



Effect of BCG Danish and oral polio vaccine on neonatal mortality in newborn babies weighing less than 2000 g in India: multicentre open label randomised controlled trial (BLOW2)

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ABSTRACT

OBJECTIVE

To test the effect on all cause neonatal mortality (aged ≤ 28 days) of a dose of BCG Danish vaccine and oral polio vaccine (OPV) administered to newborn babies weighing <2000 g.

DESIGN

Multicentre, open label, randomised controlled trial

SETTING

Three tertiary neonatal intensive care units (NICUs) in southeast India.

POPULATION

Newborn babies weighing <2000 g. 7067 were assessed for eligibility and 5420 were randomised (2714 BCG-OPV, 2706 control).

INTERVENTIONS

Newborns were randomised 1:1 to receive 0.1 mL of BCG Danish intradermally plus OPV, either within 48 hours of admission to the NICU (early vaccination group) or delayed until at least the time of discharge

(control group). Stratification was by NICU, sex, and birth weight (<1000 g, 1000–1499 g, 1500–1999 g).

MAIN OUTCOME MEASURES

The primary outcome was all cause neonatal mortality. The main secondary outcome, included a third of the way through the trial, was neonatal mortality due to infection.

RESULTS

Of the 5420 newborn babies randomised at a median age of 0.9 days and with a median birth weight of 1560 g, nine were lost to follow-up. Deaths occurred in 238 (8.8%) of 2714 newborn babies in the early vaccination group and 273 (10.1%) of 2706 in the control group. In intention-to-treat Cox survival analysis stratified by NICU and adjusted for postnatal age, birth weight, sex, and gestational age, neonatal mortality per person year was 1.29 in the early vaccination group and 1.50 in the control group (adjusted hazard ratio 0.83, 95% confidence interval (CI) 0.69 to 0.98; $P=0.03$). The number needed to treat to prevent one death was 21 (95% CI 10 to 245). Infection related neonatal mortality per person year was 0.40 in the early vaccination group and 0.73 in the control group (adjusted hazard ratio 0.53, 95% CI 0.40 to 0.70). No deaths from tuberculosis occurred, and no serious adverse effects were associated with vaccination.

CONCLUSIONS

In newborn babies weighing <2000 g in intensive care, BCG-OPV administered at a median age of 0.9 days reduced all cause neonatal mortality owing to a decrease in deaths due to infections other than tuberculosis (a non-specific or off-target effect). A substantial reduction in neonatal mortality could be achieved if a skilled administrator vaccinated a high proportion of newborn babies in high mortality settings on the day of, or soon after, birth.

TRIAL REGISTRATION

Clinical Trials Registry India CTRI/2017/01/007676.

Introduction

Of the 5.19 million deaths in children younger than 5 years worldwide in 2019, 97.9% occurred in low-middle income countries that are not members of the Organisation for Economic Co-operation and Development (OECD).¹ If the mortality rate in under 5s globally had been the same as that in OECD countries in 2019, the number of deaths would have been 0.97 million rather than 5.19 million.¹ Despite a substantial decrease in mortality in under 5s worldwide, neonatal

WHAT IS ALREADY KNOWN ON THIS TOPIC

Observational studies and randomised trials have suggested that the Danish strain of BCG given soon after birth might reduce neonatal mortality from infections other than tuberculosis

Three trials of BCG Danish in newborn babies in Guinea-Bissau found no statistically significant effect on their primary outcome, which were mortality in the first year of life in two trials and mortality in the first month of life in one trial; however, they showed a reduction in mortality in the first month of life in a meta-analysis

Two other trials of the Danish or Japanese strains of BCG in Guinea-Bissau were stopped early because of slow recruitment, and two large trials of BCG Russian in India found no effect on neonatal mortality

WHAT THIS STUDY ADDS

In this randomised controlled trial, BCG Danish and oral polio vaccine given within 48 hours of birth reduced neonatal mortality in very ill newborns weighing <2000 g, with the number needed to treat to prevent one death being 21 (95% confidence interval 10 to 245)

For regions with a high prevalence of tuberculosis, the World Health Organization (WHO) should consider recommending BCG vaccination to all newborn babies as soon as possible after birth, and not only to those who are healthy, clinically stable, and >31 weeks' gestation

To reduce neonatal mortality in low-middle income countries, a vial of BCG should be opened even if only one infant needs to be vaccinated, and the WHO indicator for timely administration of BCG should be changed from 12 months to 28 days of age

(first 28 days of life) mortality has reduced little, and now accounts for 47% of deaths in under 5s.¹ Interventions that reduce neonatal mortality are urgently needed.²

The BCG vaccine may have non-specific (off-target, heterologous) effects that reduce the severity of other infections as well as tuberculosis.³⁻⁴ These effects are due, at least in part, to heterologous immunity and “trained” innate immune responses mediated by epigenetic reprogramming of monocytes.⁴⁻⁸ The beneficial non-specific effects of BCG could last at least 1-2 years,⁹ probably depend on the strain,^{3,10} and appear to be enhanced if there is existing immunity, such as maternal BCG scar or BCG revaccination,^{3,9,11} but might be reversed by subsequent administration of a non-live vaccine such as diphtheria-tetanus-pertussis vaccine.³

In 2023, 323 million doses of BCG were required worldwide, and the most common strains used were those prequalified by the World Health Organization (WHO): Danish 1331, Bulgarian SL-222 (a Russian strain), Russian I, and Tokyo 172-1.¹² WHO recommends BCG vaccination at birth in countries where tuberculosis is endemic.¹³ In newborn babies with low birth weight, however, administration of BCG is often delayed^{14,15}; therefore it is ethical to compare early versus delayed BCG vaccination in these babies. In three randomised trials in newborns weighing <2500 g in Guinea-Bissau, none had a statistically significant effect on the primary outcome from the administration of BCG Danish vaccine (Statens Serum Institut, Copenhagen) at discharge from hospital, but meta-analysis showed a reduction in neonatal mortality of 38% (95% CI 17% to 54%)^{14,16,17}; this reduction was due to fewer infection related deaths. The trials in Guinea-Bissau are important because they were the first randomised trials designed to test the effect of BCG on all cause neonatal mortality. Two subsequent trials in Guinea-Bissau were both stopped early: one suggested that the BCG Danish vaccine had no effect on in-hospital mortality,¹⁸ and the other found a possible reduction in mortality up to age 60 days with the BCG Tokyo vaccine.¹⁹

However, in contrast with the trials of the BCG Danish vaccine in Guinea-Bissau, in two large trials in three neonatal intensive care units (NICUs) in India (BLOW1), we found that BCG Russian vaccine (Serum Institute of India, Pune) with or without oral polio vaccine (OPV) given soon after birth did not reduce neonatal mortality in newborns with birth weight <2000 g.¹⁵ The four strains of BCG that Unicef distributes to low and middle income countries differ noticeably in bacterial viability, RNA content, and immunological effects,²⁰ and the vaccines might therefore have different specific and non-specific effects.¹⁰ Soon after completion of our trials of BCG Russian, and because this vaccine may be less effective than BCG Danish,^{10,20} we performed a trial in newborn babies weighing <2000 g (BLOW2) to test the effect of BCG Danish and OPV in the same three NICUs that had tested BCG Russian using the same trial design. OPV was given with the BCG Danish

vaccine because this is standard practice in India. We used a 0.1 mL dose of BCG because this is longstanding practice in the neonatal unit of the Jawaharlal Institute of Postgraduate Medical Education and Research, and it is widely used in India.²¹⁻²⁶

Methods

Trial design

BLOW2 was a multicentre randomised trial in three large NICUs in the south east of India: Jawaharlal Institute of Postgraduate Medical Education and Research in Pondicherry (almost all inborn babies; inborn babies are born in the same hospital as the NICU, outborn babies are born elsewhere), the Institute of Child Health and Hospital for Children in Chennai (almost all outborn babies), and the adjacent Institute of Obstetrics and Gynaecology in Chennai (almost all inborn babies). At the Jawaharlal Institute, recruitment, randomisation, and vaccination took place from 1 February 2017 to 30 April 2022, and at the Institute of Child Health and Institute of Obstetrics and Gynaecology from 1 August 2018 to 30 April 2022; collection of outcome data concluded on 19 June 2022. The protocol was registered prospectively with the Clinical Trials Registry India.

Patients

Newborns were eligible for the BLOW2 trial if their birth weight was <2000 g and they were admitted to one of the three NICUs. Newborn babies were excluded if they were aged <60 minutes or >21 days; were unlikely to survive for 24 hours; had a major life threatening malformation; had already been vaccinated with BCG; the mother or baby were known to have HIV, hepatitis B, herpes zoster, or immunodeficiency; the mother was aged <18 years; or the family refused consent. All mothers with cough for two weeks or more antenatally, with or without other symptoms suggestive of tuberculosis (fever for two weeks or more, haemoptysis, unexplained substantial weight loss, or night sweats) underwent a sputum smear examination and a nucleic acid amplification test.

Sample size, randomisation, and masking

In our earlier trial of the BCG Russian vaccine and OPV,¹⁵ the mortality rate in the delayed vaccination (control) group was 17.6%. For the current trial, using the Stata version 14.1 sampsi command, we estimated that a total of 510 deaths would be required: with a mortality rate of 15% in the control group, 1998 infants would be required in each group to have 95% power to detect a 30% reduction in mortality using BCG and OPV with an $\alpha < 0.01$; this implied 300 deaths in the control group (1998×0.15) and 210 deaths in the early vaccination group ($1998 \times 0.15 \times 0.7$). The sample size was specified as the number of deaths, so that changes in mortality rates automatically adjusted the number of newborns randomised.

Written informed consent was obtained from a parent before enrolment. The newborn babies were randomised using a specially written computer

program that checked eligibility, allocated treatment, and recorded baseline and outcome data. Randomisation was 1:1 and stratified by NICU, sex, and birth weight (<1000 g, 1000-1499 g, 1500-1999 g), and with a block size that varied randomly from 2-8 newborn babies. Enrolment was stopped on 30 April 2022 when 510 babies had died. The BCG vaccine causes an obvious skin lesion, so it was not possible to mask staff to group assignment because it would have been unethical to cover the upper arm of these high risk babies for their entire stay in intensive care; the caregivers who assessed whether the baby died or survived were not blinded.

Procedures

At Jawaharlal Institute, a research nurse recruited and vaccinated newborn babies and recorded all data; at the Institute of Child Health and Institute of Obstetrics and Gynaecology, a research assistant recruited newborn babies and recorded all data, and an immunisation nurse vaccinated the participants. Babies in the early vaccination group received 0.1 mL of BCG Danish (GreenSignal Bio Pharma, Chennai) intradermally within 48 hours of admission to the NICU, along with 0.1 mL (two drops) of bivalent 1&3 OPV (Bharat Biotech International, Hyderabad; or Bio-Med, Ghaziabad). At Jawaharlal Institute, at the time of discharge, babies in the early vaccination group received hepatitis B vaccine, and babies in the control group received BCG, OPV, and hepatitis B vaccine. At the Institute of Child Health and Institute of Obstetrics and Gynaecology, babies in the early vaccination group received hepatitis B vaccine at about 6 weeks of age, and babies in the control group received BCG, OPV, and hepatitis B vaccine. Information about feeding was not collected.

Outcomes

The primary outcome was neonatal mortality in the first 28 days of life, with censoring at the time of loss to follow-up or any vaccination before 28 days (other than BCG and OPV in the early vaccination group), adjusted for age and survival dependence between twins or triplets, the randomisation stratification variables NICU, sex, and birth weight, and, if they contributed to the Cox model, gestation, season, maternal BCG scar, and whether the baby was born in the study hospital or elsewhere. A substantial proportion of babies in the control group were, however, given BCG and OPV before 28 days of age at about the time of discharge from hospital, and few deaths occurred between discharge and 28 days of age; consequently, the Data and Safety Monitoring Board recommended that the main analysis not be censored for BCG and OPV in the control group because this would cause bias by removing time at low risk of death (from discharge to 28 days of age) in the control group but not in the early vaccination group. Secondary outcomes were deaths caused by infection and deaths in newborn babies of mothers with a BCG scar. When this trial was planned, preliminary evidence suggested that

the BCG vaccine might protect newborns only if their mother had a BCG scar²⁷; therefore, the protocol stated that if vaccination reduced mortality only when the mother had a BCG scar, the trial would be extended and the primary outcome would be neonatal mortality in newborn babies of mothers with a BCG scar. The secondary outcome of death from infection was not specified in the original protocol but was added a third of the way through the trial on the advice of the Data and Safety Monitoring Board because a study of BCG vaccine in newborns in Guinea-Bissau suggested that vaccination might reduce mortality from infectious rather than non-infectious causes,¹⁷ a finding that two subsequent studies supported.^{18 28} The treating clinician assigned the cause of death from a prespecified list: congenital malformation, hyaline membrane disease, infection, intraventricular haemorrhage, necrotising enterocolitis, other, and unknown. Infection (synonymous with sepsis in this study) was defined as one or more clinical signs suggestive of infection with either a microbial isolate on blood culture, or a sterile blood culture plus at least two abnormal infection screen markers: C reactive protein >1.14 µmol/L (120 mg/L), absolute neutrophil count <1500/mm³, total leucocyte count <5000/mm³, erythrocyte sedimentation rate >15 mm/hour, or immature to total neutrophil ratio >0.2.²⁹

An independent Data and Safety Monitoring Board reviewed the conduct of the trial (see list of members in section S10 of the supplementary file). The chief investigators signed the final version 5.0 of the statistical analysis plan on 17 November 2022. The statistical analysis plan was posted on the Madras Medical College website (www.mmc.ac.in). However, this website stopped functioning in early 2023; subsequently, all versions of the protocol, Data and Safety Monitoring Board documents, and statistical analysis plan were posted at <https://osf.io/3492q/>.

Statistical analysis

All statistical tests were two sided, and we considered a P value <0.05 to be significant. Intention-to-treat analysis was performed using Stata version 18, with Cox proportional hazard models stratified by NICU with age as the underlying time so that the analysis controlled for age, with variance adjusted for survival dependence between twins or triplets, and censoring at loss to follow-up. The prespecified binomial covariates early BCG-OPV vaccination, sex, inborn or outborn status, season (wet or dry), and maternal BCG scar were tested for proportional hazards in Kaplan-Meier analysis and graphs. We assessed the potential for non-linear relations in the continuous variables gestational age and weight using fractional polynomials. The proportional hazards assumption of the Cox model was tested using Stata's time-dependent covariable command with log(time), and by using Schoenfeld residuals, log-log plots, and separately estimated Kaplan-Meier curves. Kaplan-Meier survival plots used time since enrolment as the underlying time. We did not adjust the 95% confidence intervals

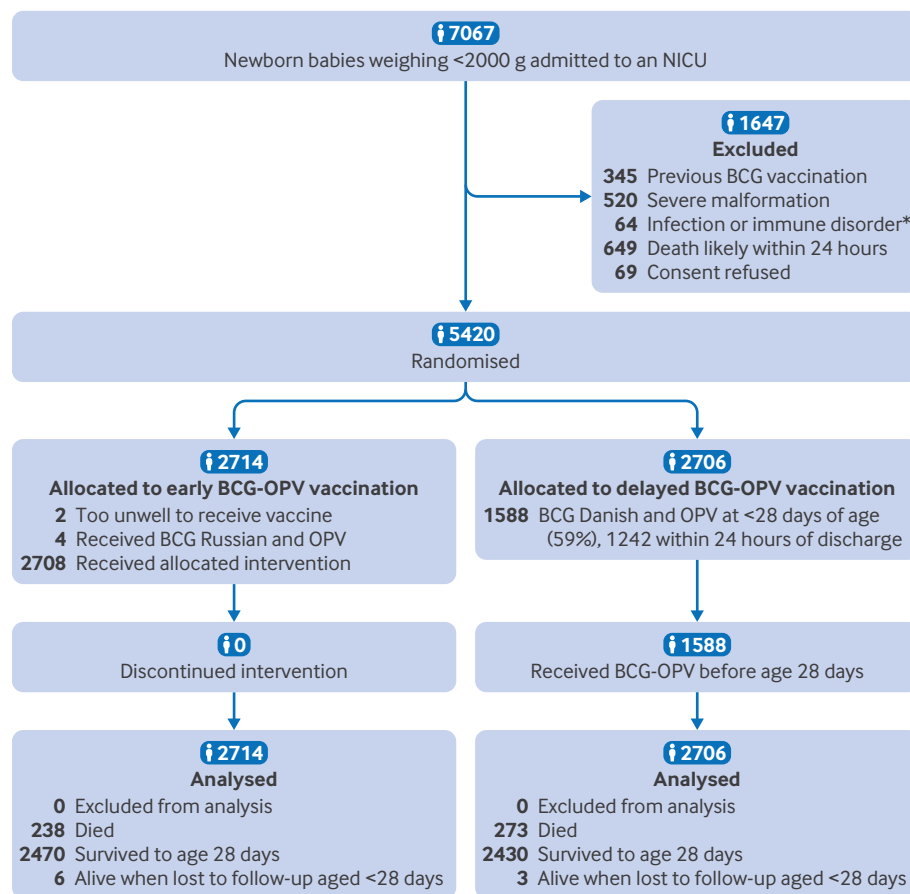


Fig 1 | Study flow chart. *Mother or baby with known HIV, hepatitis B, herpes zoster, or immune deficiency. NICU=neonatal intensive care unit; OPV=oral polio vaccine

(CIs) and P values of the secondary outcomes for multiplicity. A current meta-analysis was performed using the Stata metan command with a random effects model and the search terms listed in section S13 of the supplementary file.

Patient and public involvement

No families or members of the public were involved in the conduct of the trial because the required funding and staff were not available.

Results

Of 7067 newborn babies with a birth weight <2000 g admitted to the three study NICUs, 1647 were excluded according to the protocol criteria. Overall, 5420 newborn babies were randomised (fig 1): 2714 to the early BCG-OPV group of whom 238 (8.8%) died and 2706 to the control group of whom 273 (10.1%) died. As maternal BCG scar showed no statistically significant effect on mortality, enrolment ceased after 510 deaths. The conduct of the trial was slowed by the covid-19 pandemic and by occasional shortages of the BCG Danish vaccine. Table 1 shows the baseline characteristics of the participants.

Of the 2463 children in the early vaccination group examined at about 6 weeks of age, 2398 (97.4%) had

a visible skin reaction to BCG (see supplementary table S1). No serious side effects were found with BCG; in particular, there was no axillary ulceration or disseminated BCG.

A protocol violation occurred in 10 babies. In the early vaccination group, four babies were treated with the BCG Russian (Serum Institute of India) strain rather than the BCG Danish strain (none died), two were not vaccinated because they were thought to be too ill (both died), and one was entered twice (but analysed only once); all these babies were included in the early vaccination group in the analysis. In the control group, one twin and one triplet were randomised separately to their siblings, and one baby was initially recorded as male rather than female.

Examination of fractional polynomial powers suggested using birth weight without transformation but, for gestational age, using $(10/\text{gestational-age})^2$. Caesarean section, maternal BCG scar, whether inborn or outborn, and season did not contribute to the Cox model, and hence the final model with age as the underlying time consisted of early BCG-OPV, sex, birth weight, and transformed gestational age stratified by NICU with variance adjusted for multiple births (table 2). No statistically significant interactions were found between BCG and the other variables in the Cox model.

Table 1 | Baseline characteristics of newborn babies weighing <2000 g assigned to receive BCG Danish and OPV within 48 hours of admission (intervention) or delayed at least to discharge (control). Values are number (percentage) unless stated otherwise

Characteristics	Early BCG-OPV (n=2714)	Control (n=2706)
Sex:		
Male	1389 (51)	1382 (51)
Female	1325 (49)	1324 (49)
Median (IQR) age at enrolment (days)	0.86 (0.54-1.46)	0.86 (0.56-1.38)
Median (IQR) birth weight (g)	1560 (1320-1745)	1560 (1320-1745)
Birth weight (g):		
<1000	133 (5)	129 (5)
1000-1499	996 (37)	994 (37)
1500-1999	1585 (58)	1583 (58)
Median (IQR) gestation (weeks)	33.4 (31.7-35.4)	33.6 (31.7-35.4)
Premature (<37 week)	2374 (87)	2349 (87)
Mean (SD) Apgar score:		
1 minute	6.71 (1.52)	6.76 (1.48)
5 minutes	8.04 (1.13)	8.06 (1.11)
Born in study hospital	2460 (91)	2454 (91)
Maternal BCG scar	1601 (59)	1658 (61)
Vaginal delivery	1523 (56)	1476 (55)
Twin or triplet	210 (7.7)	197 (7.3)
Born in wet season	1431 (53)	1425 (53)
Study hospital:		
JIMPER	1440 (53)	1432 (53)
ICH	252 (9.3)	249 (9.2)
IOG	1022 (38)	1025 (38)

ICH=Institute of Child Health and Hospital for Children, Chennai; IOG=Institute of Obstetrics and Gynaecology, Chennai; IQR=interquartile range; JIMPER=Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry; NICU=neonatal intensive care unit; OPV=oral polio vaccine; SD=standard deviation.

Proportional hazards were confirmed for early BCG-OPV, sex, birth weight, and transformed gestational age.

As recommended by the Data and Safety Monitoring Board, the control group was not censored for BCG-OPV administered before 28 days of age in the main analysis (table 2). The adjusted hazard ratio for death before 28 days of age for early vaccination compared with control in the primary Cox model was 0.83 (95% CI 0.69 to 0.98; P=0.03). Figure 2 shows the probability

of death in both study groups. The number needed to treat to prevent one death was 21 (95% CI 10 to 245).

Of the 2706 newborns in the control group, 1588 (59%) were vaccinated with BCG Danish and OPV before 28 days of age, at a median of 4.5 hours before discharge in the 1423 babies discharged before 28 days of age.

Table 3 shows the times from birth and enrolment to BCG-OPV vaccination, death, and discharge. Six newborns in the early vaccination group and three in

Table 2 | Outcome data in newborn babies weighing <2000 g assigned to receive BCG Danish and OPV within 48 hours of admission (intervention) or delayed at least to discharge (control)

	Mortality rate (deaths/total person years); No		Adjusted hazard ratio (95% CI)	P value*
	Early BCG-OPV	Control		
Deaths ≤28 days (primary outcome)	1.29 (238/184); 2714	1.50 (273/182); 2706	0.83 (0.69 to 0.98)	0.03
Deaths from infection	0.40 (73/184); 2707	0.73 (133/182); 2702	0.53 (0.40 to 0.70)	<0.001†
Deaths from non-infectious cause	0.89 (164/184); 2707	0.77 (139/182); 2702	1.10 (0.88 to 1.39)	0.41†
Deaths in hospital	2.33 (237/102); 2714	2.69 (269/100); 2706	0.83 (0.70 to 0.99)	0.04†
Deaths ≤28 days; controls censored when BCG-OPV given ≤28 days (original primary outcome)	1.29 (238/184); 2714	2.25 (273/121); 2706	0.78 (0.65 to 0.93)	0.006‡
Per protocol analysis	1.28 (236/184); 2702	2.26 (275/122); 2709	0.77 (0.65 to 0.92)	0.004§
Time of death after enrolment (days):				
0-2	2.53 (56/22.1); 2714	3.41 (75/22.0); 2706	0.72 (0.51 to 1.03)	0.07¶
3-6	3.48 (99/28.4); 2658	4.13 (116/28.1); 2631	0.84 (0.64 to 1.10)	0.21¶
7-27	0.62 (83/134); 2553	0.62 (82/132); 2512	0.91 (0.67 to 1.24)	0.55¶

CI=confidence interval; OPV=oral polio vaccine.

*Cox regression stratified by neonatal intensive care unit and adjusted for age, birth weight, gestational age, and sex. Six babies in the early BCG-OPV group and three in the control group were lost to follow-up before 28 days of age and censored from the time of last contact.

†Secondary outcome not adjusted for multiplicity.

‡Biased estimate because low risk time from discharge to 28 days was removed from vaccinated controls.

§Biased estimate because low risk time from discharge to 28 days was removed from vaccinated controls, and two babies who died were randomised to BCG-OPV but were not vaccinated because they were considered too ill were moved from the BCG-OPV group to the control group in the per protocol analysis.

¶Prespecified descriptive outcome not adjusted for multiplicity.

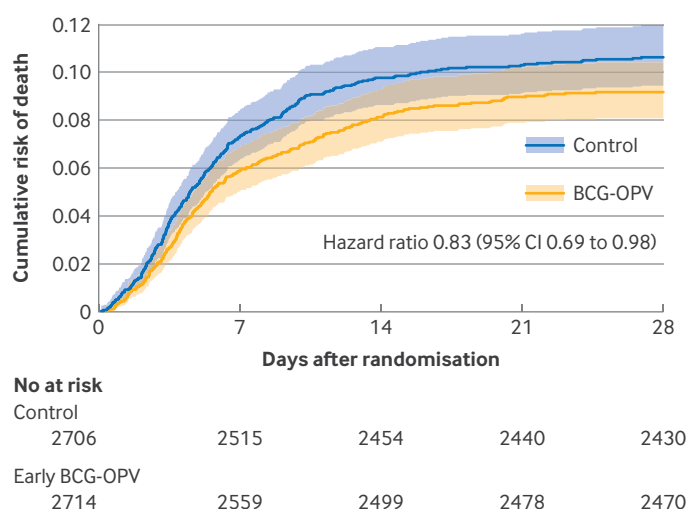


Fig 2 | Kaplan-Meier probability of survival up to 28 days after enrolment in newborn babies weighing <2000 g. Values not adjusted for age, sex, birth weight, gestation, or multiple births. Babies were at risk until they died or were lost to follow-up before 28 days of age. Shading represents 95% CIs. CI=confidence interval; OPV=oral polio vaccine

the control group were lost to follow-up before 28 days of age; survival to 28 days of age was determined in the other 5411 babies either at the follow-up visit or by mobile phone. Table 4 shows the causes of death.

The adjusted hazard ratio for the secondary outcome of death due to infection in the early vaccination group versus control group was 0.53 (95% CI 0.40 to 0.70); the confidence interval was not adjusted for multiplicity (table 2). The crude mortality rate due to infection was 73 (2.7%) of 2707 in the early vaccination group and 133 (4.9%) of 2702 in the control group, with an adjusted number needed to treat of 7 (95% CI 4 to 15). A blood culture result was positive for micro-organisms in 90 (44%) of the 206 babies with death due to infection: 23 (32%) of 73 babies in the early vaccination group and 67 (50%) of 133 in the control group (see supplementary table S2). *Klebsiella pneumoniae* was identified in 9.6% (7/73) of the isolates in the early vaccination group and 31% (41/133) in the control group; post hoc ratio 0.31 (95% CI 0.15 to 0.66), $P<0.001$. No deaths were due to tuberculosis.

Table 5 shows the interaction between the adjusted effect of early vaccination and eight prespecified

variables; none of these interactions were statistically significant. The supplementary file includes mortality by month (see supplementary table S3), days from randomisation to death by sex (table S4), mortality by age of enrolment (table S5), mortality by maternal BCG scar and sex (table S6), mortality by maternal BCG scar and whether inborn or outborn (table S7), BCG skin reactions at about 6 weeks of age (tables S1 and S8), blood culture results in babies who died (table S2), and type of OPV (table S9).

A random effects meta-analysis of the four smaller trials of the effect of Danish BCG on all cause neonatal mortality in Guinea-Bissau,^{14 16-18} and this trial in India provided an estimate for neonatal mortality of 0.77 (95% CI 0.64 to 0.92; $P=0.004$) and 0.71 (0.55 to 0.90; $P=0.005$) in the first three days of life (see section S12 of the supplementary file).

Discussion

This randomised trial in newborn babies weighing <2000 g in three NICUs in India found that BCG Danish and OPV administered at a median of 0.9 days after birth reduced all cause neonatal (≤ 28 days) mortality by 17% and, without adjustment for multiplicity, mortality due to infection by 47%. Only nine of the 5420 babies were lost to follow-up. Overall, 511 deaths occurred compared with 285 in all previous trials combined.

Interpretation of results and comparison with other studies

As in the four smaller trials of the effect of vaccination with BCG Danish on neonatal mortality in Guinea-Bissau,^{14 16-18} we found a trend towards BCG Danish with OPV having a greater effect in babies who were born at term rather than prematurely, inborn rather than outborn, female rather than male, born vaginally rather than by caesarean section, and born to a mother with a BCG scar; however, our trial was not powered to detect an effect of covariates, and none of these trends was statistically significant. Both our trial and the trials in Guinea-Bissau suggested an effect of the BCG Danish vaccine on mortality in the first three days after it was administered; this is consistent with the evidence that BCG rapidly induces “trained” innate immune responses.^{5 6} Recent data from The Gambia showed that abnormalities in gene expression are present in some apparently healthy newborn babies who later

Table 3 | Time (days) to receive BCG Danish and OPV within 48 hours of admission (intervention) or delayed at least to discharge (control) in newborn babies weighing <2000 g from birth and enrolment. Values are median (interquartile range) unless stated otherwise

	Early BCG-OPV; No	Control; No
Birth to enrolment	0.86 (0.54-1.46); 2714	0.86 (0.56-1.38); 2706
Birth to vaccination	0.93 (0.58-1.68); 2712	12.6 (7.76-20.0); 1588
Birth to death	6.41 (4.24-11.3); 238	5.99 (3.95-9.91); 273
Birth to discharge	11.8 (7.75-17.5); 2005	12.0 (7.78-18.0); 2010
Enrolment to vaccination	0.03 (0.01-0.07); 2712	11.8 (6.96-18.9); 1588
Enrolment to death	5.08 (3.14-9.59); 238	4.65 (2.80-8.23); 273
Enrolment to discharge	10.2 (6.27-16.2); 2005	10.5 (6.29-17.0); 2010

OPV=oral polio vaccine.

Table 4 | Causes of death in newborn babies weighing <2000 g assigned to receive BCG Danish and OPV within 48 hours of admission (intervention) or delayed at least to discharge (control). Values are number (percentage) unless stated otherwise

	Early BCG-OPV (n=2714)	Control (n=2706)	BCG to control ratio	Total
Infection	73 (31)	133 (49)	0.55*	206 (40)
Hyaline membrane disease	82 (34)	64 (23)	1.28	146 (29)
Intraventricular haemorrhage	22 (9)	18 (7)	1.22	40 (8)
Necrotising enterocolitis	6 (3)	4 (1)	1.50	10 (2)
Congenital malformation	3 (1)	0 (0)	-	3 (1)
Asphyxia	10 (4)	1 (0)	9.97	11 (2)
Other	41 (17)	52 (19)	0.79	93 (18)
Unknown	1 (0)	1 (0)	1.00	2 (0)
Total deaths	238 (100)	273 (100)		511 (100)

OPV=oral polio vaccine.

*For example, for infection, $(73/2714)/(133/2706)=0.55$ (adjusted hazard ratio 0.53, 95% confidence interval 0.40 to 0.70).

Because the percentages for the early BCG-OPV group and control group must both add to 100%, the lower percentage of deaths from infection in the early BCG-OPV group means that the percentage of non-infectious deaths in the early BCG-OPV-group must be higher overall than in the control group.

develop sepsis³⁰; combined with the evidence that early administration of BCG Danish is most beneficial in the first few days of life,^{14 16 17} the findings suggest that in some newborns BCG might be treating and not just preventing sepsis.

Our trial differs in important ways from the four other smaller randomised trials with an unvaccinated control group that tested the effect of BCG Danish on neonatal mortality in Guinea-Bissau.^{14 16-18} Firstly, we administered 0.1 mL of the vaccine rather than 0.05 mL, and the manufacturers differed. Secondly, our trial was larger, with 511 deaths compared with 285 deaths

in the four Guinea-Bissau trials combined. Thirdly, all but five of the deaths in our trial occurred in the NICU and the mortality rate in controls was 10.1%, whereas three of the four trials in Guinea-Bissau randomised newborn babies at the time of discharge from hospital and included some babies born at home, with a mortality of 3.7% in controls. Fourthly, we studied high risk newborns weighing <2000 g, whereas the Guinea-Bissau trials studied newborn babies weighing up to 2500 g (or more in the fourth trial). Fifthly, only 49% of deaths in our control group were caused by infection, and 30% were from hyaline membrane

Table 5 | Interaction of prespecified risk factors with effect on neonatal mortality in newborn babies weighing <2000 g assigned to receive BCG Danish and OPV within 48 hours of admission (intervention) or delayed at least to discharge (control)

	Mortality rate (deaths/total person years); No		Hazard ratio (95% CI)*	P for interaction with BCG-OPV*
	Early BCG-OPV	Control		
Birth weight (g)				
<1000	9.67 (60/6.2); 133	12.7 (67/5.3); 129	0.75 (0.53 to 1.08)	0.81†
1000-1499	1.99 (130/65); 996	2.31 (148/64); 994	0.86 (0.68 to 1.09)	
1500-1999	0.43 (48/113); 1585	0.52 (58/112); 1583	0.82 (0.56 to 1.21)	
Gestational weeks‡				
<31.8	3.55 (148/42); 689	3.82 (159/42); 703	0.89 (0.71 to 1.12)	0.82†
31.8-33.6	0.69 (34/49); 705	1.24 (56/45); 662	0.56 (0.37 to 0.87)	
33.7-35.4	0.56 (26/47); 664	0.56 (27/49); 683	0.92 (0.54 to 1.56)	
>35.4	0.65 (30/46); 656	0.67 (31/46); 658	0.95 (0.58 to 1.57)	
Maturity (weeks)				
Premature (<37)	1.43 (228/160); 2374	1.62 (254/157); 2349	0.84 (0.70 to 1.01)	0.47
Term (≥37)	0.41 (10/24); 340	0.76 (19/25); 357	0.63 (0.29 to 1.36)	
Sex				
Male	1.57 (146/93); 1389	1.69 (155/92); 1382	0.88 (0.71 to 1.11)	0.36
Female	1.01 (92/91); 1325	1.32 (118/90); 1324	0.75 (0.56 to 0.99)	
Birth location				
Study hospital	1.22 (207/170); 2460	1.48 (246/167); 2454	0.81 (0.67 to 0.97)	0.45
Elsewhere	2.12 (31/15); 254	1.81 (27/15); 252	0.99 (0.60 to 1.64)	
Maternal BCG scar				
Present	1.22 (133/109); 1601	1.40 (157/112); 1658	0.78 (0.62 to 0.99)	0.47
Absent	1.40 (105/75); 1110	1.68 (116/69); 1047	0.89 (0.69 to 1.15)	
Mode of delivery				
Caesarean section	1.23 (100/81); 1191	1.24 (104/84); 1230	0.92 (0.70 to 1.21)	0.31
Vaginal delivery	1.34 (138/103); 1523	1.73 (169/98); 1476	0.77 (0.61 to 0.96)	
Season				
Wet: Jul to Dec	1.33 (128/96); 1431	1.52 (145/95); 1425	0.82 (0.64 to 1.05)	0.97
Dry: Jan to Jun	1.25 (110/88); 1283	1.49 (128/86); 1281	0.83 (0.65 to 1.06)	

CI=confidence interval; OPV=oral polio vaccine.

*Cox regression stratified by neonatal intensive care unit and adjusted for age, birth weight, gestational age, and sex.

†Interaction of continuous variables, weight, and (10/gestational-age)², with the effect of BCG-OPV on mortality.

‡Four groups with approximately equal numbers in each group.

disease or intraventricular haemorrhage (conditions unlikely to be influenced by BCG-OPV), whereas 63% of deaths in the control groups in the Guinea-Bissau trials were due to infection.

A high proportion of deaths in newborn babies born at term in low-middle income countries are caused by infection,³¹ which suggests that BCG Danish and OPV administered at birth to such babies might have an even greater effect on all cause neonatal mortality than the 17% reduction seen in newborn babies weighing <2000 g in our trial, where 30% of deaths in controls were caused by hyaline membrane disease or intraventricular haemorrhage. A random effects meta-analysis of the four smaller trials of the effect of BCG Danish on neonatal mortality^{14 16-18} plus this trial yielded an estimated 23% reduction in all cause mortality when newborns with low birth weight were vaccinated with BCG Danish soon after birth (see supplementary table 12b). In addition, a randomised trial in Uganda found that BCG Danish given soon after birth reduced the incidence of doctor diagnosed non-tuberculous infection by 29% up to 6 weeks of age.³² Consequently, strong evidence suggests that BCG Danish with or without OPV reduces all cause mortality by protecting against infections other than tuberculosis—a non-specific or off-target effect.

Strengths and weaknesses of this study

The secondary outcome of death from infection was not specified in the original protocol but was added a third of the way through the trial on the advice of the Data and Safety Monitoring Board because a study of BCG Danish in newborn babies in Guinea-Bissau suggested that BCG reduced mortality from infectious rather than non-infectious causes,¹⁷ a finding supported by two subsequent studies.^{18 28} In our study, infection was one of the prespecified causes of death. We used a standard definition of neonatal infection,²⁹ and its performance was supported by the high proportion of newborns with a positive blood culture result: 90 (44%) of the 206 babies with mortality due to infection had a positive blood culture result (see supplementary table 2): 23 (32%) of 73 babies in the early vaccination group and 67 (50%) of 133 in the control group.

As skin lesions due to BCG are obvious, a double blind trial could not be performed as it was not ethical to keep the upper arm of these high risk babies covered during their entire stay in intensive care. In addition, we did not want babies in the control arm to miss receiving BCG because mothers thought their child had been vaccinated. It is unlikely that the lack of blinding influenced the result: our two previous open label randomised trials of BCG Russian made by the Serum Institute of India with or without OPV had a total of 879 deaths and were performed in the same NICUs as this trial and with similar designs, and no difference in neonatal mortality was found between the intervention and control groups (combined hazard ratio 0.98),¹⁵ which suggests that BCG vaccination with an obvious skin reaction did not bias the findings.

It is possible that OPV administered with the early dose of BCG contributed to the reduction in mortality in our trial, but this is unlikely because no reduction was seen in our earlier trial when OPV was given with BCG Russian (adjusted hazard ratio 1.01), which was similar to the adjusted hazard ratio of 0.95 for BCG Russian without OPV.¹⁵ It is, however, possible that OPV enhances the non-specific effects of an active strain of BCG but has no effect when given with a strain of BCG with little or no non-specific effects. WHO raised concerns about the manufacturing standards of both BCG Danish (GreenSignal Bio Pharma) and OPV (Bharat Immunological and Biologicals) during the trial, but no serious side effects were found, and mortality was reduced when vaccination with both occurred soon after birth. No serious side effects were found in the BCG-OPV group in the trial despite being administered at an intradermal dose of 0.1 mL, double the dose WHO recommends.³³

Overall, 1588 (59%) of the 2706 babies in the control group were vaccinated with BCG and OPV just before discharge or later, so death in hospital compares BCG and OPV administered soon after birth with mostly unvaccinated time in the control group, and the risk adjusted hazard ratio for death in hospital was 0.83, which is the same as the primary endpoint of 0.83 for death up to 28 days of age without censoring for BCG-OPV vaccination before 28 days of age in the control group. This suggests that the high vaccination rate before 28 days of age in the control group did not affect the outcome of this trial.

Implications for policy and future research

Our findings provide additional evidence that different strains of BCG might have different non-specific effects, and that BCG Danish might have stronger non-specific effects than BCG Russian.^{10 20} Our two previous large randomised trials found that intradermal doses of 0.05 mL of BCG Russian (Serum Institute of India) without OPV and 0.1 mL with OPV had no statistically significant effect on neonatal mortality (combined adjusted hazard ratio 0.98)¹⁵; our current trial was performed soon after those trials, in the same NICUs using the same staff and a similar trial design, and BCG Danish with OPV was associated with a statistically significant 17% reduction in neonatal mortality. These findings suggest an urgent need for head-to-head comparisons of BCG strains.¹⁰

In regions with a high prevalence of tuberculosis, WHO recommends BCG vaccination for newborns with gestational age <37 weeks or with birth weight <2500 g, providing the gestational age is >31 weeks and the babies are healthy and clinically stable.³³ The gestational age of 20% of the newborn babies in our trial was, however, ≤31 weeks, and none were healthy or clinically stable. Our findings suggest that, regardless of gestational age, unstable and very ill newborns derive substantial benefit from BCG Danish and OPV administered as soon as possible after birth. WHO should therefore consider modifying its recommendation. In addition, to increase the number

of newborn babies given BCG soon after birth, the WHO indicator for BCG vaccination should be changed from the percentage immunised at age 12 months to the percentage immunised at age 1 month, or even at age 1 week.

Conclusion

Combined with findings of the four randomised trials in Guinea-Bissau,^{14 16-18} and the trial in Uganda,³² our findings suggest that full implementation of the WHO policy of BCG vaccination at birth in countries where tuberculosis is endemic might substantially reduce neonatal mortality. At present, however, much of this potential benefit is lost. Firstly, 75% of deaths in newborn babies occur in the first week of life, but only 37% of newborns in countries where tuberculosis is endemic receive BCG by 1 week of age³⁴; consequently, the common practice that a vial of BCG is opened only if 10-12 infants need to be vaccinated should change so that a vial is opened even if only one child needs vaccinating.³⁵ Secondly, a high proportion of BCG doses—71% in 2014³⁶—were the BCG Russian strain, which might be less effective than the Danish and Japanese strains.^{10 15} Thirdly, many vaccinated infants do not develop a BCG scar, suggesting that routine immunisation with BCG in high burden countries often fails to achieve intradermal administration,³⁷ a difficult task given the thin skin of newborn babies. This suggests that a substantial reduction of neonatal mortality could be achieved if a high proportion of newborns in high mortality settings received BCG and OPV in the first week of life from a skilled administrator, perhaps using BCG Danish or Japanese rather than BCG Russian.

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Contributors: BA, BM, BVB, CK, FS, KJ, MS, and SPN conceived the trial and contributed to the design of the trial and the protocol. BA, CK, BVB, SPN, FS, and MS were project administrators. FS, BM, and PDR performed data curation and validated the data. Assisted by FS, BM wrote the computer program that determined eligibility, allocated treatment, and recorded the demographic and outcome data. PDR ran the trial at the Jawaharlal Institute of Postgraduate Medical Education

and Research supervised by BVB and BA, CK supervised the trial staff at the Institute of Child Health, and MS supervised the trial staff at the Institute of Obstetrics and Gynaecology. BA, CK, BVB, MS, SPN, and FS allocated resources. The independent Data Safety and Monitoring Board provided oversight. FS directly accessed and verified the data, and FS and SPN performed the statistical analysis and wrote the first draft of the paper. All authors reviewed the manuscript and suggested edits. The full trial data and the Stata analysis file were made available to all authors. All authors read and approved the final version of the manuscript and share responsibility for the decision to submit for publication. FS and SPN are the guarantors. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing interests: All authors have completed the ICMJE uniform disclosure form at <https://www.icmje.org/disclosure-of-interest/> and declare: no external support from any organisation for the submitted work; no financial relationships with any organisation that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: Before randomisation began at each site, the trial was approved by the Jawaharlal Institute of Postgraduate Medical Education and Research Ethics and Scientific Advisory Committees (JIP/IEC/2016/25/831), and for the Institute of Child Health and Institute of Obstetrics and Gynaecology by the Institutional Ethics Committee of the Madras Medical College (No 02052018). The protocol was registered prospectively with the Clinical Trials Registry India (CTRI/2017/01/007676).

Data sharing: The Stata statistical analysis code is included in the supplementary file. Deidentified individual patient data are stored at <https://doi.org/10.7303/syn68349250> and are fully accessible after registration with synapse.org.

Transparency: The corresponding author (FS) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that discrepancies from the study as registered have been explained.

Dissemination to participants and related patient and public communities: The results of the trial will be presented to the Government of India to suggest that all newborn babies, even if they are unwell, should be vaccinated with BCG 0.1 mL intradermally, preferably using the Danish strain, on the day of birth or soon after, rather than being delayed in newborn babies weighing <2000 g, and a vial of BCG should be opened even if only one infant requires vaccination. In addition, the results will be presented at the PEDICON (paediatric) and NEOCON (neonatal) meetings in India, and at the Optimunize international vaccine conference at the University of Cambridge in 2026. The results will be shared with neonatologists in India by social media, and the Jawaharlal Institute of Postgraduate Medical Education and Research will arrange for publicity on social media, radio, and television, and in newspapers.

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Supplementary information: Additional information