of timing of BCG vaccination. Unfortunately, we did not have HIV status for mothers or children, and we were therefore not able to assess whether HIV status influenced our results.

TST was only assessed for a subsample of our population, and we had scarce data in some groups. Our data did not allow for conclusions as to whether timing of BCG may have an impact on latent TB infection assessed by PPD reactions above 10mm or 15mm.

Comparison with other studies

The vaccine efficacy of BCG on preventing TB has been widely debated as different studies have yielded very different effects[20]. However, a meta-analysis of BCG's effect on TB found higher vaccine efficacy when BCG was given early in life[2]. Our results support that BCG in early life has beneficial effects, and furthermore suggest that timing of BCG is important. We did not study the vaccine efficacy on TB, and the results are therefore not directly comparable, but our findings support that BCG should be administered early in life.

Increasing evidence supports that BCG aside from the specific effects also have NSEs[6,21]. Most studies assessing the effect of BCG on all-cause mortality compared BCG-vaccinated children with un-vaccinated children[6,22,23] and randomised trials of BCG-Denmark have found beneficial effects in the neonatal period[8]. It should be noted that two recent trials testing the effect of BCG-Russia in India found no effect[24]; the different results may be related to different BCG strains[24,25]. A previous study from the 1990s in Guinea-Bissau found that BCG was associated with stronger beneficial effects if provided in the first week of life[26]. Our study supports that the timing of BCG is important, not only because children vaccinated later are deprived of beneficial effects early in life,

but also that there are benefits beyond the initial early phase. Similar benefits have been observed for other live vaccines (OPV[27], MV[28]).

In line with our study, data from the same setting between 1996 and 1998 indicate that TB exposure was associated with higher mortality in children aged 0-5 years[29]. We also found that TB exposure was associated with higher mortality, but less so among children with neonatal BCG, due to no excess mortality among boys with neonatal BCG. It is generally accepted that TB-exposure can result in increased mortality[9,30], and it is recommended to provide preventive TB treatment for children younger than 5 years of age[31], however, a policy-practice gap remains, and the policy is rarely implemented[32,33]. A recent modelling study estimated that more than 80,000 TB deaths could be averted globally in children younger than 5 years by altering coverage of household contact management from zero to full coverage[34]. Our data suggest that part of this effect (in boys) may also be obtained by emphasising BCG at birth.

Interpretation and implications

Our conclusions were robust to sensitivity analyses and adjusting for available potential confounders. However, if BCG vaccination was delayed mainly for frail children, this could bias our results. To adjust for indicators of frailty, we conducted sensitivity analyses excluding twins, and stratifying by LBW status. Excluding twins or stratifying by LBW status did not alter the main conclusions. Assessing the effect of neonatal BCG by LBW limited the analysis to the 63% of the TB-exposed children and 65% of the TB-unexposed children with information on birthweight, and should thus be interpreted with caution. As a whole, the sensitivity analyses did not suggest that frailty in children with no neonatal BCG explained our results.

Currently, timing of BCG is not captured as part of the WHO/UNICEF coverage estimates[5], and although BCG is planned to be given at birth in many countries, BCG is often delayed[4,5]. Our results support that timing of BCG is important. WHO already recommends that BCG should be provided as soon as possible after birth[1], but we need more focus on barriers for neonatal BCG vaccination. Since policies are frequently implemented according to donor's priorities, vaccine donors should be involved in creating incentives for neonatal BCG vaccination. Currently, vaccine donors evaluate performance of vaccine programmes by 12 months coverage estimates and vaccine wastage targets[35]. Including neonatal BCG vaccination as a performance indicator in addition to 12 months coverage would provide countries with an incentive to strive for timely vaccination.

Conclusion

Neonatal BCG was associated with reduced mortality in TB-exposed and TB-unexposed children, supporting that timing of BCG is important for all-cause mortality. Neonatal BCG should be emphasised and barriers for neonatal BCG vaccination should be removed.

Authors' contributions

SMT and ABF conceived the idea for the study and planned the analyses. CB, AR, PA and ABF supervised the demographic surveillance data collection, AR and ABF supervised and cleaned the PPD data, VFG, FR and CW supervised the data registration of TB patients. SMT analysed the data, and wrote the first manuscript draft with input from PK, PA and ABF. All authors received and approved the final manuscript.

Funding

This work was supported by Augustinus Foundation. The Bandim Health Project received support from the Novo Nordisk Foundation, DANIDA; EU [ICA 4-CT-2002-10053], the Danish National Research Foundation via Research Center for Vitamins and Vaccines [DNRF108], and Karen Elise Jensen Foundation. The sponsors had no role in designing the study, the data collection, data analysis, data interpretation or writing the paper.

Acknowledgments

We wish to thank all children, mothers and TB patients contributing with information to the present study. Furthermore, we would like to thank the dedicated staff working at BHP in Guinea-Bissau.

Conflicts of interest

All authors have no interests to declare.

References

1. World Health Organisation. BCG vaccines: WHO position paper - February 2018. *Weekly Epidemiological Journal* 2018; **93**: 73-96.
2. Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **58**(4): 470-80.
3. VanderEnde K, Gacic-Dobo M, Diallo MS, Conklin LM, Wallace AS. Global Routine Vaccination Coverage - 2017. *MMWR Morbidity and mortality weekly report* 2018; **67**(45): 1261-4.
4. Clark A, Sanderson C. Timing of children's vaccinations in 45 low-income and middle-income countries: an analysis of survey data. *Lancet* 2009; **373**(9674): 1543-9.
5. Thysen SM, Fisker AB, INDEPTH working group on Vaccines and Child Survival. Selecting the right indicators to ensure optimised implementation of BCG vaccination policy. *Vaccine* 2018; **36**(24): 3406-7.
6. Higgins JP, Soares-Weiser K, Lopez-Lopez JA, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 2016; **355**: i5170.
7. Uthayakumar D, Paris S, Chapat L, Freyburger C, Poulet H, De Luca K. Non-specific effects of vaccines illustrated through the BCG example: From observations to demonstrations. *Front Immunol* 2018.
8. Biering-Sørensen S, Aaby P, Lund N, et al. Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. *Clinical Infectious Diseases* 2017; **65**(7): 1183-90.
9. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2013; **41**(1): 140-56.
10. World Health Organisation. Global Tubeculosis Report 2018. Licence: CC BY-NC-SA 3.0 IGO., 2018.
11. Jenkins HE. Global Burden of Childhood Tuberculosis. *Pneumonia (Nathan)* 2016; **8**.
12. Enarson DA. Use of tuberculin skin test in children. *Paediatric Respiratory Reviews* 2004; **5**(Supp A): 135-7.
13. Lemvik G, Rudolf F, Vieira F, et al. Decline in overall, smear-negative and HIV-positive TB incidence while smear-positive incidence stays stable in Guinea-Bissau 2004-2011. *Trop Med Int Health* 2014; **19**(11): 1367-76.
14. Gomes VF, Andersen A, Lemvik G, et al. Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau: a prospective cohort study. *BMJ open* 2013; **3**(3).
15. Lemvik G. Active TB among adults and latent TB among children in Guinea-Bissau - risk factors and adherence to preventive therapy: Aarhus University; 2013.
16. Roth AE, Benn CS, Ravn H, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. *BMJ* 2010; **340**: c671.
17. Bouros D, Zeros G, Panaretos C, Vassilatos C, Siafakas N. Palpation vs pen method for the measurement of skin tuberculin reaction (Mantoux test). *Chest* 1991; **99**(2): 416-9.
18. Menzies D. What Does Tuberculin Reactivity after Bacille Calmette-Guérin Vaccination Tell Us? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2000; **31**.
19. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guérin vaccination on tuberculin skin test measurements. *Thorax* 2002; **57**: 804-9.
20. Abubakar I, Pimpin L, Ariti C, et al. Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis. *Health technology assessment* 2013; **17**(37): 1-372, v-vi.
21. Arts RJW, Moorlag S, Novakovic B, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell host & microbe* 2018; **23**(1): 89-100 e5.

22. Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 2000; **321**(7274): 1435-8.
23. Aaby P, Jensen H, Gomes J, Fernandes M, Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. *Int J Epidemiol* 2004; **33**(2): 374-80.
24. Jayaraman K, Adhisivam B, Nallasivan S, et al. Two Randomized Trials of the Effect of BCG-Russia Alone or With Oral Polio Vaccine on Neonatal Mortality in Infants Weighing <2000 G in India. *Pediatr Infect Dis J* 2018.
25. Curtis N. BCG Vaccination and All-Cause Neonatal Mortality. *Pediatr Infect Dis J* 2019; **38**(2): 195-7.
26. Roth A, Jensen H, Garly M-L, et al. Low Birth Weight Infants and Calmette-Guérin Bacillus Vaccination at Birth. *Pediatr Infect Dis J* 2004; **23**(6): 544-50.
27. Lund N, Andersen A, Hansen AS, et al. The Effect of Oral Polio Vaccine at Birth on Infant Mortality: A Randomized Trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2015; **61**(10): 1504-11.
28. Aaby P, Martins CL, Garly ML, et al. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 2010; **341**: c6495.
29. Gomes VF, Andersen A, Wejse C, et al. Impact of tuberculosis exposure at home on mortality in children under 5 years of age in Guinea-Bissau. *Thorax* 2011; **66**(2): 163-7.
30. Marais BJ. Preventing tuberculosis in household contacts crucial to protect children and contain epidemic spread. *The Lancet Global Health* 2018.
31. World Health Organization. Latent TB Infection: Updated and consolidated guidelines for programmatic management. http://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239-eng.pdf;jsessionid=822A28C5C42D1A1A1262F556FC0CEC7C?sequence=1: World Health Organization, 2018.
32. Hill P, Rutherford M, Audas R, van Crevel R, Graham S. Closing the policy-practice gap in the management of child contacts of tuberculosis cases in developing countries. *PLoS Med* 2011.
33. Gomes VF, Wejse C, Oliveira I, et al. Adherence to isoniazid preventive therapy in children exposed to tuberculosis: a prospective study from Guinea-Bissau. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2011; **15**(12): 1637-43.
34. Dodd PJ, Yuen CM, Becerra MC, Revill P, Jenkins HE, Seddon JA. Potential effect of household contact management on childhood tuberculosis: a mathematical modelling study. *The Lancet Global Health* 2018.
35. GAVI. Gavi 2016-2020 strategy indicators. 2016.

Figure 1: Timeline of study period

Table 1. Baseline characteristics of children with and without neonatal BCG by registered TB-exposure status.

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children				TB-unexposed children			
	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value
Number	3022	2605 (86.2%)	417 (13.8%)		36399	30532 (83.9%)	5867 (16.1%)	
Sex[1]				0.849				.35
Male	1574	1355 (52.0%)	219 (52.5%)		18421	15485 (50.7%)	2936 (50.1%)	
Female	1448	1250 (48.0%)	198 (47.5%)		17977	15047 (49.3%)	2930 (49.9%)	
Twin[2]				<0.0001				<0.0001
Yes	99	70 (2.7%)	29 (7.0%)		1233	826 (2.7%)	407 (7.0%)	
No	2921	2534 (97.3%)	387 (93.0%)		35114	29669 (97.3%)	5445 (93.0%)	
Season of birth				0.376				0.024
Rainy season	1474	1279 (49.1%)	195 (46.8%)		17795	15006 (49.1%)	2789 (47.5%)	
Dry season	1548	1326 (50.9%)	222 (53.2%)		18604	15526 (50.9%)	3078 (52.5%)	
Birth location[3]				<0.0001				<0.0001
Home	902	721 (27.7%)	181 (43.6%)		10030	7463 (24.5%)	2567 (44.1%)	
Health centre	1329	1259 (48.4%)	70 (16.9%)		16171	15285 (50.2%)	886 (15.2%)	
Hospital	647	512 (19.7%)	135 (32.5%)		7923	6001 (19.7%)	1922 (33.0%)	
Other	139	110 (4.2%)	29 (7.0%)		2179	1727 (5.7%)	452 (7.8%)	
Year of birth				0.550				<0.0001
2000-2006	1010	866 (33.2%)	144 (34.5%)		11410	9430 (30.9%)	1980 (33.7%)	
2007-2011	999	856 (32.9%)	143 (34.3%)		12654	10625 (34.8%)	2029 (34.6%)	
2012-2017	1013	883 (33.9%)	130 (31.2%)		12335	10477 (34.3%)	1858 (31.7%)	
Number of pregnancies[4]				0.694				<0.0001
1	838	723 (27.8%)	115 (27.6%)		10972	9198 (30.1%)	1774 (30.3%)	
2 - 3	1249	1083 (41.6%)	166 (39.8%)		15316	12987 (42.6%)	2329 (39.7%)	
4 or more	934	798 (30.6%)	136 (32.6%)		10095	8336 (27.3%)	1759 (30.0%)	
Maternal age[5]	2939	26.5 (6.25)	25.6 (6.40)	0.005	34712	26.2 (6.22)	25.3 (6.38)	<0.0001
Education of caretaker[6]				<0.0001				<0.0001
0 year	772	639 (26.4%)	133 (36.4%)		9747	7637 (26.5%)	2110 (39.8%)	

1-4 years	384	330 (13.6%)	54 (14.8%)		4530	3686 (12.8%)	844 (15.9%)	
+4 years	1629	1451 (60.0%)	178 (48.8%)		19865	17523 (60.7%)	2342 (44.2%)	
Suburb				0.138				<0.0001
Bandim	1426	1214 (46.6%)	212 (50.8%)		16010	13194 (43.2%)	2816 (48.0%)	
Belem / Mindara	502	445 (17.1%)	57 (13.7%)		5556	4877 (16.0%)	679 (11.6%)	
Cuntum	1094	946 (36.3%)	148 (35.5%)		14833	12461 (40.8%)	2372 (40.4%)	
Ethnicity[7]				0.001				<0.0001
Pepel	819	685 (26.7%)	134 (32.4%)		10007	8161 (27.0%)	1846 (31.7%)	
Balanta	290	244 (9.5%)	46 (11.1%)		2947	2447 (8.1%)	500 (8.6%)	
Mandinga / Fula	858	726 (28.3%)	132 (31.9%)		10943	9038 (29.9%)	1905 (32.7%)	
Manjaco / Mancanha	596	538 (21.0%)	58 (14.0%)		6464	5688 (18.8%)	776 (13.3%)	
Others	417	373 (14.5%)	44 (10.6%)		5743	4941 (16.3%)	802 (13.8%)	
Socioeconomic factors								
Type of roof[8]				0.330				<0.0001
Straw	93	77 (3.0%)	16 (3.9%)		922	728 (2.4%)	194 (3.3%)	
Hard	2919	2520 (97.0%)	399 (96.1%)		35422	29769 (97.6%)	5653 (96.7%)	
Toilet[9]				<0.0001				<0.0001
None	11	6 (0.2%)	5 (1.2%)		95	75 (0.2%)	20 (0.3%)	
Latrine	2618	2240 (86.3%)	378 (91.1%)		30210	24959 (81.9%)	5251 (89.8%)	
Inside	381	349 (13.4%)	32 (7.7%)		6007	5428 (17.8%)	579 (9.9%)	
Electricity[10]				<0.0001				<0.0001
Yes	950	859 (33.0%)	91 (21.9%)		12841	11437 (37.5%)	1404 (24.0%)	
No	2067	1742 (67.0%)	325 (78.1%)		23505	19060 (62.5%)	4445 (76.0%)	

[1] Missing information for 0 TB-exposed children and 1 TB-unexposed child.
[2] Missing information for 2 TB-exposed children and 52 TB-unexposed children.
[3] Missing information for 5 TB-exposed children and 96 TB-unexposed children.
[4] Missing information for 1 TB-exposed children and 16 TB-unexposed children.
[5] Missing information for 83 TB-exposed children and 1687 TB-unexposed children.
[6] Missing information for 237 TB-exposed children and 2257 TB-unexposed children.
[7] Missing information for 42 TB-exposed children and 295 TB-unexposed children.
[8] Missing information for 10 TB-exposed children and 55 TB-unexposed children.
[9] Missing information for 12 TB-exposed children and 87 TB-unexposed children.
[10] Missing information for 5 TB-exposed children and 53 TB-unexposed children.

Table 2. Baseline characteristics of children registered as exposed to and not exposed to TB

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children versus TB-unexposed children			
	Total	TB-exposed (n %)	TB-unexposed (n %)	p-value
Number	39421	3022 (7.7%)	36399 (92.3%)	
Sex[1]				0.119
Male	19995	1574 (52.1%)	18421 (50.6%)	
Female	19425	1448 (47.9%)	17977 (49.4%)	
Twin[2]				0.739
Yes	1332	99 (3.3%)	1233 (3.4%)	
No	38035	2921 (96.7%)	35114 (96.6%)	
Season of birth				0.905
Rainy season	19269	1474 (48.8%)	17795 (48.9%)	
Dry season	20152	1548 (51.2%)	18604 (51.1%)	
Birth location[3]				0.002
Home	10932	902 (29.9%)	10030 (27.6%)	
Health centre	17500	1329 (44.1%)	16171 (44.5%)	
Hospital	8570	647 (21.4%)	7923 (21.8%)	
Other	2318	139 (4.6%)	2179 (6.0%)	
Year of birth				0.043
2000-2006	12420	1010 (33.4%)	11410 (31.1%)	
2007-2011	13653	999 (33.1%)	12654 (34.5%)	
2012-2017	13348	1013 (33.5%)	12335 (33.6%)	
Number of pregnancies[4]				<0.0001
1	11810	838 (27.7%)	10972 (30.2%)	
2 - 3	16565	1249 (41.3%)	15316 (42.1%)	
4 or more	11029	934 (30.9%)	10095 (27.7%)	
Maternal age[5]	37651	26.4 (6.28)	26.1 (6.26)	0.008
Education of caretaker[6]				0.553
0 year	10519	772 (27.7%)	9747 (28.5%)	

1-4 years	4914	384 (13.8%)	4530 (13.3%)	
+4 years	21494	1629 (58.5%)	19865 (58.2%)	
Suburb				<0.0001
Bandim	17436	1426 (47.2%)	16010 (44.0%)	
Belem / Mindara	6058	502 (16.6%)	5556 (15.3%)	
Cuntum	15927	1094 (36.2%)	14833 (40.8%)	
Ethnicity[7]				<0.0001
Pepel	10826	819 (27.5%)	10007 (27.7%)	
Balanta	3237	290 (9.7%)	2,947 (8.2%)	
Mandinga / Fula	11801	858 (28.8%)	10943 (30.3%)	
Manjaco / Mancanha	7060	596 (20.0%)	6464 (17.9%)	
Others	6160	417 (14.0%)	5743 (15.9%)	
Socioeconomic factors				
Type of roof[8]				0.067
Straw	1015	93 (3.1%)	922 (2.5%)	
Hard	38341	2919 (96.9%)	35422 (97.5%)	
Toilet[9]				<0.0001
None	106	11 (0.4%)	95 (0.3%)	
Latrine	32828	2618 (87.0%)	30210 (83.2%)	
Inside	6388	381 (12.7%)	6007 (16.5%)	
Electricity[10]				<0.0001
Yes	13791	950 (31.5%)	12841 (35.3%)	
No	25572	2067 (68.5%)	23505 (64.7%)	

[1] Missing information for 1 child
[2] Missing information for 54 children
[3] Missing information for 101 children
[4] Missing information for 17 children
[5] Missing information for 1770 children
[6] Missing information for 2494 children
[7] Missing information for 337 children
[8] Missing information for 65 children
[9] Missing information for 99 children
[10] Missing information for 58 children

Table 3. Mortality of children with and without neonatal BCG by TB-exposure status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Neonatal BCG	2605	12.2 (30/2,462)	0.29 (0.14-0.57)	0.57 (0.26-1.27)[1]
No neonatal BCG	417	35.2 (13/369)	Ref	Ref
TB-unexposed				
Neonatal BCG	32040	13.5 (533/39,608)	0.57 (0.47-0.69)	0.57 (0.47-0.69)[2]
No neonatal BCG	6105	22.8 (157/6,880)	Ref	Ref
Combined TB-exposed and TB-unexposed				
Neonatal BCG	33137	13.4 (563/42,069)	0.54 (0.45-0.65)	0.54 (0.45-0.65)[2]
No neonatal BCG	6284	23.4 (170/7,250)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.

Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.

[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis. The crude analysis excluding children with missing information on twin status, maternal age or year of birth yielded HR: 0.43 (0.19-0.93).

[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Table 4. Mortality of children with and without neonatal BCG by TB-exposure status and sex.

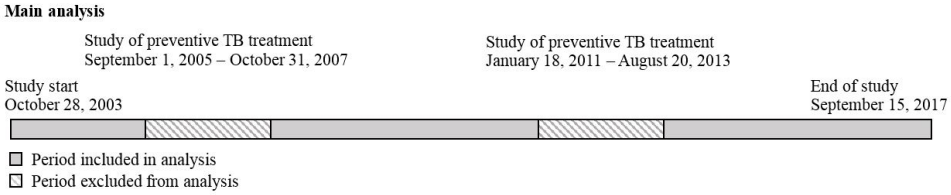
	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed boys				
Neonatal BCG	1355	8.5 (11/1,288)	0.19 (0.07-0.51)	0.23 (0.08-0.62)[1]
No neonatal BCG	219	35.6 (7/197)	Ref	Ref
TB-unexposed boys				
Neonatal BCG	16268	13.6 (275/20,166)	0.58 (0.45-0.75)	0.58 (0.45-0.75)[2]
No neonatal BCG	3072	23.0 (80/3,478)	Ref	Ref
TB-exposed girls				
Neonatal BCG	1250	16.2 (19/1,174)	0.37 (0.14-0.96)	1.61 (0.36-7.08)[1]
No neonatal BCG	198	34.7 (6/173)	Ref	Ref
TB-unexposed girls				
Neonatal BCG	15772	13.3 (258/19,442)	0.56 (0.43-0.73)	0.56 (0.43-0.73)[2]
No neonatal BCG	3032	22.6 (77/3,402)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis
[2] Adjusted HR yielded the crude analysis, and no child was excluded.

Table 5. Mortality of TB-exposed children compared with TB-unexposed children by timing of BCG and sex.

	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)
All children		
Neonatal BCG		
TB-exposed	12.2 (30/2,462)	1.11 (0.77-1.61)
TB-unexposed	13.5 (533/39,608)	Ref
No neonatal BCG		
TB-exposed	35.2 (13/369)	1.93 (1.10-3.41)
TB-unexposed	22.8 (157/6,880)	Ref
Boys		
Neonatal BCG		
TB-exposed	8.5 (11/1,288)	0.77 (0.42-1.41)
TB-unexposed	13.6 (275/20,166)	Ref
No neonatal BCG		
TB-exposed	35.6 (7/197)	1.98 (0.91-4.28)
TB-unexposed	23.0 (80/3,478)	Ref
Girls		
Neonatal BCG		
TB-exposed	16.2 (19/1,174)	1.50 (0.94-2.39)
TB-unexposed	13.3 (258/19,442)	Ref
No neonatal BCG		
TB-exposed	34.7 (6/173)	1.90 (0.83-4.35)
TB-unexposed	22.6 (77/3,402)	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
Adjusted HR are not presented as no baseline factor changed the estimates by more than 5%.

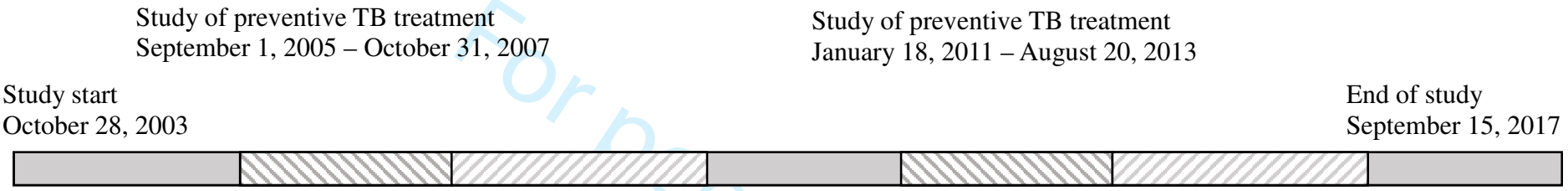


Timeline for study period

109x56mm (300 x 300 DPI)

Supplementary material for "Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children"

Supplementary Figure 1: Timeline of study period; sensitivity analysis excluding children potentially eligible for studies of preventive TB treatment



Supplementary table 1. Mortality of children with early neonatal BCG, late neonatal BCG and no neonatal BCG by TB-exposure status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed children				
Early neonatal BCG (Day 0-7)	1718	11.3 (18/1,588)	0.19 (0.08-0.44)	0.39 (0.15-0.98)[1]
Late neonatal BCG (Day 8-28)	887	13.7 (12/874)	0.45 (0.20-1.01)	0.90 (0.36-2.25)[1]
No neonatal BCG	417	35.2 (13/369)	Ref	Ref
TB-unexposed children				
Early neonatal BCG (Day 0-7)	21310	13.7 (357/25,981)	0.59 (0.48-0.74)	0.59 (0.48-0.74)[2]
Late neonatal BCG (Day 8-28)	10730	12.9 (176/13,627)	0.55 (0.44-0.68)	0.55 (0.44-0.68)[2]
No neonatal BCG	6105	22.8 (157/6,880)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis
[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 2. Sensitivity analyses comparing mortality of children with and without neonatal BCG for TB-exposed and TB-unexposed children.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
Extended follow-up: Mortality between 28 days and 5 years of life[1]				
TB-exposed				
Neonatal BCG	2902	10.9 (35/3,217)	0.30 (0.16-0.57)	0.56 (0.27-1.16)[5]
No neonatal BCG	476	29.6 (15/507)	Ref	Ref
TB-unexposed				
Neonatal BCG	32104	13.0 (574/44,114)	0.57 (0.48-0.68)	0.57 (0.48-0.68)[6]
No neonatal BCG	6109	21.9 (171/7,821)	Ref	Ref
Only children with a registered BCG vaccine are included in the analysis[2]				
TB-exposed				
Neonatal BCG	2605	12.2 (30/2,462)	0.29 (0.14-0.61)	0.62 (0.26-1.49)[5]
No neonatal BCG	356	34.4 (11/320)	Ref	Ref
TB-unexposed				
Neonatal BCG	32040	13.5 (533/39,608)	0.62 (0.51-0.76)	0.62 (0.51-0.76)[6]
No neonatal BCG	5247	20.6 (125/6,075)	Ref	Ref
Excluding children who were twins[3]				
TB-exposed				
Neonatal BCG	2534	11.7 (28/2,392)	0.33 (0.16-0.69)	0.63 (0.26-1.51)[5]
No neonatal BCG	387	32.1 (11/343)	Ref	Ref
TB-unexposed				
Neonatal BCG	31141	13.2 (506/38,473)	0.59 (0.48-0.72)	0.59 (0.48-0.72)[6]
No neonatal BCG	5668	21.6 (138/6,387)	Ref	Ref
Excluding children potentially eligible for studies with preventive TB treatment for children (Supplementary Figure 1)[4]				
TB-exposed				
Neonatal BCG	1461	16.4 (23/1,399)	0.46 (0.18-1.20)	0.95 (0.31-2.97)[5]
No neonatal BCG	258	27.5 (6/218)	Ref	Ref
TB-unexposed				
Neonatal BCG	22416	15.3 (418/27,312)	0.57 (0.46-0.70)	0.57 (0.46-0.70)[6]
No neonatal BCG	4580	25.7 (129/5,026)	Ref	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Extending follow-up to 5 years included 356 additional TB-exposed children (7 deaths) and 68 additional TB-unexposed children (55 deaths) in the analysis.

[2] Limiting the analysis to children with a registered BCG vaccine excluded 61 TB-exposed children (2 deaths) and 858 TB-unexposed children (32 deaths) from the analysis.
[3] Limiting the analysis to children who are not twins excluded 101 TB-exposed children (4 deaths) and 1336 TB-unexposed children (46 deaths) from the analysis.
[4] Limiting the analysis to children who had not been eligible for studies of preventive TB treatment (living in the study area and below 5 years of age in the study period) excluded 1303 TB-exposed children (14 deaths) and 11149 TB-unexposed children (143 deaths) from the analysis (Supplementary Figure 1).
[5] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children.
[6] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 3. Mortality of children with and without neonatal BCG by TB-exposure status and low-birth-weight status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Low-birth-weight children				
Neonatal BCG	118	17.4 (2/115)	0.16 (0.03-0.78)	0.33 (0.06-1.93)[1]
No neonatal BCG	56	150.3 (7/47)	Ref	Ref
Normal-birth-weight children				
Neonatal BCG	1631	9.8 (15/1,532)	N/A	N/A
No neonatal BCG	106	9.8 (1/103)	Ref	Ref
TB-unexposed				
Low-birth-weight children				
Neonatal BCG	1341	24.9 (39/1,568)	0.70 (0.44-1.11)	0.70 (0.44-1.11)[2]
No neonatal BCG	898	33.5 (36/1,076)	Ref	Ref
Normal-birth-weight children				
Neonatal BCG	21108	12.1 (319/26,389)	0.76 (0.50-1.16)	0.76 (0.50-1.16)[2]
No neonatal BCG	1525	14.2 (26/1,828)	Ref	Ref

Limiting the analysis to children with information on birthweight excluded 1111 TB-exposed children (21 deaths) and 13273 TB-unexposed children (270 deaths) from the analysis.
All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis.
[2] Adjusted HR calculated yielded the crude analysis, and no child was excluded.

Supplementary table 4. PPD reactions at 6 and 12 months of age of children with and without neonatal BCG by TB-exposure status.

	Number of children n	Median PPD response in mm (interquartile range)	Positive PPD 10mm cut-off	Prevalence ratio 10mm cut-off	Positive PPD 15mm cut-off	Prevalence ratio 15mm cut-off
PPD response at 6 months of age						
TB-exposed children						
Neonatal BCG	48	0 (0-4.8)	3 (6.3%)	NA	0 (0.0%)	NA
No neonatal BCG	5	0 (0-3.5)	0 (0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	1248	0 (0-4.5)	94 (7.5%)	0.93 (0.51-1.70)	23 (1.8%)	1.25 (0.30-5.26)
No neonatal BCG	136	0 (0-4.5)	11 (8.1%)	Ref	2 (1.5%)	Ref
PPD response at 12 months of age						
TB-exposed children						
Neonatal BCG	5	0 (0-0)	(0.0%)	NA	0 (0.0%)	NA
No neonatal BCG	2	6 (5.5-6.5)	(0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	297	0 (0-0)	21 (7.1%)	0.90 (0.28-2.86)	6 (2.0%)	0.26 (0.07-0.98)
No neonatal BCG	38	0 (0-2.5)	3 (7.9%)	Ref	3 (7.9%)	Ref

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1+2	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4	
Objectives	3	State specific objectives, including any prespecified hypotheses	4	
Methods				
Study design	4	Present key elements of study design early in the paper	4-6	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6	
Bias	9	Describe any efforts to address potential sources of bias	5-6	
Study size	10	Explain how the study size was arrived at	6	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7 + table 1 and 2
		(b) Indicate number of participants with missing data for each variable of interest	Table 1+2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Table 3
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 3
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7 + Table 3
		(*b*) Report category boundaries when continuous variables were categorized	Table 1+2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 3-5 + Supp tables
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Neonatal BCG vaccination and child survival in TB-exposed and TB-unexposed children: a prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035595.R1
Article Type:	Original research
Date Submitted by the Author:	17-Jan-2020
Complete List of Authors:	Thysen, Sanne; University of Southern Denmark, Bandim Health Project, OPEN; Bandim Health Project, Bandim Health Project Benn, Christine Stabell; Statens Serum Institut, Bandim Health Project; University of Southern Denmark, OPEN Gomes, Victor; Bandim Health Project, ; Serum Statens Institute, Epidemiology Rudolf, Frauke; Bandim Health Project, Bandim Health Project; Aarhus University Hospital, Department of Infectious Diseases Wejse, Christian; Aarhus University, Center for Global Health (GloHAU), Department of Public Health Roth, Adam; Public Health Agency of Sweden, Dept. of Communicable Disease Control and Health Protection; Lund University, Dept. of Translational Medicine Kallestrup, Per; Aarhus University, Center for Global Health, Department of Public Health Aaby, Peter; Bandim Health Project, Fisker, Ane; Bandim Health Project,
Primary Subject Heading:	Global health
Secondary Subject Heading:	Epidemiology, Health policy, Infectious diseases, Paediatrics, Public health
Keywords:	EPIDEMIOLOGY, Public health < INFECTIOUS DISEASES, Tuberculosis < INFECTIOUS DISEASES, Paediatric infectious disease & immunisation < PAEDIATRICS, PUBLIC HEALTH

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Neonatal BCG vaccination and child survival in TB-exposed and TB-unexposed children: a prospective cohort study

Sanne M. Thysen[a,b,c,d] PhD, Christine S. Benn[a,b,c] DMSc, Victor F. Gomes[b] PhD, Frauke Rudolf[b,d] PhD, Christian Wejse[b,d,e] PhD, Adam Roth[f,g] PhD, Per Kallestrup[d] PhD, Peter Aaby[b,c] DMSc, Ane B. Fisker[a,b,c] PhD

[a] OPEN, Institute of Clinical Research, Odense University Hospital and University of Southern Denmark, J.B. Winsløwsvej 9, 5000 Odense, Denmark

[b] Bandim Health Project, Apartado 861, 1004 Bissau Codex, Guinea-Bissau

[c] Research Centre for Vitamins and Vaccines, Bandim Health Project, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark

[d] Center for Global Health (GloHAU), Aarhus University, Bartholins Allé 2, 8000 Aarhus, Denmark

[e] Dept of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

[f] Dept. of Communicable Disease Control and Health Protection, Public Health Agency of Sweden, SE-171 81 Solna, Sweden

[g] Dept. of Translational Medicine, Lund University, SE-205 02 Malmö, Sweden

Corresponding author: Sanne Marie Thysen, Bandim Health Project, INDEPTH Network; Apartado 861, 1004 Bissau Codex, Guinea-Bissau; s.thysen@bandim.org, Telephone: +45 31127825/ +245 955859948

Word count: Abstract: 249, Manuscript: 3592

Abstract

Objectives To assess the association between neonatal BCG vaccination and mortality between 28 days and 3 years of age among TB-exposed and TB-unexposed children.

Design Prospective cohort study.

Setting Bandim Health Project runs an urban Health and Demographic Surveillance site in Guinea-Bissau with registration of mortality, vaccination status and TB cases.

Participants Children entered the analysis when their vaccination card was inspected after 28 days of age, and remained under surveillance to 3 years of age. Children residing in the same house as a TB case were classified as TB-exposed from 3 months prior to case registration to the end of follow-up.

Methods Using Cox-proportional hazards models with age as underlying time scale, we compared mortality of children with and without neonatal BCG between October 2003 and September 2017.

Main outcome measure Hazard ratio (HR) for neonatal BCG compared with no neonatal BCG by TB-exposure status.

Results Among the 39,421 children who entered the analyses, 3,022 (8%) had observation time as TB-exposed. In total, 84% of children received neonatal BCG. Children with neonatal BCG had lower mortality both in TB-exposed (adjusted Hazard Ratio: 0.57 (0.26-1.27)) and in TB-unexposed children (HR: 0.57 (95% CI: 0.47-0.69)) than children without neonatal BCG. Children exposed to TB had higher mortality than TB-unexposed children if they had not received neonatal BCG.

Conclusion Neonatal BCG vaccination was associated with lower mortality among both TB-exposed and TB-unexposed children, consistent with neonatal BCG vaccination having beneficial non-specific effects. Interventions to increase timely BCG vaccination are urgently warranted.

Strengths and limitations of this study

- The study was conducted in Bandim Health Project's urban Health and Demographic Surveillance Site with continuous registration of births, deaths and vaccination status and linked with the TB surveillance database, which allowed classification of households as TB-exposed and TB-unexposed
- More than 39,000 children entered the analysis, all children entering the analysis had their vaccination status assessed after the neonatal period
- The analyses were adjusted for potential confounders, however, residual confounding cannot be excluded
- Some TB cases may be undiagnosed, leading to some TB-exposed children being classified as TB-unexposed. We expect any misclassification to be independent of timing of BCG vaccination

Introduction

Bacillus Calmette-Guérin (BCG) vaccine was developed to protect against tuberculosis (TB), and remains the only approved TB vaccine[1]. The efficacy of the BCG vaccine to protect against TB has varied between different trials[2]. However, trials of neonatal BCG vaccination consistently find that BCG is associated with reduced TB incidence[2].

BCG is recommended at birth in countries with high burden of TB[1]. According to WHO/UNICEF estimates, 88 percent of children are BCG vaccinated in countries where BCG is part of the routine vaccination programme[3]. However, WHO/UNICEF coverage estimates are based on coverage at 12 months of age, and BCG is often delayed in low-income countries[4,5]. If delayed BCG vaccination is associated with lower vaccine efficacy, delays may be critical.

Vaccines are designed to protect against specific target diseases. Increasing evidence supports that vaccines have additional effects affecting the susceptibility of untargeted infections, these have been coined non-specific effects (NSEs), and increasing evidence suggest that BCG affects mortality by more than can be explained by the protection against TB[6,7], that is BCG may have beneficial NSE. Three randomised trials in low-weight children in Guinea-Bissau found that BCG vaccination at birth was associated with 38% (17-54%) lower neonatal mortality compared with children not BCG vaccinated at birth[8]. Already 3 days after enrolment, BCG was associated with 45% (7-68%) lower mortality compared with unvaccinated children[8], suggesting that even small delays in BCG vaccination may be important for survival.

A potential effect of early BCG on childhood mortality could be due to prevention of TB, NSEs of BCG or a combination. If the effect of BCG was merely specific, we should expect strong effects among children exposed to TB[9] and no effect in children not exposed to TB.

The main objective of this study was to assess the association between neonatal BCG versus later BCG vaccination and mortality by registered exposure to TB. We furthermore assessed the association between neonatal BCG vaccination and positive TST reactions by registered exposure to TB.

Methods

Setting and study population

Bandim Health Project (BHP) runs a Health and Demographic Surveillance Site (HDSS) in six suburban districts in Bissau, the capital of Guinea-Bissau. Children are followed through tri-monthly home visits until 3 years of age. At the home visits, a field assistant collects information on vital status, measures mid-upper-arm circumference (MUAC) and registers vaccination status by transcribing information from the child's vaccination card. Each month, field assistants conduct home visits to follow pregnant women and register new births. Children above 3 years of age and adults are followed through censuses conducted every 2-5 years.

The BCG vaccines used in Guinea-Bissau have been provided by UNICEF and mainly the Russian strain has been used. However, during large periods within the study period, the BHP provided vaccines for the study area; these were the BCG-Denmark strain purchased at the Statens Serum Institut, Denmark.

Since 1996, all diagnosed TB cases above 15 years of age living in the study area have been registered and followed[10]. In 2003, a register of information on all TB patients from the study area was established, making it possible to identify houses with exposure to TB. We defined inhabitants as exposed to TB from 3 months prior to diagnosis of a TB case in the house (with several households) and until 2 weeks after diagnosis, to account for delay in diagnosis, which we, in line with previous studies[11,12], assumed was a median of 3 months. Children were classified as TB-exposed from the first registered TB exposure (3 months prior to TB diagnosis of co-inhabitant) and remained classified as TB-exposed throughout the follow-up period. We expect some TB cases to be undiagnosed. Thus, some children classified as TB-unexposed may have been exposed.

Preventive treatment to TB-exposed children is not routinely provided in Guinea-Bissau. Between September 1, 2005 and October 31, 2007, and between January 18, 2011 and August 20, 2013, studies of the effect of preventive treatment to TB-exposed children were conducted in the BHP study area[12,13]. We excluded these periods for all children in the present study (Figure 1). Thus, we included children who had their vaccination status assessed after 28 days of age between October 28, 2003 (Start of the TB registry) and September 15, 2017 excluding periods with studies of preventive TB treatment. From July 2002 to April 2004, a randomised trial of BCG-revaccination at 19 months of age was conducted in the study area, we therefore censored follow-up at 19 months of age for children eligible for the BCG-revaccination trial[14].

Between start of study and July 1, 2008, a sample of children living in the study area were TST tested using the Mantoux method with an intradermal application of 0.1 ml of PPD (2 tuberculin units RT23, Statens Serum Institut, Denmark) in the forearm at ages 6 and 12 months. The PPD reactions were measured after 48-72 hours using a ruler and ballpoint technique to measure two diameters[15]. Among children with measured PPD reactions, we assessed the effect of neonatal BCG vaccination versus later BCG vaccination on PPD reactions using cut-offs of 10mm and 15mm.

Statistical analyses

We compared baseline characteristics of children with and without neonatal BCG in TB-exposed and TB-unexposed children using chi^2 and paired t-tests. We also compared baseline characteristics of children registered and not registered as TB-exposed.

In Cox-proportional hazards models with age as underlying time scale, we compared mortality rates of children with neonatal BCG with mortality rates of children without neonatal BCG (delayed BCG or no BCG) separately for TB-exposed and TB-unexposed children, allowing for different baseline hazards according to sex and place of birth (maternity ward, health centre or home). Children entered the analysis the first time their vaccination status was assessed at a home visit after the neonatal period. Observation time was split at the first time-point, where the child was considered to be TB-exposed. Thus, TB-exposed children could contribute with observation time in the TB-unexposed group until 3 months prior to diagnosis of a co-inhabitant. To control for potential confounding, we assessed whether baseline characteristics (Table 1) changed the estimate by including the factors in the analysis one by one. We adjusted for baseline characteristics that changed the estimate by more than 5%.

In a secondary analysis, we explored whether the effect of neonatal BCG differed by timing of BCG within the neonatal period, thus we divided children with neonatal BCG in two groups, children

with early neonatal BCG (vaccinated within 7 days after birth), and children with late neonatal BCG (vaccinated between day 8 and day 28).

In sensitivity analyses, we: A) Excluded all children, who potentially had been exposed to preventive TB treatment as part of the aforementioned studies (Supplementary Figure 1). B) Restricted the analysis to children with a registered BCG vaccine (i.e. allowing a child only to contribute time at risk from the first visit at which a BCG vaccine was registered, therefore, children with no registered BCG vaccine would not enter the analysis). C) Extended follow-up to 5 years of age. D) Excluded twins, as these are more likely to be low-birth-weight (LBW) children and thus receive delayed BCG. E) Stratified the analysis by LBW status for the subset of children for whom we had information on birthweight.

BCG vaccination may cause a transient weak response to PPD[16], but reactions above 15mm would be less likely to be caused by BCG[17]. Using log-binomial regression, we compared the prevalence of positive TST in children with neonatal BCG with children without neonatal BCG among TB-exposed and TB-unexposed children, respectively. We limited comparison to children BCG vaccinated prior to TST assessment and evaluated PPD reaction at age 6 and 12 months using cut-offs of 10mm and 15mm.

Ethical considerations

The data used for this study was mostly obtained from BHP's urban HDSS, which has been following women and children since 1978, where data collection was initiated at the request of the Ministry of Health in Guinea-Bissau. Further already collected data on TST was obtained through previous studies approved by the Guinean Ethics Committee. Oral consent was obtained from mother/guardian of the children prior to TST. As no additional data was collected for this study, no ethical approval was needed.

Patient and public involvement

The communities were involved in locating households, when the HDSS was setup and contributed information allowing tracing of internal migrants between suburbs throughout the study period. No participant was involved in setting the research question or the outcome measure, nor were they involved in developing plans for recruitment, design, or implementation of the study. No participant was asked to advise on interpretation or writing up the results. The results are disseminated to the national public health institute. There are no plans to disseminate the results of the research to study participants or the community.

Results

A total of 39,421 children contributed time at risk, and among these 3,022 had observation time while living in houses of registered TB cases. Among children with a vaccination card seen after 28 days, 33,137 (84%) had received neonatal BCG. The median age of vaccination in the neonatal BCG group was 2 days (interquartile range (IQR): 1-10). Among the 6,284 children not BCG vaccinated in the neonatal period, 5,450 (87%) had a BCG vaccine registered at some point of time during the follow up period (median age of vaccination: 48 days (IQR: 36-69)).

Baseline characteristics

We compared baseline characteristics of children with and without neonatal BCG according to TB-exposure status. In this large dataset, most statistically significant differences were small absolute differences. In both groups, mothers of children with neonatal BCG were better educated, older, and had better socioeconomic status (toilet, electricity, among not TB-exposed children also type of roof). Children with neonatal BCG were more likely to be born at a health facility and less likely to be twins. Ethnic groups also varied between children with and without neonatal BCG. Among TB-unexposed children, the distribution of season of birth, year of birth, number of pregnancies and suburb differed between neonatal BCG vaccinated and not neonatal BCG vaccinated children (Table 1).

Several baseline characteristics were statistically significant different between TB-exposed and TB-unexposed children (birth location, number of pregnancies, maternal age, suburb, ethnicity, and socioeconomic factors) (Table 2).

Effect of neonatal BCG on mortality among children registered as TB-exposed

We included 3,022 TB-exposed children in the analyses, among these 2,605 (86%) were neonatally BCG vaccinated, whereas 417 (14%) were not. TB-exposed children with neonatal BCG had a mortality rate (MR) of 12.2 per 1000 person years (PYRS, 30 deaths during 2,462 PYRS), and children without neonatal BCG (delayed or no BCG) had a MR of 35.2 per 1000 PYRS (13 deaths during 369 PYRS). The hazard ratio (HR) comparing TB-exposed children with and without neonatal BCG was 0.29 (95% CI: 0.14-0.57). Adjusting for the baseline characteristics that changed the estimate by more than 5% resulted in an adjusted hazard ratio (aHR) of 0.57 (0.26-1.27) (Table 3). Neonatal BCG was beneficial for TB-exposed boys (aHR: 0.23 (0.08-0.62)), but not for TB-exposed girls (aHR: 1.61 (0.36-7.08)) (p-value=0.03) (Table 4).

The mortality was lowest among children with early neonatal BCG (aHR compared with children with no neonatal BCG: 0.39 (0.15-0.98) and an aHR of 0.90 (0.36-2.25) for children with late neonatal BCG compared with children with no neonatal BCG) (Supplementary table 1).

Extending follow-up to 5 years of age or excluding children with no registered BCG vaccination, or excluding twins from the analysis did not alter the conclusions (Supplementary table 2). Excluding children who had potentially been eligible for studies of preventive TB treatment (Supplementary figure 1) resulted in an aHR of 0.95 (95% CI: 0.31-2.97) (Supplementary table 2). Stratifying the analysis by LBW status (<2500g), had limited power (Supplementary table 3).

Effect of neonatal BCG on mortality among children registered as TB-unexposed

We included 38,145 children classified as TB-unexposed in this analysis, 32,040 (84%) were neonatally BCG vaccinated, and 6,105 (16%) were not. Children with neonatal BCG vaccination had a MR of 13.5 per 1000 PYRS (533 deaths during 39,608 PYRS) and not neonatal BCG vaccinated children had a MR of 22.8 per 1000 PYRS (157 deaths during 6,880 PYRS). Children with neonatal BCG had 43% lower mortality (HR: 0.57 (95% CI: 0.47-0.69) compared with not neonatal BCG vaccinated children (Table 3). No background factor changed the estimate by more than 5%, and thus adjusted estimates are not presented. The effect of neonatal BCG did not differ by sex (p=0.87) (Table 4).

The effect of BCG within the neonatal period was similar: HR: 0.59 (0.48-0.74) for children with early neonatal BCG compared with no neonatal BCG and HR: 0.55 (0.44-0.68) for children with late neonatal BCG (Supplementary table 1).

Extending follow-up to 5 years of age, excluding children with no registered BCG vaccine, excluding twins from the analysis or excluding children potentially eligible for studies of preventive TB treatment (Supplementary figure 1) did not alter the conclusions (Supplementary table 2). Stratifying the analysis by LBW status, indicated that neonatal BCG was associated with an HR of 0.70 (0.44-1.11) in LBW children, and an HR of 0.76 (0.50-1.16) in NBW children (Supplementary table 3).

Mortality in TB-exposed and TB-unexposed children according to vaccination status

We also examined the effect of being TB-exposed on mortality among children with neonatal BCG and children without neonatal BCG, respectively. Among children with neonatal BCG, TB-exposed children had slightly higher mortality than TB-unexposed children (HR: 1.11 (0.77-1.61)). Among not neonatally BCG-vaccinated children, TB-exposed children had higher mortality (HR: 1.93 (1.10-3.41)) (p=0.11 for interaction between neonatal BCG and TB-exposure, Table 5).

We explored whether the effect differed for boys and girls. TB-exposure was associated with higher mortality for girls both with and without neonatal BCG (HR: 1.50 (0.94-2.39) and 1.90 (0.83-4.35), respectively). In boys, TB-exposure was associated with higher mortality for children without neonatal BCG (HR: 1.98 (0.91-4.28)), whereas this was not the case for children with neonatal BCG (HR: 0.77 (0.42-1.41)) (Table 5).

Tuberculin skin test reactions

Among 53 TB-exposed children with a TST assessed at 6 months, a total of 3 (5.7%) children (all with neonatal BCG) had a PPD reaction above 10mm. Only seven TB-exposed children had a TST assessed at 12 months and no child had a PPD reaction above 15mm (Supplementary Table 4).

Among 1384 TB-unexposed children with assessed TST at 6 months, neonatal BCG did not affect PPD reactions; among the 335 children with TST assessed at 12 months fewer children with neonatal BCG had large TSTs (Supplementary Table 4).

Discussion

Main findings

Neonatal BCG was associated with lower mortality among both TB-exposed (aHR: 0.57 (95%CI: 0.26-1.27)) and TB-unexposed children (HR: 0.57 (95%CI: 0.47-0.69)), resulting in a combined HR of 0.54 (0.45-0.65), not adjusted as no factor changed the estimate by more than 5%. The results were robust to sensitivity analyses of longer follow-up, exclusion of children with no registered BCG vaccine, exclusion of children who were twins and exclusion of children potentially eligible for studies of preventive TB treatment. Children exposed to TB had higher mortality than TB-unexposed children if they had not received neonatal BCG.

Strengths and weaknesses

The study was conducted within the setup of Bandim Health Project's urban HDSS that since 1978 has followed the population in the study area with regular home visits to collect information on health status of children. Information on vaccination status and vital status is collected by experienced field assistants at home visits every third month, and hard endpoints like death are therefore unlikely to be misclassified. Only children with assessed vaccination status after 28 days of life were included in the analysis, and children only entered the analyses when the vaccination status was assessed at a home visit, thus avoiding differential misclassification of vaccination status and survival bias.

Self-selection to BCG vaccination could create healthy vaccinee bias, where early BCG is associated with lower mortality because the healthiest children receive BCG early, rather than early BCG causing healthy children. To remove potential confounding, we controlled for baseline characteristics that changed the estimate by more than 5% when included in the analyses one by one. Adjusting for available potential confounders affected the estimates for TB-exposed children, but did not alter the conclusions, neither did the sensitivity analyses conducted. Thus, self-selection for vaccination is unlikely to explain all of the effect. Since we find marked effects of neonatal BCG on all-cause mortality in both TB-exposed and TB-unexposed children, our results support that the effect of neonatal BCG is not merely due to protection against TB. However, there may be residual confounding not accounted for.

The registration of diagnosed TB cases from the study area allows for classifying children as TB-exposed. However, some TB cases will be undiagnosed, and some TB-exposed children may therefore have been misclassified as TB-unexposed. We expect any misclassification to be independent of timing of BCG vaccination. Unfortunately, we did not have HIV status for mothers or children, and we were therefore not able to assess whether HIV status influenced our results.

TB is difficult to diagnose, especially in children, and many TB cases are not diagnosed and treated[18,19]. Tuberculin skin test (TST) with purified protein derivatives (PPD) is commonly used to test for latent infection with *M. tuberculosis*[20]. A response to PPD can be due to either *M. tuberculosis* infection or BCG vaccination; however, BCG vaccination has been shown to result in lower PPD response compared with latent TB infection[20]. TST was only assessed for a subsample of our population, and we had scarce data in some groups. Our data did not allow for conclusions as to whether timing of BCG may have an impact on latent TB infection assessed by PPD reactions above 10mm or 15mm.

Comparison with other studies

The vaccine efficacy of BCG on preventing TB has been widely debated as different studies have yielded very different effects[21]. However, a meta-analysis of BCG's effect on TB found higher vaccine efficacy when BCG was given early in life[2]. Our results support that BCG in early life has beneficial effects, and furthermore suggest that timing of BCG is important. We did not study the vaccine efficacy on TB, and the results are therefore not directly comparable, but our findings support that BCG should be administered early in life.

Increasing evidence supports that BCG aside from the specific effects also have NSEs[6,22]. Most studies assessing the effect of BCG on all-cause mortality compared BCG-vaccinated children with un-vaccinated children[6,23,24] and randomised trials of BCG-Denmark have found beneficial effects

in the neonatal period[8]. It should be noted that two recent trials testing the effect of BCG-Russia in India found no effect[25]; the different results may be related to different BCG strains[25,26]. A previous study from the 1990s in Guinea-Bissau found that BCG was associated with stronger beneficial effects if provided in the first week of life[27]. Our study supports that the timing of BCG is important, not only because children vaccinated later are deprived of beneficial effects early in life, but also that there are benefits beyond the initial early phase. Similar benefits have been observed for other live vaccines (OPV[28], MV[29]).

In line with our study, data from the same setting between 1996 and 1998 indicate that TB exposure was associated with higher mortality in children aged 0-5 years[11]. We also found that TB exposure was associated with higher mortality, but less so among children with neonatal BCG, due to no excess mortality among boys with neonatal BCG. It is generally accepted that TB-exposure can result in increased mortality[9,30], and it is recommended to provide preventive TB treatment for children younger than 5 years of age[31], however, a policy-practice gap remains, and the policy is rarely implemented[32,33]. A recent modelling study estimated that more than 80,000 TB deaths could be averted globally in children younger than 5 years by altering coverage of household contact management from zero to full coverage[34]. Our data suggest that part of this effect (in boys) may also be obtained by emphasising BCG at birth.

Interpretation and implications

Our conclusions were robust to sensitivity analyses and adjusting for available potential confounders. However, if BCG vaccination was delayed mainly for frail children, this could bias our results. To adjust for indicators of frailty, we conducted sensitivity analyses excluding twins, and stratifying by LBW status. Excluding twins or stratifying by LBW status did not alter the main conclusions. Assessing the effect of neonatal BCG by LBW limited the analysis to the 63% of the TB-exposed children and 65% of the TB-unexposed children with information on birthweight, and should thus be interpreted with caution. As a whole, the sensitivity analyses did not suggest that frailty in children with no neonatal BCG explained our results.

Currently, timing of BCG is not captured as part of the WHO/UNICEF coverage estimates[5], and although BCG is planned to be given at birth in many countries, BCG is often delayed[4,5]. Our results support that timing of BCG is important. WHO already recommends that BCG should be provided as soon as possible after birth[1], but we need more focus on barriers for neonatal BCG vaccination. Since policies are frequently implemented according to donor's priorities, vaccine donors should be involved in creating incentives for neonatal BCG vaccination. Currently, vaccine donors evaluate performance of vaccine programmes by 12 months coverage estimates and vaccine wastage targets[35]. Including neonatal BCG vaccination as a performance indicator in addition to 12 months coverage would provide countries with an incentive to strive for timely vaccination.

Conclusion

Neonatal BCG was associated with reduced mortality in TB-exposed and TB-unexposed children, supporting that timing of BCG is important for all-cause mortality. Neonatal BCG should be emphasised and barriers for neonatal BCG vaccination should be removed.

Authors' contributions

SMT and ABF conceived the idea for the study and planned the analyses. CB, AR, PA and ABF supervised the demographic surveillance data collection, AR and ABF supervised and cleaned the PPD data, VFG, FR and CW supervised the data registration of TB patients. SMT analysed the data, and wrote the first manuscript draft with input from PK, PA and ABF. All authors received and approved the final manuscript.

Funding

This work was supported by Augustinus Foundation. The Bandim Health Project received support from the Novo Nordisk Foundation, DANIDA; EU [ICA 4-CT-2002-10053], the Danish National Research Foundation via Research Center for Vitamins and Vaccines [DNRF108], and Karen Elise Jensen Foundation. The sponsors had no role in designing the study, the data collection, data analysis, data interpretation or writing the paper.

Acknowledgments

We wish to thank all children, mothers and TB patients contributing with information to the present study. Furthermore, we would like to thank the dedicated staff working at BHP in Guinea-Bissau.

Conflicts of interest

All authors have no interests to declare.

Data availability

Data are available on a collaborative basis, please see www.bandim.org for further information.

References

1. World Health Organisation. BCG vaccines: WHO position paper - February 2018. *Weekly Epidemiological Journal* 2018; **93**: 73-96.
2. Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **58**(4): 470-80.
3. VanderEnde K, Gacic-Dobo M, Diallo MS, Conklin LM, Wallace AS. Global Routine Vaccination Coverage - 2017. *MMWR Morbidity and mortality weekly report* 2018; **67**(45): 1261-4.
4. Clark A, Sanderson C. Timing of children's vaccinations in 45 low-income and middle-income countries: an analysis of survey data. *Lancet* 2009; **373**(9674): 1543-9.
5. Thysen SM, Fisker AB, INDEPTH working group on Vaccines and Child Survival. Selecting the right indicators to ensure optimised implementation of BCG vaccination policy. *Vaccine* 2018; **36**(24): 3406-7.
6. Higgins JP, Soares-Weiser K, Lopez-Lopez JA, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 2016; **355**: i5170.
7. Uthayakumar D, Paris S, Chapat L, Freyburger C, Poulet H, De Luca K. Non-specific effects of vaccines illustrated through the BCG example: From observations to demonstrations. *Front Immunol* 2018.
8. Biering-Sørensen S, Aaby P, Lund N, et al. Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. *Clinical Infectious Diseases* 2017; **65**(7): 1183-90.
9. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2013; **41**(1): 140-56.
10. Lemvik G, Rudolf F, Vieira F, et al. Decline in overall, smear-negative and HIV-positive TB incidence while smear-positive incidence stays stable in Guinea-Bissau 2004-2011. *Trop Med Int Health* 2014; **19**(11): 1367-76.
11. Gomes VF, Andersen A, Wejse C, et al. Impact of tuberculosis exposure at home on mortality in children under 5 years of age in Guinea-Bissau. *Thorax* 2011; **66**(2): 163-7.
12. Gomes VF, Andersen A, Lemvik G, et al. Impact of isoniazid preventive therapy on mortality among children less than 5 years old following exposure to tuberculosis at home in Guinea-Bissau: a prospective cohort study. *BMJ open* 2013; **3**(3).
13. Lemvik G. Active TB among adults and latent TB among children in Guinea-Bissau - risk factors and adherence to preventive therapy: Aarhus University; 2013.
14. Roth AE, Benn CS, Ravn H, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. *BMJ* 2010; **340**: c671.
15. Bouros D, Zeros G, Panaretos C, Vassilatos C, Siafakas N. Palpation vs pen method for the measurement of skin tuberculin reaction (Mantoux test). *Chest* 1991; **99**(2): 416-9.
16. Menzies D. What Does Tuberculin Reactivity after Bacille Calmette-Guérin Vaccination Tell Us? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2000; **31**.
17. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guérin vaccination on tuberculin skin test measurements. *Thorax* 2002; **57**: 804-9.
18. World Health Organisation. Global Tubeculosis Report 2018. Licence: CC BY-NC-SA 3.0 IGO., 2018.
19. Jenkins HE. Global Burden of Childhood Tuberculosis. *Pneumonia (Nathan)* 2016; **8**.
20. Enarson DA. Use of tuberculin skin test in children. *Paediatric Respiratory Reviews* 2004; **5**(Supp A): 135-7.
21. Abubakar I, Pimpin L, Ariti C, et al. Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis. *Health technology assessment* 2013; **17**(37): 1-372, v-vi.

22. Arts RJW, Moorlag S, Novakovic B, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell host & microbe* 2018; **23**(1): 89-100 e5.
23. Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 2000; **321**(7274): 1435-8.
24. Aaby P, Jensen H, Gomes J, Fernandes M, Lisse IM. The introduction of diphtheria-tetanus-pertussis vaccine and child mortality in rural Guinea-Bissau: an observational study. *Int J Epidemiol* 2004; **33**(2): 374-80.
25. Jayaraman K, Adhisivam B, Nallasivan S, et al. Two Randomized Trials of the Effect of BCG-Russia Alone or With Oral Polio Vaccine on Neonatal Mortality in Infants Weighing <2000 G in India. *Pediatr Infect Dis J* 2018.
26. Curtis N. BCG Vaccination and All-Cause Neonatal Mortality. *Pediatr Infect Dis J* 2019; **38**(2): 195-7.
27. Roth A, Jensen H, Garly M-L, et al. Low Birth Weight Infants and Calmette-Guérin Bacillus Vaccination at Birth. *Pediatr Infect Dis J* 2004; **23**(6): 544-50.
28. Lund N, Andersen A, Hansen AS, et al. The Effect of Oral Polio Vaccine at Birth on Infant Mortality: A Randomized Trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2015; **61**(10): 1504-11.
29. Aaby P, Martins CL, Garly ML, et al. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. *BMJ* 2010; **341**: c6495.
30. Marais BJ. Preventing tuberculosis in household contacts crucial to protect children and contain epidemic spread. *The Lancet Global Health* 2018.
31. World Health Organization. Latent TB Infection: Updated and consolidated guidelines for programmatic management. http://apps.who.int/iris/bitstream/handle/10665/260233/9789241550239-eng.pdf;jsessionid=822A28C5C42D1A1A1262F556FC0CEC7C?sequence=1: World Health Organization, 2018.
32. Hill P, Rutherford M, Audas R, van Crevel R, Graham S. Closing the policy-practice gap in the management of child contacts of tuberculosis cases in developing countries. *PLoS Med* 2011.
33. Gomes VF, Wejse C, Oliveira I, et al. Adherence to isoniazid preventive therapy in children exposed to tuberculosis: a prospective study from Guinea-Bissau. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2011; **15**(12): 1637-43.
34. Dodd PJ, Yuen CM, Becerra MC, Revill P, Jenkins HE, Seddon JA. Potential effect of household contact management on childhood tuberculosis: a mathematical modelling study. *The Lancet Global Health* 2018.
35. GAVI. Gavi 2016-2020 strategy indicators. 2016.

Figure 1: Timeline of study period

Table 1. Baseline characteristics of children by neonatal BCG and registered TB-exposure status.

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children				TB-unexposed children			
	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value	Total	Neonatal BCG (n %)	No neonatal BCG (n %)	p-value
Number	3022	2605 (86.2%)	417 (13.8%)		36399	30532 (83.9%)	5867 (16.1%)	
Sex[1]				0.849				.35
Male	1574	1355 (52.0%)	219 (52.5%)		18421	15485 (50.7%)	2936 (50.1%)	
Female	1448	1250 (48.0%)	198 (47.5%)		17977	15047 (49.3%)	2930 (49.9%)	
Twin[2]				<0.0001				<0.0001
Yes	99	70 (2.7%)	29 (7.0%)		1233	826 (2.7%)	407 (7.0%)	
No	2921	2534 (97.3%)	387 (93.0%)		35114	29669 (97.3%)	5445 (93.0%)	
Season of birth				0.376				0.024
Rainy season	1474	1279 (49.1%)	195 (46.8%)		17795	15006 (49.1%)	2789 (47.5%)	
Dry season	1548	1326 (50.9%)	222 (53.2%)		18604	15526 (50.9%)	3078 (52.5%)	
Birth location[3]				<0.0001				<0.0001
Home	902	721 (27.7%)	181 (43.6%)		10030	7463 (24.5%)	2567 (44.1%)	
Health centre	1329	1259 (48.4%)	70 (16.9%)		16171	15285 (50.2%)	886 (15.2%)	
Hospital	647	512 (19.7%)	135 (32.5%)		7923	6001 (19.7%)	1922 (33.0%)	
Other	139	110 (4.2%)	29 (7.0%)		2179	1727 (5.7%)	452 (7.8%)	
Year of birth				0.550				<0.0001
2000-2006	1010	866 (33.2%)	144 (34.5%)		11410	9430 (30.9%)	1980 (33.7%)	
2007-2011	999	856 (32.9%)	143 (34.3%)		12654	10625 (34.8%)	2029 (34.6%)	
2012-2017	1013	883 (33.9%)	130 (31.2%)		12335	10477 (34.3%)	1858 (31.7%)	
Number of pregnancies[4]				0.694				<0.0001
1	838	723 (27.8%)	115 (27.6%)		10972	9198 (30.1%)	1774 (30.3%)	
2 - 3	1249	1083 (41.6%)	166 (39.8%)		15316	12987 (42.6%)	2329 (39.7%)	
4 or more	934	798 (30.6%)	136 (32.6%)		10095	8336 (27.3%)	1759 (30.0%)	
Maternal age[5]	2939	26.5 (6.25)	25.6 (6.40)	0.005	34712	26.2 (6.22)	25.3 (6.38)	<0.0001
Education of caretaker[6]				<0.0001				<0.0001
0 year	772	639 (26.4%)	133 (36.4%)		9747	7637 (26.5%)	2110 (39.8%)	

1-4 years	384	330 (13.6%)	54 (14.8%)		4530	3686 (12.8%)	844 (15.9%)	
+4 years	1629	1451 (60.0%)	178 (48.8%)		19865	17523 (60.7%)	2342 (44.2%)	
Suburb				0.138				<0.0001
Bandim	1426	1214 (46.6%)	212 (50.8%)		16010	13194 (43.2%)	2816 (48.0%)	
Belem / Mindara	502	445 (17.1%)	57 (13.7%)		5556	4877 (16.0%)	679 (11.6%)	
Cuntum	1094	946 (36.3%)	148 (35.5%)		14833	12461 (40.8%)	2372 (40.4%)	
Ethnicity[7]				0.001				<0.0001
Pepel	819	685 (26.7%)	134 (32.4%)		10007	8161 (27.0%)	1846 (31.7%)	
Balanta	290	244 (9.5%)	46 (11.1%)		2947	2447 (8.1%)	500 (8.6%)	
Mandinga / Fula	858	726 (28.3%)	132 (31.9%)		10943	9038 (29.9%)	1905 (32.7%)	
Manjaco / Mancanha	596	538 (21.0%)	58 (14.0%)		6464	5688 (18.8%)	776 (13.3%)	
Others	417	373 (14.5%)	44 (10.6%)		5743	4941 (16.3%)	802 (13.8%)	
Socioeconomic factors								
Type of roof[8]				0.330				<0.0001
Straw	93	77 (3.0%)	16 (3.9%)		922	728 (2.4%)	194 (3.3%)	
Hard	2919	2520 (97.0%)	399 (96.1%)		35422	29769 (97.6%)	5653 (96.7%)	
Toilet[9]				<0.0001				<0.0001
None	11	6 (0.2%)	5 (1.2%)		95	75 (0.2%)	20 (0.3%)	
Latrine	2618	2240 (86.3%)	378 (91.1%)		30210	24959 (81.9%)	5251 (89.8%)	
Inside	381	349 (13.4%)	32 (7.7%)		6007	5428 (17.8%)	579 (9.9%)	
Electricity[10]				<0.0001				<0.0001
Yes	950	859 (33.0%)	91 (21.9%)		12841	11437 (37.5%)	1404 (24.0%)	
No	2067	1742 (67.0%)	325 (78.1%)		23505	19060 (62.5%)	4445 (76.0%)	

[1] Missing information for 0 TB-exposed children and 1 TB-unexposed child.
[2] Missing information for 2 TB-exposed children and 52 TB-unexposed children.
[3] Missing information for 5 TB-exposed children and 96 TB-unexposed children.
[4] Missing information for 1 TB-exposed children and 16 TB-unexposed children.
[5] Missing information for 83 TB-exposed children and 1687 TB-unexposed children.
[6] Missing information for 237 TB-exposed children and 2257 TB-unexposed children.
[7] Missing information for 42 TB-exposed children and 295 TB-unexposed children.
[8] Missing information for 10 TB-exposed children and 55 TB-unexposed children.
[9] Missing information for 12 TB-exposed children and 87 TB-unexposed children.
[10] Missing information for 5 TB-exposed children and 53 TB-unexposed children.

Table 2. Baseline characteristics of children registered as exposed to and not exposed to TB

TB-exposed children are only represented in the TB-exposed group despite some children also contribute observation time as not TB-exposed to avoid comparing a child with itself.

	TB-exposed children versus TB-unexposed children			
	Total	TB-exposed (n %)	TB-unexposed (n %)	p-value
Number	39421	3022 (7.7%)	36399 (92.3%)	
Sex[1]				0.119
Male	19995	1574 (52.1%)	18421 (50.6%)	
Female	19425	1448 (47.9%)	17977 (49.4%)	
Twin[2]				0.739
Yes	1332	99 (3.3%)	1233 (3.4%)	
No	38035	2921 (96.7%)	35114 (96.6%)	
Season of birth				0.905
Rainy season	19269	1474 (48.8%)	17795 (48.9%)	
Dry season	20152	1548 (51.2%)	18604 (51.1%)	
Birth location[3]				0.002
Home	10932	902 (29.9%)	10030 (27.6%)	
Health centre	17500	1329 (44.1%)	16171 (44.5%)	
Hospital	8570	647 (21.4%)	7923 (21.8%)	
Other	2318	139 (4.6%)	2179 (6.0%)	
Year of birth				0.043
2000-2006	12420	1010 (33.4%)	11410 (31.1%)	
2007-2011	13653	999 (33.1%)	12654 (34.5%)	
2012-2017	13348	1013 (33.5%)	12335 (33.6%)	
Number of pregnancies[4]				<0.0001
1	11810	838 (27.7%)	10972 (30.2%)	
2 - 3	16565	1249 (41.3%)	15316 (42.1%)	
4 or more	11029	934 (30.9%)	10095 (27.7%)	
Maternal age[5]	37651	26.4 (6.28)	26.1 (6.26)	0.008
Education of caretaker[6]				0.553
0 year	10519	772 (27.7%)	9747 (28.5%)	

1-4 years	4914	384 (13.8%)	4530 (13.3%)	
+4 years	21494	1629 (58.5%)	19865 (58.2%)	
Suburb				<0.0001
Bandim	17436	1426 (47.2%)	16010 (44.0%)	
Belem / Mindara	6058	502 (16.6%)	5556 (15.3%)	
Cuntum	15927	1094 (36.2%)	14833 (40.8%)	
Ethnicity[7]				<0.0001
Pepel	10826	819 (27.5%)	10007 (27.7%)	
Balanta	3237	290 (9.7%)	2,947 (8.2%)	
Mandinga / Fula	11801	858 (28.8%)	10943 (30.3%)	
Manjaco / Mancanha	7060	596 (20.0%)	6464 (17.9%)	
Others	6160	417 (14.0%)	5743 (15.9%)	
Socioeconomic factors				
Type of roof[8]				0.067
Straw	1015	93 (3.1%)	922 (2.5%)	
Hard	38341	2919 (96.9%)	35422 (97.5%)	
Toilet[9]				<0.0001
None	106	11 (0.4%)	95 (0.3%)	
Latrine	32828	2618 (87.0%)	30210 (83.2%)	
Inside	6388	381 (12.7%)	6007 (16.5%)	
Electricity[10]				<0.0001
Yes	13791	950 (31.5%)	12841 (35.3%)	
No	25572	2067 (68.5%)	23505 (64.7%)	

[1] Missing information for 1 child
[2] Missing information for 54 children
[3] Missing information for 101 children
[4] Missing information for 17 children
[5] Missing information for 1770 children
[6] Missing information for 2494 children
[7] Missing information for 337 children
[8] Missing information for 65 children
[9] Missing information for 99 children
[10] Missing information for 58 children

Table 3. Mortality of children with and without neonatal BCG by TB-exposure status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Neonatal BCG	2605	12.2 (30/2,462)	0.29 (0.14-0.57)	0.57 (0.26-1.27)[1]
No neonatal BCG	417	35.2 (13/369)	Ref	Ref
TB-unexposed[2]				
Neonatal BCG	32040	13.5 (533/39,608)	0.57 (0.47-0.69)	
No neonatal BCG	6105	22.8 (157/6,880)	Ref	
Combined TB-exposed and TB-unexposed[2]				
Neonatal BCG	33137	13.4 (563/42,069)	0.54 (0.45-0.65)	
No neonatal BCG	6284	23.4 (170/7,250)	Ref	

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.

Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.

[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis. The crude analysis excluding children with missing information on twin status, maternal age or year of birth yielded HR: 0.43 (0.19-0.93).

[2] No factor changed the estimate by more than 5%, and adjusted HR are therefore not presented.

Table 4. Mortality of children with and without neonatal BCG by TB-exposure status and sex.

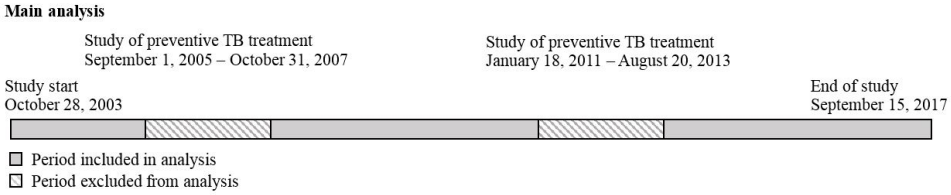
	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed boys				
Neonatal BCG	1355	8.5 (11/1,288)	0.19 (0.07-0.51)	0.23 (0.08-0.62)[1]
No neonatal BCG	219	35.6 (7/197)	Ref	Ref
TB-unexposed boys[2]				
Neonatal BCG	16268	13.6 (275/20,166)	0.58 (0.45-0.75)	
No neonatal BCG	3072	23.0 (80/3,478)	Ref	
TB-exposed girls				
Neonatal BCG	1250	16.2 (19/1,174)	0.37 (0.14-0.96)	1.61 (0.36-7.08)[1]
No neonatal BCG	198	34.7 (6/173)	Ref	Ref
TB-unexposed girls[2]				
Neonatal BCG	15772	13.3 (258/19,442)	0.56 (0.43-0.73)	
No neonatal BCG	3032	22.6 (77/3,402)	Ref	

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis
[2] No factor changed the estimate by more than 5%, and adjusted HR are therefore not presented.

Table 5. Mortality of TB-exposed children compared with TB-unexposed children by timing of BCG and sex.

	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)
All children		
Neonatal BCG		
TB-exposed	12.2 (30/2,462)	1.11 (0.77-1.61)
TB-unexposed	13.5 (533/39,608)	Ref
No neonatal BCG		
TB-exposed	35.2 (13/369)	1.93 (1.10-3.41)
TB-unexposed	22.8 (157/6,880)	Ref
Boys		
Neonatal BCG		
TB-exposed	8.5 (11/1,288)	0.77 (0.42-1.41)
TB-unexposed	13.6 (275/20,166)	Ref
No neonatal BCG		
TB-exposed	35.6 (7/197)	1.98 (0.91-4.28)
TB-unexposed	23.0 (80/3,478)	Ref
Girls		
Neonatal BCG		
TB-exposed	16.2 (19/1,174)	1.50 (0.94-2.39)
TB-unexposed	13.3 (258/19,442)	Ref
No neonatal BCG		
TB-exposed	34.7 (6/173)	1.90 (0.83-4.35)
TB-unexposed	22.6 (77/3,402)	Ref

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
Adjusted HR are not presented as no baseline factor changed the estimates by more than 5%.

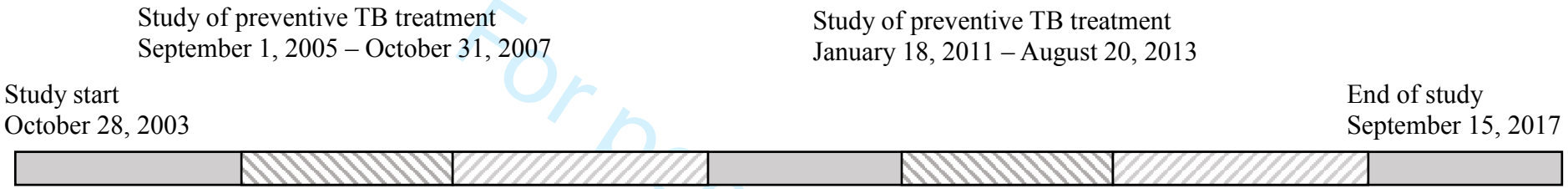


Timeline for study period

109x56mm (300 x 300 DPI)

Supplementary material for "Neonatal BCG vaccination is associated with better child survival than delayed BCG vaccination for both TB-exposed and TB-unexposed children"

Supplementary Figure 1: Timeline of study period; sensitivity analysis excluding children potentially eligible for studies of preventive TB treatment



Supplementary table 1. Mortality of children with early neonatal BCG, late neonatal BCG and no neonatal BCG by TB-exposure status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed children				
Early neonatal BCG (Day 0-7)	1718	11.3 (18/1,588)	0.19 (0.08-0.44)	0.39 (0.15-0.98)[1]
Late neonatal BCG (Day 8-28)	887	13.7 (12/874)	0.45 (0.20-1.01)	0.90 (0.36-2.25)[1]
No neonatal BCG	417	35.2 (13/369)	Ref	Ref
TB-unexposed children[2]				
Early neonatal BCG (Day 0-7)	21310	13.7 (357/25,981)	0.59 (0.48-0.74)	
Late neonatal BCG (Day 8-28)	10730	12.9 (176/13,627)	0.55 (0.44-0.68)	
No neonatal BCG	6105	22.8 (157/6,880)	Ref	

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis
[2] No factor changed the estimate by more than 5%, and adjusted HR are therefore not presented.

Supplementary table 2. Sensitivity analyses comparing mortality of children with and without neonatal BCG for TB-exposed and TB-unexposed children.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
Extended follow-up: Mortality between 28 days and 5 years of life[1]				
TB-exposed				
Neonatal BCG	2902	10.9 (35/3,217)	0.30 (0.16-0.57)	0.56 (0.27-1.16)[5]
No neonatal BCG	476	29.6 (15/507)	Ref	Ref
TB-unexposed[6]				
Neonatal BCG	32104	13.0 (574/44,114)	0.57 (0.48-0.68)	
No neonatal BCG	6109	21.9 (171/7,821)	Ref	
Only children with a registered BCG vaccine are included in the analysis[2]				
TB-exposed				
Neonatal BCG	2605	12.2 (30/2,462)	0.29 (0.14-0.61)	0.62 (0.26-1.49)[5]
No neonatal BCG	356	34.4 (11/320)	Ref	Ref
TB-unexposed[6]				
Neonatal BCG	32040	13.5 (533/39,608)	0.62 (0.51-0.76)	
No neonatal BCG	5247	20.6 (125/6,075)	Ref	
Excluding children who were twins[3]				
TB-exposed				
Neonatal BCG	2534	11.7 (28/2,392)	0.33 (0.16-0.69)	0.63 (0.26-1.51)[5]
No neonatal BCG	387	32.1 (11/343)	Ref	Ref
TB-unexposed[6]				
Neonatal BCG	31141	13.2 (506/38,473)	0.59 (0.48-0.72)	
No neonatal BCG	5668	21.6 (138/6,387)	Ref	
Excluding children potentially eligible for studies with preventive TB treatment for children (Supplementary Figure 1)[4]				
TB-exposed				
Neonatal BCG	1461	16.4 (23/1,399)	0.46 (0.18-1.20)	0.95 (0.31-2.97)[5]
No neonatal BCG	258	27.5 (6/218)	Ref	Ref
TB-unexposed[6]				
Neonatal BCG	22416	15.3 (418/27,312)	0.57 (0.46-0.70)	
No neonatal BCG	4580	25.7 (129/5,026)	Ref	

All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Extending follow-up to 5 years included 356 additional TB-exposed children (7 deaths) and 68 additional TB-unexposed children (55 deaths) in the analysis.

[2] Limiting the analysis to children with a registered BCG vaccine excluded 61 TB-exposed children (2 deaths) and 858 TB-unexposed children (32 deaths) from the analysis.
[3] Limiting the analysis to children who are not twins excluded 101 TB-exposed children (4 deaths) and 1336 TB-unexposed children (46 deaths) from the analysis.
[4] Limiting the analysis to children who had not been eligible for studies of preventive TB treatment (living in the study area and below 5 years of age in the study period) excluded 1303 TB-exposed children (14 deaths) and 11149 TB-unexposed children (143 deaths) from the analysis (Supplementary Figure 1).
[5] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children.
[6] No factor changed the estimate by more than 5%, and adjusted HR are therefore not presented.

Supplementary table 3. Mortality of children with and without neonatal BCG by TB-exposure status and low-birth-weight status.

	Number of children n	MR per 1000 PYRS (deaths / PYRS)	Hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
TB-exposed				
Low-birth-weight children				
Neonatal BCG	118	17.4 (2/115)	0.16 (0.03-0.78)	0.33 (0.06-1.93)[1]
No neonatal BCG	56	150.3 (7/47)	Ref	Ref
Normal-birth-weight children				
Neonatal BCG	1631	9.8 (15/1,532)	N/A	N/A
No neonatal BCG	106	9.8 (1/103)	Ref	Ref
TB-unexposed[2]				
Low-birth-weight children				
Neonatal BCG	1341	24.9 (39/1,568)	0.70 (0.44-1.11)	
No neonatal BCG	898	33.5 (36/1,076)	Ref	
Normal-birth-weight children				
Neonatal BCG	21108	12.1 (319/26,389)	0.76 (0.50-1.16)	
No neonatal BCG	1525	14.2 (26/1,828)	Ref	

Limiting the analysis to children with information on birthweight excluded 1111 TB-exposed children (21 deaths) and 13273 TB-unexposed children (270 deaths) from the analysis.
All hazard ratios were obtained from Cox-proportional hazards models with age as underlying age, allowing different baseline hazards by sex and birth location.
Abbreviations: MR: Mortality rate, PYRS: Person years, CI: Confidence interval.
[1] Adjusted HR calculated adjusting for twin status, maternal age, and year of birth among TB-exposed children, excluding 85 children (4 deaths) from the analysis.
[2] No factor changed the estimate by more than 5%, and adjusted HR are therefore not presented.

Supplementary table 4. PPD reactions at 6 and 12 months of age of children with and without neonatal BCG by TB-exposure status.

	Number of children n	Median PPD response in mm (interquartile range)	Positive PPD 10mm cut-off	Prevalence ratio 10mm cut-off	Positive PPD 15mm cut-off	Prevalence ratio 15mm cut-off
PPD response at 6 months of age						
TB-exposed children						
Neonatal BCG	48	0 (0-4.8)	3 (6.3%)	NA	0 (0.0%)	NA
No neonatal BCG	5	0 (0-3.5)	0 (0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	1248	0 (0-4.5)	94 (7.5%)	0.93 (0.51-1.70)	23 (1.8%)	1.25 (0.30-5.26)
No neonatal BCG	136	0 (0-4.5)	11 (8.1%)	Ref	2 (1.5%)	Ref
PPD response at 12 months of age						
TB-exposed children						
Neonatal BCG	5	0 (0-0)	(0.0%)	NA	0 (0.0%)	NA
No neonatal BCG	2	6 (5.5-6.5)	(0.0%)	Ref	0 (0.0%)	Ref
TB-unexposed children						
Neonatal BCG	297	0 (0-0)	21 (7.1%)	0.90 (0.28-2.86)	6 (2.0%)	0.26 (0.07-0.98)
No neonatal BCG	38	0 (0-2.5)	3 (7.9%)	Ref	3 (7.9%)	Ref

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1+2	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4	
Objectives	3	State specific objectives, including any prespecified hypotheses	4	
Methods				
Study design	4	Present key elements of study design early in the paper	4-6	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6	
Bias	9	Describe any efforts to address potential sources of bias	5-6	
Study size	10	Explain how the study size was arrived at	6	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7 + table 1 and 2
		(b) Indicate number of participants with missing data for each variable of interest	Table 1+2
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Table 3
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 3
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7 + Table 3
		(*b*) Report category boundaries when continuous variables were categorized	Table 1+2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 3-5 + Supp tables
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.