Randomized controlled trials of early versus late BCG vaccination

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Abstract

Background: Bacillus Calmette–Guérin (BCG) vaccination may have non-specific effects (NSE), i.e. effects on childhood morbidity and mortality that go beyond its effect on the risk of childhood tuberculosis (TB). Though the available literature is mostly from observational studies, and is fraught with controversy, it does suggest that BCG vaccination at birth may protect young infants in high mortality populations against serious infections other than TB. Yet other studies indicate that giving BCG later in infancy may induce a stronger immunity to BCG and Mycobacterium tuberculosis (MTB) as well as to non-MTB and non-BCG antigens. An ongoing trial estimates the effect of deferring BCG vaccination till 14 weeks of age in HIV-1 exposed (HE) infants, who experience a disproportionately high risk of severe bacterial infections. The current generic protocol is intended as a basis to develop protocols for trials that compare BCG vaccination at birth with BCG vaccination at 14 weeks of age in all infants, both in HIV-1 unexposed (HU) babies and in those whose mothers are infected with HIV-1.

Methods: Individually randomized controlled trial comparing BCG vaccination within 24 hours of birth with vaccination at 14 weeks of age. The main study outcomes include death in the first 14 weeks of life, death in the remaining part as well as during the entire infancy, and a subsample, production of Tumor Necrosis Factor, Interleukin (IL)–1b, IL-6 and Interferon-γ in response to mycobacterial and non-mycobacterial antigens.

Discussion: A well-timed BCG vaccination could have important NSEs in infants. This trial could inform the development of programmatically appropriate timing of BCG vaccination for infants in low and middle income countries and yield a NSE benchmark against which new TB vaccines should be compared.

Key words: BCG, vaccination, Non-specific effects, trial
Introduction

**BCG vaccination and non-specific effects**

Several observational studies suggest that routine childhood vaccines may have effects on childhood morbidity and mortality that are separate from their effects on the incidence of the diseases they target [1-8]. Importantly, the *Bacillus Calmette–Guérin* (BCG) vaccine may have effects on morbidity and mortality that cannot be restricted to its protective effect against tuberculosis (TB) [9-13]. A case-control study among infants in Guinea-Bissau showed that infants who had received the BCG vaccine were at least 60% less likely to have acute lower respiratory tract infections (ALRI) and respiratory syncytial virus infections [14] than those who had not been vaccinated. A small Malawian study indicated that the likelihood of septicaemia among infants with a BCG scar was lower than that among infants without a scar [15]. A Senegalese prospective study analyzing data from two birth cohorts (a total of 11,369 children under two years of age) found an association between a combination of BCG/ DTP administered simultaneously and reduced mortality [1]. A secondary analysis of data from a randomized controlled trial among low birth weight preterm infants indicated that there may be an association between early BCG vaccination and lower perinatal mortality, but not lower infant mortality [11]. In a birth cohort study in Uganda, we showed that children between 1 month and 5 years of age who had received BCG had a substantially lower mortality than those who had not [16]. In fact, the apparent beneficial effect of BCG was even stronger in the neonates but we decided not to display this potentially important finding because of the possibility that some of the association could have been due to reverse causality, as vaccinators could have refrained from giving BCG to some of the sick babies. Other observational studies among older children, adolescents and adults have shown mixed results for the effects of the BCG vaccine against atopy [17-20] and helminthic infections [21-23]. Non-specific effects (NSEs) of vaccines, including of BCG, are now receiving increased attention, all the more so when the incidence of the TB is decreasing worldwide [24].

The WHO recently established a Strategic Advisory Group of Experts (SAGE) on non-specific effects of vaccines [25]. Substantial resources and efforts are currently being invested to develop and evaluate new vaccines against TB, aiming to confer substantial protection not only against primary TB, as does BCG, but also against reactivated TB and thereby cavernous lung TB, which sustains the spread of *Mycobacterium tuberculosis* (MTB). If such new vaccines are to replace BCG child health vaccination programs, their efficacy against primary TB will first need to be assessed, BCG being the likely comparator. However, before engaging in such trials it is important to obtain a benchmark for any non-specific benefical effects that BCG may have in terms of disease risk and mortality reduction, so that the overall health benefits, not only the protection against TB, can be assessed.

Studies in several low and middle income countries show that, although WHO and most national guidelines state that BCG is to be given as soon as possible after birth, and coverage is reported to be high (http://tinyurl.com/BCGCoverage), timeliness of
BCG vaccination varies widely (Clark A, Sanderson C (2009). Timing of children’s vaccinations in 45 low-income and middle-income countries: an analysis of survey data. Lancet 373: 1543–1549. Thus, in the Gambia, for example, a country with very high vaccination coverage, 40% to 50% of the babies in the rural areas had not received their BCG vaccine by the end of their first month of life, while ??% had not been vaccinated at 3 months of age (Victoria to enter reference: Scott et al, Plos One 2014; 9: e107280). In Kenya, the corresponding figures are ??% and ??%, respectively.

Methodological challenges in existing studies
The literature on NSEs of vaccines is fraught with controversy [9]. Much of this controversy stems from interpretational challenges of the observational studies carried out in high mortality areas, and in heavily burdened health care systems [26, 27]. It has been argued that children with a lower morbidity and mortality are more likely to be vaccinated than higher risk children, resulting in a spurious non-specific protective effect of vaccines in such studies [26, 27]. There is also the possibility of survival bias if death (a common study outcome) is associated with missing vaccination cards. This has been observed in some retrospective studies in Africa where child deaths were quickly followed by destruction or loss of vaccination cards [1, 26, 28, 29]. These selection challenges are best alleviated by well-designed and executed randomized controlled trials. However, as most childhood vaccines are part of routine national and international child health programmes, it has been considered unethical to randomize children to not receive BCG. For this reason, the literature on NSEs has continued to be overshadowed by controversy and continues to be dependent on initial studies carried out several decades ago when morbidity and mortality patterns as well as the vaccines themselves were different from what they are now [26, 27].

Immunological reprogramming induced by BCG
In order to strengthen the understanding of the mechanisms underlying any NSEs of BCG, it is important that upcoming trials of NSEs identify any immunological reprogramming induced by the vaccine, programming which has been recently suggested to be responsible for NSEs [30]. The immunological responses thought to account for the NSEs of BCG may in broad terms be considered to involve two processes: trained innate immunity and heterologous immunity. Trained immunity is a relatively novel concept that described the functional reprogramming of innate immune cells [such as monocytes, macrophages or natural killer (NK) cells] after an infection or vaccination [31]. The molecular mechanism mediating trained immunity is represented by epigenetic modifications due to changes in histone methylation and acetylation, leading to a looser chromatin structure and increased gene transcription [32]. Similar epigenetic reprogramming has been described in monocytes of individuals vaccinated with BCG [33]. In addition to trained immunity, a second important immunological mechanism that is likely to be involved in NSEs is heterologous immunity: the capacity of memory T-lymphocytes to respond in an IL-12/IL-18-dependent manner with increased production of cytokines upon stimulation with different stimuli [34].

Timing of BCG vaccination
A small trial in South Africa in which newborn HIV-1 unexposed infants were randomized to receive BCG at birth or at 10 weeks of age, showed that deferring the vaccination resulted in increased numbers of BCG specific CD4 T cells, most importantly polyfunctional T cells co-expressing IFN-γ, TNF-α and IL-2, at one year of age [35]. A recent study among HIV-1 exposed children reported robust T-cellular responses to Bordetella pertussis and tetanus toxoid in infants in whom BCG vaccination was deferred to 8 weeks of age [36]. It is unknown, whether these effects of deferred BCG administration translate into enhanced protection against TB or, for that matter, against other serious infections, i.e. enhanced NSEs.

The most appropriate timing for BCG vaccination that maximizes both specific and possible non-specific effects is thus presently unknown. This uncertainty compels us to study the NSEs of BCG with a design that overcomes the methodological challenges of observational studies.

**Incidence of young infant TB in high mortality areas**

If infants in an RCT are to be randomized to receive deferred BCG vaccination, it is crucial that their risk of TB is kept at a minimum and that they are brought to appropriate care should they or another family member develop any signs of TB.

**Purpose and rationale of studies this generic protocol is intended to motivate**

Having the above considerations in mind, and provided infants at high risk of TB are excluded from upcoming studies, we believe that there is clinical equipoise between early and deferred BCG vaccination. This generic protocol suggests approaches to inform the development of programmatically appropriate scheduling of infant BCG vaccination by measuring the safety, potential benefits or disadvantages of deferring BCG vaccination to 14 weeks of age. The long term cost-implications and cost-effectiveness of the alternatives have never been investigated, so our protocol also includes suggestions for how to estimate cost-effectiveness of alternative vaccination regimens.

**Hypotheses and Objectives**

**Hypotheses**

a) Compared to deferring BCG vaccination till 14 weeks of age, BCG administered within the first 24 hours of birth leads to at least a 15% relative mortality risk\(^3\) reduction in infants less than 14 weeks of age.

b) Compared to deferring BCG vaccination till 14 weeks of age, BCG administered within the first 24 hours of birth leads to at least a 15% relative risk reduction of severe illness\(^4\) in infants less than 14 weeks of age.

\(^3\) Death caused by violent injury or burns will not count towards the mortality risk estimates.

\(^4\) Among children <2 months of age, severe illness (other than TB) will be defined as illness that is associated with any of the following danger signs observed or verified by a study clinician: inability to feed or vomiting of everything, lethargy or unconsciousness, severe lower chest in-
c) Deferred BCG results in at least a 30% higher production of TNF, IL-1β, IL-6, IL-10, IL-17, IL-22 and IFN-γ in response to mycobacterial (from *Mycobacterium tuberculosis* and PPD) and non-mycobacterial (from *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*) antigens compared to when BCG is administered at birth.

**Specific objectives**

**Primary objectives:** To compare

1) the risk of death in the first 14 weeks of life, and the
2) the risk of severe illness in the first 14 weeks of life,
3) production of TNF, IL-1β, IL-6, IL-10, IL-17, IL-22 and IFN-γ in response to mycobacterial (from *M. tuberculosis* and PPD) and non-mycobacterial (from *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*) antigens at birth (before BCG vaccination) and at 1, 14, 15 and 28 weeks of age among infants administered BCG at birth (early BCG) with those administered BCG at 14 weeks of age (deferred BCG).

**Secondary objectives:**

A. Among infants to compare

1) the mortality risk from 48 hours after randomization till 14 weeks of life in infants administered early BCG with those receiving deferred BCG
2) the risk of severe illness from 48 hours after randomization till 14 weeks of life in infants administered early BCG with those receiving deferred BCG
3) infant mortality between those receiving early versus deferred BCG

B. through life cycle modelling long term aggregate health benefits and cost-implications of the delivery strategies, as well as their cost-effectiveness.

[To increase the diagnostic specificity, the trials could also capture “Clinical sepsis”

Further, to increase diagnostic specificity and to describe the bacterial drawing, axillary temperature of ≥37.5°C or <35.5°C, grunting, cyanosis, convulsions or a history of convulsions, and/or results in hospitalization and/or results in death.

Among children ≥2 months of age, severe illness (other than TB) will be defined as illness that: is associated with at least one of the following danger signs observed or verified by a study clinician: inability to drink or breastfeed lethargy or unconsciousness, vomiting of all feeds, convulsions or a history of convulsions, and/or results in hospitalization and/or results in death. Hospitalization and death resulting from violent injury or burns will not contribute to the severe illness definition.

5In infants from whom a blood culture is not done or in whom a blood culture was negative, “Clinical sepsis” is signaled by one of the study physicians having initiated treatment for sepsis or confirmed that such treatment was appropriate and/or by a positive Septic screen. A positive Septic screen is the presence of any two of the following: total leucocyte count <5000/cmm; absolute neutrophil count
etiology of “confirmed sepsis”, a blood culture could be taken from all children with severe illness. This should be carefully considered and discarded as an option if we believe that the required closer contact with the families and babies induces Hawthorne effects and/or if we think that it’ll make the trial too complex and costly.

Although it is unclear whether BCG scarring is a marker of immune responsiveness in general and it may be that such scarring is unrelated to protection against TB, it should be captured as a marker of “vaccine take”. The trials should also examine the effect of the timing of BCG administration on infant growth, as it is an (albeit suboptimal) indicator of overall infant health and development.

**Intervention & co-interventions**

The study intervention will be an intra-dermal administration of 0.05 ml Tubervac® BCG vaccine from the Serum Institute of India or the BCG Vaccine "SSI"® from Statens Serum Institut in Copenhagen, Denmark. After randomization within 24 hours of birth, infants in one trial limb will receive the vaccine immediately (early BCG) while infants in the other limb will receive the vaccine at 14 weeks of age (deferred BCG). Babies who are to receive a deferred BCG vaccine will receive a placebo injection at birth, while those who receive BCG at birth will also receive a placebo injection at 14 weeks of age. In order to ensure batch consistency between those who are vaccinated at birth and at 14 weeks of age, each site must ensure that sufficient amounts of each vaccine batch is kept in stock so that the babies who are to receive the deferred vaccine gets the same batch number as their peers randomized to be vaccinated at birth. A photograph of the injection site in all infants will document the wheal or lack thereof.

**Key outcome measures**

**Primary outcome measures**

1) Death in the first 14 weeks of life
2) Severe illness in the first 14 weeks of life
3) In a subset: TNF, IL-1β, IL-6 and IFN-γ in response to mycobacterial (*M. tuberculosis* and PPD) and non-mycobacterial pathogens (*E. coli, C. albicans* and *S. aureus*) at 1, 14, 15 and 28 weeks of age

**Secondary outcome measures**

1) Death from 48 h after randomization to 14 weeks of life [based on considerations from immunologists, this could be moved to become the first primary outcome]

<1500/cmm; band cell:neutrophil ratio >0.2; micro-ESR >15 mm at 1 hour and both of two C-reactive protein serum levels >1mg/dl in specimens taken 24 h-48 h apart.
2) Severe illness from 48 h after randomization to 14 weeks of life [based on considerations from immunologists, this could be moved to become the second primary outcome]
3) Death in the 14th to the 52nd weeks of life
4) Severe illness in the 14th to the 52nd weeks of life
5) Death in infancy
6) Severe illness in infancy
7) Growth up to 52 weeks of life
8) BCG scar 12 weeks post vaccination

Study setting
The study setting should be described in detail, and should include the study population, also with respect to the prevalence of HIV infection in women of reproductive age and among infants. Adequate enrollment requires that women are recruited during pregnancy, so that recruitment after the birth of the babies can benefit from the study having been explained to them well in advance of their giving birth.

Selection of participants, inclusion & exclusion criteria
Pregnant women between 28 and 40 weeks of gestation attending antenatal care (ANC) should be approached by the study team (figure 1). These women should be informed about the trial, its purpose, benefits, risks and their potential eligibility. The study team should encourage the women to deliver at the study health clinics. Randomization of infants and, in those randomized to receive early BCG, the vaccination, will take place in or very close to the labour or postnatal units and within 24 hours of birth.

Inclusion criteria
A baby born at a participating study clinic will be included if s/he:
1) has a mother that is of legal age for participation in clinical research studies or is an emancipated minor
2) has a mother/caregiver that resides within the study area, is not intending to move out of the area in the next 4 months and is likely to be traceable for up to 12 months
3) has a mother/caregiver that gives informed consent to trial participation

Exclusion criteria
A new-born child will be excluded if s/he has:
1) serious congenital malformation(s)
2) severe illness requiring hospitalization
3) a birth weight < 2.0 kg
4) a mother participating in another research study on the day of enrolment or a mother who will participate in another research study within the next 3 months.
5) a mother or other household member with symptoms and signs of TB on the day of enrolment
6) a severely ill mother with a condition or conditions requiring hospitalization
Trial size estimates for the primary hypotheses

Hypothesis a) and b)
The outcome in this hypothesis is death in the first 14 weeks of life. Based on an estimated risk of death ranging from 3% to 1% in the first 14 weeks of life [ref needed], 90% power and a risk ratio of 0.85, the required sample size per trial limb ranges from 30,003 to 88,468 infants or 60,006 and 176,936 infants in the entire trial. Taking into account a maximal of 10% attrition, the total number of infants required will range from 65,500 to 192,000.

Total trial sample size* (i.e. both study limbs); alternative assumptions

<table>
<thead>
<tr>
<th>Risk of death</th>
<th>Power</th>
<th>0.1%</th>
<th>0.3%</th>
<th>0.5%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Ratio=0.8</td>
<td>80%</td>
<td>726,828</td>
<td>242,658</td>
<td>145,826</td>
<td>73,204</td>
<td>36,896</td>
<td>24,798</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>966,316</td>
<td>322,614</td>
<td>193,874</td>
<td>97,322</td>
<td>49,092</td>
<td>32,968</td>
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<td>Risk Ratio=0.85</td>
<td>80%</td>
<td>1,318,336</td>
<td>440,188</td>
<td>264,560</td>
<td>132,844</td>
<td>66,994</td>
<td>45,052</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>1,755,918</td>
<td>586,294</td>
<td>352,372</td>
<td><strong>176,936</strong></td>
<td>89,230</td>
<td><strong>60,006</strong></td>
</tr>
<tr>
<td>Risk Ratio=0.90</td>
<td>80%</td>
<td>3,025,128</td>
<td>1,010,190</td>
<td>607,208</td>
<td>304,980</td>
<td>153,888</td>
<td>103,542</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>4,036,308</td>
<td>1,347,856</td>
<td>810,172</td>
<td>402,900</td>
<td>205,324</td>
<td>138,150</td>
</tr>
</tbody>
</table>

*Max 10% for attrition to be added.

Hypothesis b)
The outcome in this hypothesis is severe illness in the first 14 weeks of life. Based on an estimated risk of severe illness ranging from 5% to 1% in the first 14 weeks of life, 90% power and a risk ratio of 0.85, the required sample size per trial limb ranges from 16,025 to 88,468 infants, or 32,050 and 176,936, infants in the entire trial. Taking into account a maximal of 10% attrition, the total number of infants required will range from 36,000 to 195,000.

Total trial sample size* (i.e. both study limbs); alternative assumptions

<table>
<thead>
<tr>
<th>Frequency of the severe illness</th>
<th>Power</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>4%</th>
<th>5%</th>
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<tbody>
<tr>
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<td>80%</td>
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<td>15,030</td>
<td>9,392</td>
<td>7,092</td>
<td>5,714</td>
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<tr>
<td></td>
<td>90%</td>
<td>39,966</td>
<td>20,122</td>
<td>12,572</td>
<td>9,496</td>
<td>7,912</td>
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<tr>
<td>Risk Ratio=0.8</td>
<td>80%</td>
<td>73,204</td>
<td>36,896</td>
<td>24,798</td>
<td>16,932</td>
<td>13,658</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>97,322</td>
<td>49,092</td>
<td>32,968</td>
<td>22,502</td>
<td>18,150</td>
</tr>
<tr>
<td>Risk Ratio=0.85</td>
<td>80%</td>
<td>132,844</td>
<td>66,994</td>
<td>45,052</td>
<td>29,828</td>
<td>24,074</td>
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<tr>
<td></td>
<td>90%</td>
<td><strong>176,936</strong></td>
<td>89,230</td>
<td>60,006</td>
<td>39,712</td>
<td><strong>32,050</strong></td>
</tr>
<tr>
<td>Risk Ratio=0.90</td>
<td>80%</td>
<td>304,980</td>
<td>153,888</td>
<td>103,542</td>
<td>78,384</td>
<td>63,302</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>402,900</td>
<td>205,324</td>
<td>138,150</td>
<td>104,584</td>
<td>84,460</td>
</tr>
</tbody>
</table>

*Max 10% for attrition to be added.
Hypothesis c)
The outcomes for this hypothesis is the production of relevant cytokines in response to mycobacterial and non-mycobacterial antigens. Based on previous studies [33, 37] we expect that comparisons involving 500 children in each trial limb, but not less than 100 per country, will be sufficient to detect biologically relevant differences. However, if mediation analyses are to ascribe causal immunological pathways, the sample size for the corresponding assays and thereby blood draws will need to match that of the clinical outcome they are intended to explain.

**Randomization**
Infants of HIV-1 uninfected mothers fulfilling the other inclusion criteria will be randomized within 24 hours of birth using pre-prepared randomisation lists. Randomization is to be stratified by clinic using permuted blocks of varying size (4, 6 or 8) where eligible infants will be allocated in a 1:1 ratio to one of the two following limbs: BCG vaccination within 24 hours of birth (early BCG) or BCG vaccination at week 14 (deferred BCG). All computer generated randomization lists, should be prepared for each clinic by an independent scientist and kept by an off-site study statistician/epidemiologist. Concealment until the mother-infant pair has been deemed eligible for final inclusion will be ensured using an application running on one dedicated Android cell phone at each clinic.

**Blinding/masking**
In order to protect randomization, a placebo injection will be given to the babies (in the early vaccination trial limb at 14 weeks of age, in the deferred BCG trial limb at birth). Still, because a high proportion of babies who receive BCG will develop a pustule and scar, it is vitally important that the personnel that record outcomes are rigorously trained and to the extent possible should blinded to which trial limb the children belong to. Further, the vaccine should be administered by routine health workers at the health centers that regularly administer the BCG vaccine as well as other childhood vaccines in order to further mask the staff that record the outcomes.

**Implementation plan, staff recruitment and training**

**Field organization**
Recruitment at each of the health facilities should be by an adequate number of full time research assistants (nurses) and a part-time research assistant. The country coordinator should be responsible for the day-to-day running of the trial, and will report to the country principle investigator (PI) and country co-PI who will ensure the overall scientific integrity of the trial. In each country, the trial should also employ a data manager that will oversee data quality. Considering the large sample size and the need to encompass populations from different high infant mortality areas, the trial is likely to be undertaken as a multi-country study. All study personnel must receive intensive training on study procedures before commencement of the trial and refresher training during the conduct of the trial and at least one initial and one mid-trial joint training session for all country PIs, co-PIs and supervisors must be conducted. Efforts must be made to ensure congruence in study implementation by using identical
Standard Operating Procedures (SOPs), also for training of personnel. The study SOPs should be available at clinics and used during training and study trial implementation, while the Study protocol should not be kept at the clinics, nor used in the training of the study personnel, this to reduce the likelihood of bias in study outcome assessment.

**Screening of pregnant women**
All pregnant women between 28 and 40 weeks of gestation attending antenatal care at the designated clinics should be informed about the trial. Specifically, they will be informed that if they choose to participate in the, their infants will be randomized to get the BCG vaccine or a placebo injection within 24 hours of birth and then placebo or BCG at 14 weeks of age. Consenting women will be assessed clinically and with a hemoglobin estimation as well as examined for TB. A careful history deliberately unveiling any TB patients in the infant’s home environment must also be undertaken. Pregnant women who are found to be eligible during screening and who consent to participate can be recruited into the trial. During this initial recruitment, data on baseline characteristics and other social-demographic variables are to be collected.

**Postnatal screening**
The infant will be assessed for other inclusion and exclusion criteria, and if eligible, randomized within 24 hours of birth. At birth, cord blood should be collected for the child’s full blood count at baseline. A sample of this cord blood should be stored for future investigations.

**Collection of data on baseline features and potential confounders**
Data on relevant baseline characteristics will be collected during pregnancy and at birth. This will include indicators of socio-demographic characteristics (maternal education and age, food insecurity, income, employment etc.), health (illness history, parity, clinical examination, hemoglobin, BMI), postpartum complications, sex of the child, infant feeding practices, and whether the mother received tetanus toxoid during pregnancy as per national recommendations.

**Follow up and subject retention**
Follow up visits at the study clinic shall be scheduled on day 2, 6, 10, 14, 15, 26 and 52 after birth (table 1 and figure 2). At each of these visits, the outcome assessors shall record whether the baby has died or is alive, and if so, whether (s)he has had severe illness since the last contact. When a study infant has died, the 2014 WHO verbal autopsy questionnaire and narrative interview will be employed to capture the likely cause of perinatal or child death [38]. Further, any other adverse events (localized abscess formation, suppurative lymphadenitis and disseminated BCG infection) should be recorded, anthropometry and on potential confounding variables (see above). SMS reminders should be sent to each participant before each clinic visit. Those who do not send a confirmation back by SMS should be called up. All mothers or those without a cell phone, shall be given a cell phone to facilitate contact with study staff in case of an illness or other important child events.

Home visits shall be made for participants missing clinic visits while those coming to their scheduled clinic visits shall have their transport fees reimbursed, this will reduce losses to follow up. An alternative is to arrange for pick-up in the homes, at least for
those who cannot use public transport. Data on illness, hospital visits and hospitalization shall be collected from interviews with the mother, with more detailed information obtained from the hospital records. Illness and hospitalization data shall be based on recall between a previous visit and a current visit.

Upon enrolment and on subsequent visits, mothers and other caregivers should be informed about symptoms and signs of severe illness, and should be encouraged to contact the study clinic in case the baby gets any of these symptoms. Each mother should be provided with a durable plastic-protected note book, in which she should be instructed and trained to note down all relevant events in her child. The front-page of the book shall have clear reminders that she needs to contact the study team in case the child visits a health clinic, a physician or is admitted to a hospital. A child who comes to a clinic with such symptoms should be examined by a study nurse/midwife. If the child does not show up after the mother/caregiver has informed the study team of such signs or for a scheduled visit, the study nurse/midwife should make a visit to the child’s house. If the nurse/midwife classifies the condition as possible severe illness, a study physician should be immediately called to examine the child. If the physician classifies the child as having severe illness, he or she should collect a blood specimen for blood culture (using BACTEC) and a septic screen (CRP, TLC, differential count, and band cell:neutrophil count ratio, micro-ESR) as soon as possible. A repeat specimen should be collected again for CRP within 24 to 48 hours. The study team should do its utmost to obtain specimens for such a septic screen from as many infants with signs of severe illness as possible, and the proportion from whom such specimens are collected must exceed 90%.

Length and weight shall be measured at the scheduled study visits, based on the WHO guidelines, and z-scores calculated using WHO child growth standards [39].

Data collection and management
All trial procedures should be standardized. Training and refresher sessions should be scheduled before and during the trial. Using e.g. ODK (https://opendatakit.org), pre-coded electronic case report files (CRF)/questionnaires with range and consistency checks should be used. The data capture tool currently used in the ongoing trial on BCG vaccination in HIV exposed babies will be made freely available to WHO from the Ugandan PI. The CRFs should be designed, completed, stored and relayed according to GCP principles. Entered data shall be checked for consistency and any errors corrected within 24 hours of being entered by study supervisors and, if there are queries, returned to the research assistants for correction within another 24 hours after feedback. The CRFs and supporting documentation must be kept securely, in locked cabinets or password protected computers, as study source documents for review and/or audit during or after the study. Source documents and files should not be destroyed without specific written permission from the country PI. Only the country PI, Co-PI, study coordinator and authorised study personnel shall have access to CRFs and supporting documents that should be kept on password secured computers to ensure participant confidentiality. To ensure correct operation according to SOPs, all system users should be trained in their use and evaluated on a regular basis. The outcome data collection team should be kept separate from the intervention implementation team, i.e.
those vaccinating the babies and the hypotheses and study objectives should not be discussed with the clinic staff, to minimize information bias. Once a year, the data from each country should be anonymized and transferred to the overall trial PI who will compile appropriate tables for the Data Monitoring Committee (DMC)’s annual assessment of study progress.

**Clinical laboratory tests**
In the appropriately sized subsample of babies, at week 14 and week 52, blood should be drawn by venipuncture full/complete blood counts. A heel prick specimen will be collected in all babies in countries where the prevalence of HIV-1 infection among women of reproductive age exceeds 3% to diagnose HIV-1 infection in children. TLC, differential count, band cell:neutrophil count ratio, CRP, micro-ESR will be captured as part of the septic screen in babies with clinical severe illness from whom also a blood culture specimen will be drawn and worked up, whenever possible.

**Functional immunology assessment**
Both induction of trained immunity and heterologous immunity should be characterized. Blood cells shall be stimulated for 48 hours with a cell lysate of BCG or *M. tuberculosis*, and IFN-γ production estimated by ELISA as a measure of classical adaptive immunity. Using the cell culture medium (RPMI) as a control stimulus, trained innate immunity shall be assessed by stimulation of blood for 24 hours by unrelated non-specific stimuli: lipopolysaccharide (LPS), heat-killed *E. coli*, *C. albicans*, or *S. aureus* at 1, 15, and 28 weeks of age. Production of the monocyte-derived cytokines TNF, IL-1β and IL-6 shall be assessed by ELISA. Genome-wide gene transcription shall be assessed in the unstimulated and stimulated whole blood by RNA sequencing. Heterologous immunity shall be assessed by measurement of both Th1 (IFN-γ) and Th17 (IL-17, IL-22) cytokines, in blood stimulated for 96 hours with the non-specific stimuli described above. The anti-inflammatory cytokine IL-10 shall be assessed as well in the 48h stimulation time point. Protein and mRNA assessment shall be the main immunological readouts.

**Quality assurance and quality control**
The WHO Good Laboratory Practice Guidelines [40] should be used as the reference when establishing quality control laboratory procedures. Haematological, biochemical and immunology assays and, in countries where HIV-1 prevalence exceeds 3%, HIV-1 serology and CD4 counts, shall be submitted to a stringent quality assessment programme. On-going training and monitoring shall be in place during the trial.

**Handling losses, withdrawals and protocol deviations**

**Protocol deviations**
If a protocol violation such as an inadequate informed consent, inappropriate concealment or wrongful enrolment of an under-age mother or an infant with an exclusion criterion occurs, the country PI shall inform the trial PI who again communicates this information to the DMC and the country ethics committee as soon as possible and no later than 5 working days after the event. Detailed documentation mentioning the dates and reasons of these protocol deviations should be kept by the
country- and trial-PI. If continuing the child in the trial puts the health of the mother or her child at risk, it may be necessary to abandon follow-up, and, if so, his/her data included in time-to-event analyses up to the time he/she was removed from the trial.

**Plan of analysis**
Continuous variables with right-skewed distributions should be log-transformed. Means with standard deviations should be used to summarize symmetrically distributed continuous variables while medians with interquartile ranges should be used for non-normally distributed continuous variables, and percentages for categorical variables. Between-group comparisons for continuous variables that are symmetrically distributed should be made using t-tests or, if adjustment for other variables are required, linear regression, while the Wilcoxon rank sum test should be used to compare continuous variables where even their log-transformed values remains non-symmetrically distributed. Group comparisons for categorical variables should use chi-square tests and regression with generalized linear models of the binomial family with a log link (‘relative risk regression’) or logistic regression. Two-sided statistical tests and 95% confidence intervals for descriptive results, effect estimates and safety estimates should be used. All relevant data; from both scheduled and unscheduled visits should be included in the analysis.

**Primary analysis:** All randomized children shall be included in the primary, i.e. intention-to-treat, analysis. The main study outcomes are death and severe illness (of which death is one defining outcome) during the first 14 weeks of life and immunological readouts. Although the sample size estimations for the former two are based on specified relative risks, children that are lost to follow-up should be included in survival analyses, censoring them during periods when data could not be recorded. The survival analyses shall include Kaplan-Meier plots and log-rank tests for descriptive statistics. Cox proportional hazards regression models shall be used to estimate the effect of the intervention on child death. For this outcome, data shall be censored for periods during which a child is lost to follow-up.

For the severe illness outcome, the final analysis should also use incidence density and Poisson regression or, in case of overdispersion, negative bimomial regression analyses, to estimate incidence rate ratios (IRR), enabling the capture of more than one illness event per child. To take into account multiple study events occurring in the same child in time-to-event analyses, the Cox regression models shall utilize generalized estimating equations (GEE) or in other ways account for dependency between repeated events. The effect of the intervention on the occurrence of severe illness will be estimated using both relative [relative risks (RR), IRR and hazard ratios (HR)] and absolute (risk differences and incidence rate differences (IRD)) measures of effect. In the event the ‘relative risk regression’ model fails to converge, risk ratios can be obtained from logistic regression [41] or from a modified Poisson regression model with robust variance [42, 43].

Also autoregressive correlation approaches should be used to account for multiple and possibly correlated observations within the same study participant. These methods take the correlation structures into account and thereby the fact that measurements taken
closer in time for an individual are likely to be more correlated than two measurements taken farther apart for that same individual. The effect in percent is $100 \times (1 - \text{RR})$, $100 \times (1 - \text{IRR})$ or $100 \times (1 - \text{HR})$. The prevalence of BCG scarring should be compared between the two study limbs using the above mentioned approaches, as appropriate. T-tests and or linear regression should be used to compare the TNF and IFN-$\gamma$ responses between the trial limbs. Adjustment for potential confounders should be done in the unlikely event that there is a combination of: 1) baseline imbalances between trial limbs, 2) the variables for which there are such baseline differences are strongly associated with the study event or 3) they cause a $\geq 5\%$ difference in the effect measures when they are added to the main model. Full case analyses should be default, but appropriate imputations should be considered for missing data.

In addition to the intention-to-treat analysis where outcomes should be compared according to the random allocation, instrumental variable analyses can be conducted in an attempt to estimate biological/causal effects of the actual receipt of the vaccine. In these analyses, random allocation should be the instrument. To enable such analyses, actual receipt as well as the age of BCG vaccination should be captured in all participants. Also per protocol and as treated analyses can be considered.

Secondary analysis (to be modified based on which secondary outcomes one decides to include):

1) As a hypothesis generating effort to understand the latency period after which BCG may induce NSE, estimate the protection against severe illness during the first 14 weeks of life on a sliding scale starting from randomization until 7 completed days post-randomization.

2) Safety will be analysed according to type, frequency and severity of adverse events (AEs) that occur in children during the trial.

3) Sub-group analysis for potential effect measure modification will be on the strata defined by low (<2,500 g) or normal ($\geq$2,500 g) birth weight, the babies’ sex and whether the mother has HIV-1 infection or not, in addition to other baseline characteristics for which there are reasons may interact with early versus deferred BCG administration. Such subgroup/interaction analyses must be defined based on available literature and considerations before embarking on the analyses; it is not be driven by study data, and must be described in a detailed analysis plan which is made publically available before data analysis is embanked upon.

4) Mediational analysis with BCG scarring and, if adequate sample size, immunological radouts, as the mediator(s) for death and for severe illness.

5) Survival analyses using non-parametric and parametric methods to extrapolate over 5 years the incidence of NSE and death for use in economic evaluation.

**Plan of analysis for economic evaluation**

A Markov life-cycle decision model should be developed to model long term aggregate health and cost implications of the delivery strategies, and to compare their cost-effectiveness. The decision model should have two limbs, one for early, the other for
deferred BCG administration (Figure 3). A Markov life-cycle should be attached to each trial limb, in which infants are followed in weekly cycles from 0-14 weeks, monthly cycles between 14 and 52 weeks, and thereafter yearly cycles. The model should track a relevant cohort of infants until they are 5 years old.

The main focus of the model should be severe illness (including death) during the first year of life, and will rely on prospectively collected trial data (table 3). For each cycle (weekly, monthly or annual) children may experience disease, or they may remain healthy.

The model represents a simplification of clinical reality, and a simplifying assumption is that BCG-induced protection against severe illnesses occurs only during infancy. This reflects the availability of primary data and follow-up from the controlled trial. Modelling of the health outcomes may be divided into three phases; (i) the observation period, (ii) the period of assumed but gradually waning impact of the vaccine (<5 years), and (iii) the post-vaccine period, where no further vaccination effects are assumed. Extrapolation is required to capture probable health benefits during the second of these phases, but the actual functional shape and other assumptions about continued treatment benefits need to be informed by data through survival analyses, and cannot be decided ex-ante. Transition probabilities are assumed to depend on vaccination strategy, and will be informed by the trial. More specifically, transition probabilities, i.e. probabilities of moving between the health states, will be calculated based on hazard functions from the survival analyses (above). After the extrapolation period, children are not assumed to be protected from NSEs by early or deferred BCG, but are assumed to experience background mortality for their remaining life time. Long term health effects should be aggregated using disability adjusted life years (DALYs) as the instrument, while incremental cost-effectiveness should be expressed in terms of US dollars per DALY averted. Costs should be collected from the perspective of the relevant national health care system, and include those related to providing the BCG vaccine as well as cost savings related to NSEs in the short and long term.

**AE reporting/ Clinical and safety monitoring**
An Adverse Event (AE) shall be defined as any harmful manifestation occurring in a trial participant, whether this manifestation is related or not to the study BCG vaccine. Potential adverse events include localized abscess formation at the injection site, suppurative lymphadenitis and disseminated BCG infection.

**AE monitoring, recording and reporting**
Potential AEs shall be carefully monitored throughout the trial with specific questionnaires. The mothers shall be invited and encouraged to consult the study clinic in case of any disease or symptoms that arise between visits. AEs shall be investigated at each follow-up visit and can be reported spontaneously or in response to general, non-directed discussions with the attending midwife/researcher or physician/researcher. All AEs, regardless of seriousness, severity, or presumed relationship to study therapy, shall be recorded using medical terminology in the source document and on the AE page. Whenever possible, diagnoses shall be given when signs and symptoms are due to a common aetiology. Investigators shall record their
opinion concerning the relationship of the AE to BCG vaccination on the AE page. All AEs shall be followed up until resolution or until a stable clinical end-point is reached. All measures required for AE management and the ultimate outcome of the AE shall be recorded in the source document and reported on the AE page.

**AE reporting**

When the investigator, or trained doctor, becomes aware that a serious AE (SAE) has occurred, the appropriate reporting form shall be completed, and a copy emailed to the DMC and the local Institutional Review Board within 48 hours.

**Management of SAE**

In case of an SAE, the mother shall be encouraged to immediately use the dedicated cell phone to contact the research unit and or to bring the infant immediately to the research unit. Infants shall be seen by one of the research midwives/ paediatricians and appropriate medical or surgical interventions shall be provided. Insurance coverage shall be made by a prespecified Insurance company, and if any participant is harmed as a result of the BCG vaccine (within 2 years of the vaccination) she/he shall be appropriately compensated.

**Monitoring**

**Data Monitoring Committee (DMC):** A group of independent scientists with expert knowledge and experience in paediatrics, immunology, TB and statistics shall form a DMC. After meeting shortly before trial start, it shall periodically and at least annually, review and assess available study data for safety, conduct and efficacy. The board shall advise the project management on study continuation, modification or termination based on its reviews and pre-established stopping rules. The DMC is to perform an interim analysis for safety taking into account the DAMOCLES group recommendations [44, 45] when approximately half of all the expected deaths have been recorded.

**Auditing:** The study shall be audited by an independent auditor or monitored by (a) participating scientist(s) not involved in the day-to-day running of the trial before study start (site-readiness) and then once a year.

**Protocol amendments:** Important protocol modifications (such as those resulting from changes to eligibility criteria, outcomes, and planned analyses) shall be communicated to and discussed among the investigators, discussed with the DMC and, when appropriate the ethics committees. Such modifications shall also be reflected in amendments to the description in the trials registry, e.g. www.clinicaltrials.gov.

**Dissemination of study findings:** The study findings should be communicated in scientific conferences and published in peer-reviewed scientific journals. Further, the investigators should prepare contextualized evidence briefs for policy to relevant stakeholders including the authorities in which the trial(s) is/are undertaken, such as the Ministry of Health, National Drug Authority, National Academy of Sciences etc. as well as development partners supporting the Expanded Programme on Immunization (EPI), WHO (Vaccines and Biologicals), and UNICEF. The investigators should, also in collaboration with the WHO, prepare plain language summaries and press releases for consumer groups and mass media, respectively. Finally, the investigators should
engage the clinic staff and study participants and their communities to inform them of the trial findings.

**Ethical considerations**

This trial will compare BCG take and risk of severe illness and death among children receiving the vaccine shortly after birth to such a risk in infants receiving BCG at 14 weeks of age.

The main ethical concerns specific to deferring BCG vaccination are:

1) Acquisition of TB before BCG has engendered a protective immune response
2) Higher mortality and incidence of severe illness during the first 14 weeks of life

To address the first concern, the study will exclude children with household members that have signs or symptoms of TB, or have a diagnosis of TB. The risk of TB infection among trial babies will therefore be negligible [46]. In addition, the study will continue to actively screen and refer suspected cases to the national TB clinics until the participating infants have received BCG (at 14 weeks of age). The study will support diagnosis and initiation of treatment at the TB clinics, of any of the participants’ household members with signs and symptoms of TB from randomization until 14 weeks of age. Moreover, the study team will collaborate closely with the families to identify, treat and actively follow up the babies should there be a history of exposure to active TB or should they develop any symptoms indicative of TB. Should our study show that deferred vaccination is beneficial in terms of reducing mortality and/or protecting the babies against severe illness and it in the long run leads to a policy shift, with children from households without TB being vaccinated late, our findings will inform subsequent programmes and would thereby contribute to improved infant health. Should early vaccination induce beneficial NSEs in the first 14 weeks of life and deferred BCG have similar effects in the latter part of infancy, one would need to consider, in a new trial, assessing whether a dual dosing schedule, i.e. at birth and at 14 weeks of age, may enhance child infant health and survival beyond only vaccinating the infants at birth. An alternative strategy is to redesign this study to become a three-limbed trial, randomizing babies to be vaccinated: (1) at birth or (2) at 14 weeks of age or (3) at birth and 14 weeks of age.

To mitigate the second concern, appropriate diagnostic procedures and treatment shall be provided according to national guidelines to all severely ill children. Ethics permission to conduct the study will be obtained from all relevant local and international ethical committees/bodies. Written individual informed consent in vernacular will be obtained from each of the participating mothers by trained study staff. The consent process will explain the nature of the study, the risks and benefits of participating in the study, the intervention and that intervention allocation is by a random process. In situations requiring translation or in cases where the mother is unable to read and write, the consent process will take place in the presence of an independent third person, who will act as a witness and also co-sign the consent form. Additional consent will be obtained from study participants for the collection and storage of blood specimens for ancillary studies.
Confidentiality of information and the right of the participant to withdraw from the study at any time during the study will be explained to the mothers. All study staff will be trained on participant confidentiality and autonomy.

**Discussion**

The proposed placebo-controlled randomized trial compares the effect BCG vaccination at birth with BCG vaccination at 14 weeks of age on 1) Death in the first 14 weeks of life; 2) Severe illness in the first 14 weeks of life, 3) 5) TNF, IL-1b, IL-6, IL-17, IL-22 and IFN-γ in response to mycobacterial and non-mycobacterial pathogens, and 4) Severe illness or death alone in 14-52 weeks of life and throughout infancy.

The study circumvents the methodological challenges of earlier observational studies that reported an association between BCG vaccination and NSEs. The exclusion criteria combined with the random allocation protected by a placebo injection in the babies randomized to receive a deferred BCG and large sample size will substantially reduce the likelihood that children with higher morbidity end up in either group. There will be no survival bias linked to missing vaccination cards as BCG will be administered by study staff before outcomes are recorded. This study will be further strengthened by an exploration of immunological mechanisms for the vaccine’s hypothesised NSEs. The study will explore the trained innate immunity and heterologous immunity processes that underlie the immunological responses thought to account for the NSEs of BCG. While there is a possibility of NSEs of BCG vaccination, the most appropriate timing of the vaccine that maximizes these NSEs is unclear. It has been posited that a deferred vaccine given when the immune system is more mature than it is at birth could result in better outcomes.

This trial, comparing NSEs when the vaccine is deferred with NSEs when the vaccine is given at birth could inform the development of programatically appropriate scheduling of BCG vaccination. This, in-turn, could importantly impact morbidity and mortality among infants.

**List of Abbreviations**

- **AE** – Adverse Event
- **ALAT** – Alanine aminotransferase
- **ASAT** – Aspartate aminotransferase
- **BCG** – Bacille Calmette Guerin
- **CISMAC** – Centre for Intervention Science in Maternal and Child Health
- **CD 4+** – Positive to Cluster of Differentiation 4
- **CD 8+** – Positive to Cluster of Differentiation 8
- **dBCG** – Disseminated BCG
- **DAIDS** – Division of Acquired Immunodeficiency Syndrome
- **DNA** – Deoxyribonucleic acid
- **DSMB** – Data Safety and Monitoring Board now also called Data Monitoring Committee (DMC)
- **DPT** – Diphtheria Pertussis Tetanus
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<tr>
<td>eMTCT</td>
<td>Elimination of Mother to child transmission of HIV-1</td>
</tr>
<tr>
<td>FBC</td>
<td>Full blood count</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon Gamma</td>
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<tr>
<td>IL-x</td>
<td>Interleukin (x refers to number)</td>
</tr>
<tr>
<td>HE</td>
<td>HIV exposed</td>
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<tr>
<td>HEU</td>
<td>HIV Exposed Uninfected</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>LMIC</td>
<td>Low and Middle Income Country</td>
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<td>MTCT</td>
<td>Mother To Child Transmission of HIV-1</td>
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<td>NDA</td>
<td>National Drug Authority</td>
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<tr>
<td>NK</td>
<td>Natural Killer</td>
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<tr>
<td>NSE</td>
<td>Non Specific Effect</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PPD</td>
<td>Purified Protein Derivative</td>
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<tr>
<td>SAE</td>
<td>Severe Adverse Events</td>
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<td>SAGE</td>
<td>Strategic Advisory Group of Experts</td>
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<td>SoMREC</td>
<td>School of Medicine Research and Ethics Board</td>
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<td>TB</td>
<td>Tuberculosis</td>
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<td>TNF α</td>
<td>Tumor Necrosis Factor alpha</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
References


25. SAGE Working Group on non-specific effects of vaccines (March 2013 - June 2013) [http://www.who.int/immunization/sage/sage_wg_non_specific_effects_vaccines_march2013/en/]


04 August 2017 Version for external consultation
38. The 2014 WHO verbal autopsy instrument
Table 1: Time schedule for study procedures

<table>
<thead>
<tr>
<th></th>
<th>Maternity Screening</th>
<th>Postnatal Screening (≤24 h of birth)</th>
<th>W 2</th>
<th>W 6</th>
<th>W 10</th>
<th>W 14</th>
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<td><strong>Maternal Lab tests</strong></td>
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<td>Eligibility of infant</td>
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<td>Randomization</td>
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<td>Clinical assessment, AE, SAE &amp; anthropometry</td>
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<td><strong>Lab tests on infants:</strong></td>
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<td>Subsample: Blood cells stimulated with MTB/BCG, PPD, E. coli, S. aureus and C. albicans antigens</td>
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*a Mother or caregiver interview including a focus on TB history in household members;  
b Procedure conducted on cord blood specimens

Table 3: Economic evaluation data requirements and sources

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
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<tbody>
<tr>
<td><strong>Epidemiology</strong></td>
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<tr>
<td>Age specific incidence of NSE</td>
<td>Primary data (for each condition)</td>
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<td>Background mortality</td>
<td>Secondary data (national life table)</td>
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<td>Disease specific CFRs for NSE</td>
<td>Primary data</td>
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<td><strong>Effectiveness</strong></td>
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<tr>
<td>Disease specific efficacy NSE</td>
<td>Primary data</td>
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<tr>
<td>Uptake of intervention</td>
<td>Secondary data (effective coverage of national vaccine program)</td>
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<td><strong>Aggregate health</strong></td>
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<td>Years of life lost</td>
<td>Secondary data (national life table)</td>
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<td>Disease weights</td>
<td>Secondary data (Burden of disease study)</td>
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<td><strong>Costs</strong></td>
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<td>Intervention costs</td>
<td>Primary data (prospectively costed)</td>
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<td>Treatment costs NSE</td>
<td>Primary data (retrospectively costed)</td>
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Figure 1: BCG trial participant screening, enrolment and follow-up

Prenatal Assessment
(Antenatal care clinics starting at 28 weeks of gestation)
1. Weeks of gestation
2. Signs/ symptoms of TB
3. Antenatal screening consent

Postnatal Assessment
(Postnatal unit, ≤24 hours after birth)
Assessment of the mother and the child for
1. Inclusion criteria
2. Exclusion criteria

Enrolment and Randomization
(Postnatal unit/study clinic ≤24 hours after birth)

Follow up to one year of age
(Study clinic)
Figure 2: Flow chart of study participants

- Approached in study sites
- Recruited pregnant mothers
  - Not eligible
  - Refusals
- Enrolled mother-infant pairs
  - Stillbirths
  - Maternal death
  - Infant deaths
  - Twins
  - Other
  - Total
- Intervention arm
  - Follow up 14 weeks
    - Lost to follow-up
  - Follow up 28 weeks
    - Lost to follow-up
  - Follow up 52 weeks
    - Excluded from analysis
    - Analyzed
- Control arm
  - Follow up 14 weeks
    - Lost to follow-up
  - Follow up 28 weeks
    - Lost to follow-up
  - Follow up 52 weeks
    - Excluded from analysis
    - Analyzed
Figure 3: The decision model