Bacillus Calmette-Guérin immunisation at birth and morbidity among Danish children
A prospective, randomised, clinical trial

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Published in:
Contemporary Clinical Trials

DOI:
10.1016/j.cct.2015.04.006

Publication date:
2015

Document version
Publisher’s PDF, also known as Version of record

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Citation for published version (APA):
Background: Studies from low-income countries report positive non-specific effects of early Bacillus Calmette-Guérin (BCG) immunisation on childhood health and survival. Neonatal immunisation with BCG may prime the immune system and offer partial protection against other infectious and possibly allergic diseases. The potential clinical value of these non-specific effects has not yet been examined in a large randomised trial in high-income countries.

Methods: The Danish Calmette Study is a multicentre randomised clinical trial conducted between October 2012 and November 2015. Within the first 7 days of life, infants were randomly assigned to intra-dermal vaccination with BCG or no intervention. At 3 and 13 months of age structured telephone interviews and clinical examinations of the children were conducted. In a subgroup of children blood samples were drawn and stool samples collected at age 4 days, 3 and 13 months. Thymus index was assessed by ultrasound in a subgroup at randomisation and at 3 months. The primary study outcome is hospitalisation within the first 15 months of life as assessed in Danish health registers. Secondary outcomes include infectious disease hospitalisations, wheezing, eczema, use of prescribed medication, growth, development, thymus index, T- and B-cell subpopulations assessed by flow cytometry, in vitro cytokine responses and specific antibody responses to other vaccines. Adverse reactions were registered.
1. Introduction

The Bacillus Calmette-Guérin vaccine (BCG) is administered to infants in more than 100 countries to protect against the most severe forms of tuberculosis [1]. Due to a declining incidence of tuberculosis, BCG was withdrawn from the Danish Childhood Immunisation Program in the 1980s.

Many observational studies from low-income settings have reported positive non-specific effects of neonatal BCG immunisation on childhood health and survival [2–5]. One study from West Africa found reduced sensitisation to allergens among BCG-immunised children [5]. Recently, two randomised trials among low-birth-weight children observed a significantly lower mortality in the group BCG-immunised at birth than those who received the usual delayed BCG [6,7]. Morbidity and mortality due to infectious diseases in early infancy are high in West Africa while tuberculosis is very uncommon in infancy. Therefore, a protective mechanism has to be non-specific.

During the past three decades eczema and asthma have become the most common chronic diseases among children in high-income countries [8]. Research suggests that a skewed immunologic stimulation, possibly caused by increased hygiene and fewer infections, may be part of the explanation for the increased prevalence of these diseases [9]. BCG strongly stimulates the immune system, resulting in an interferon gamma (IFN-γ) response [10] that may counterbalance the Th helper-cell (Th)-2 response found in atopic individuals. It has therefore been hypothesised that BCG could have a protective effect on atopic diseases, but results from several retrospective studies have been inconsistent [11–14]. According to a meta-analysis from 2014 BCG vaccination might have exerted a protective effect against non-atopic asthma, although it did not protect against allergic sensitisation [15]. Apart from a small randomised trial from the Netherlands [16], no randomised trials of BCG’s effect on allergic diseases have been conducted. The Dutch trial found significantly reduced use of medication for eczema among the BCG-immunised children [16].

In the present trial we aimed to test the effect of BCG on hospitalisation and morbidity including allergic diseases in high-income settings in a large clinical trial with patient-relevant outcomes. Our main hypothesis was that neonatal BCG vaccination would be associated with a 20% reduction in hospitalisation during the first 15 months of life.

2. Methods/design

The Danish Calmette Study is a multicentre randomised clinical trial with 1:1 allocation of newborns within 7 days of birth to either BCG vaccination or no intervention. The inclusion criteria were gestational age of at least 32 weeks, a birth weight of at least 1000 g, and a signed consent form from the parents. Exclusion criteria were maternal intake of immune modulating medicine during pregnancy or signs of severe illness or major malformation in the newborn. Only parents fluent in Danish were included, as questionnaires in Danish were used.

3. Setting

From October 2012 to November 2013 all parents planning to give birth at Rigshospitalet, Hvidovre Hospital, and Kolding Hospital received a letter in the 2nd or 3rd trimester of pregnancy with information about the Danish Calmette Study and an invitation to participate in the study. Invited parents were contacted by phone and offered further information either on the phone or, on request, at a personal meeting. We obtained ethical permission to inform the women by phone.

4. Interventions

Within 7 days of birth participants were randomised to either the intervention group (BCG immunisation) or to the control group using an electronic case report form (e-crf). Randomisation was stratified by sex, hospital and gestational age (≥37 weeks of gestation versus <37 weeks). Block sizes were 2–4–6 in random order. Twins and triplets were allocated to the same randomisation group based on the sex of the first born child. The random allocation sequence was generated by the e-crf shortly before the vaccination and was concealed to all study staff until the intervention was assigned. Specially trained midwives and study health professionals at the three trial sites assigned participants to intervention and vaccinated children in the BCG group intra-dermally with BCG vaccine, SSI strain 1331, in the standard dose of 0.05 ml in the upper, lateral part of the left shoulder. The children randomised to the control group were not given a placebo vaccine and the parents were not blinded to the intervention because no other injection can mimic the typical reaction following a BCG vaccination.


Before randomisation of their child the parents received written and oral information about the most common adverse reactions after BCG vaccination (Table 1).

<table>
<thead>
<tr>
<th>Uncommon (≥1/1000, &lt;1/100)</th>
<th>Systemic: Headache, fever.</th>
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<tr>
<td></td>
<td>Local: Enlargement of regional lymph node &gt;1 cm. Ulceration with a discharging ulcer at the site of injection.</td>
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<tr>
<td>Rare (&lt;1/1000)</td>
<td>Systemic: Disseminated BCG complications as osteitis or osteomyelitis. Allergic reactions, including anaphylactic reactions.</td>
</tr>
<tr>
<td></td>
<td>Local: Suppurative lymphadenitis, abscess formation.</td>
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</tbody>
</table>
In case of suspected adverse reactions other than the expected redness, swelling, skin sore, swollen regional lymph nodes and a scar, the parents were instructed to contact the study staff as soon as possible, so the case could be reported and medical advice be given and, if needed, the child referred to clinical control and treatment. The Data Safety and Monitoring Board (DSMB) of the Danish Calmette Study regularly evaluated the patterns of adverse reactions.

5. Outcomes

The primary outcome of the Danish Calmette Study is hospitalisation in the first 15 months of life. The secondary outcomes include infectious disease hospitalisations, atopic dermatitis, asthmatic bronchitis, use of prescribed medication, development, anthropometrics measured at 3 and 13 months, immunological outcomes (T- and B-cell subpopulations assessed by flow cytometry and cytokine production in stimulated whole blood) measured at 4 days of age, 3 and 13 months and thymus volume as measured by ultrasound scanning (thymus index) and the ratio between thymus volume and infant body weight (thymus/weight index) measured at inclusion and at 3 months. We also investigated which factors affect parents' decision concerning participation in the Danish Calmette Study and whether their experience with the BCG vaccination influenced their attitude towards future vaccinations.

6. Data collection

6.1. Background information

A structured telephone interview was conducted prior to randomisation focusing on demographics and risk factors such as atopic disposition and parental smoking habits. All telephone interviews, including the later mentioned follow-up interviews, were performed by specially trained study staff. All members of the study staff had a medical background as either doctors, nurses, midwives, medical students or midwife students.

6.2. Registry-based data

Hospitalisations were identified using the Danish National Patient Registry [17]. All Danish citizens have a unique social security number (CPR) that can be used to link data at an individual level. The Danish Register of Medicinal Product Statistics contains CPR-number-based information about sale of all prescribed medicines in Denmark. The included children were followed in these registries until 15 months of age for hospitalisations, total medication use, including use of antibiotics, anti-asthmatics, and topical medication against eczema.

6.3. Clinical and paraclinical data

6.3.1. At inclusion

Thymic scans: At the time of randomisation, just before vaccination, thymus index (a volume estimate) and thymus/weight index (the ratio between thymus volume and infant body weight) were assessed using a transsternal ultrasonic method [18] on a subpopulation of 300 children (thymus cohort), including the 150 children who delivered a blood sample (see below).

6.3.2. 4-day follow-up

Blood samples: At Hvidovre Hospital blood samples were obtained from 150 children (blood sample cohort), 75 children from each randomisation group, 4 days after randomisation. The samples were used for immunological analyses, including total leucocyte count, assessment of T- and B-cell subpopulations (recent thymic emigrants, naïve cells, central memory cells, effector memory cells, late differentiated cells, Th17 cells, Tc17 cells, regulatory B-cells, regulatory T-cells, chronic activated cells, apopotic cells) measured by six-colour flow cytometry in whole blood and examination of the production of cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IFN-γ, IL-10, IL-17, IL-22) using whole blood stimulated in vitro with other pathogens, RPMI (medium alone), lipopolysaccharide (LPS), phytohaemagglutinin (PHA), Escherichia coli (E. coli), Streptococcus pneumonia (S. pneu), Candida albicans (C. alb), and BCG. Stool samples for microbiome analyses were obtained from a subset of participants in the blood sample cohort.

6.3.3. 3-month follow-up

Structured telephone interviews were conducted at age 3 months to obtain parent-reported data on illness episodes, vaccinations, anthropometrics and nutrition. At the end of the telephone interview the e-crf revealed a few supplementary questions to parents of the BCG-vaccinated children about the reaction at the vaccination site and the information received about adverse reactions. All children were invited for a clinical examination at the study facility.

The clinical examination was conducted by specially trained study staff (doctors and nurses) and included: anthropometrics (weight, height and head circumference), heart and lung auscultation, and examination for eczema and wheeze. In case of atopic dermatitis, the Scoring Atopic Dermatitis (SCORAD) form was used [19]. Parents were instructed not to reveal the randomisation group of their child when interviewed on the phone and when they attended the clinical examination. Before performing the clinical examination we asked the parents to cover the typical site of vaccination using a plaster. At Hvidovre Hospital blood samples were drawn from the blood sample cohort and the immunological analyses were repeated. Stool samples were obtained from a subset of participants in the blood sample cohort and thymus index and thymus/weight index were assessed in the thymus cohort.

6.3.4. 13-month follow-up

The structured telephone interview from the 3-month follow-up was repeated, supplemented with questions concerning the use of healthcare services. To measure the children's psychomotor development, their parents were asked to fill out a standardised, validated online Ages and Stages Questionnaire (ASQ) [20]. The children were invited for a clinical examination.

The clinical examination assessed the same outcomes as at 3 months of age, supplemented with measuring of skin folds, abdominal circumference and mid upper arm circumference (MUAC). At the very end of the last clinical examination the
length and width of the BCG scar was measured after the
parents had removed the plaster.

At Hvidovre Hospital blood samples were drawn from
the blood sample cohort and in addition from 150 other
children, thus providing 300 blood samples, 150 from each
randomisation group. The immunological analyses were
repeated, supplemented by analyses for specific antibody
responses to other vaccines (Diphtheria, tetanus, Bordetella
pertussis, Haemophilus influenzae type b and S. pneu) and
analyses for allergic sensitisation (specific immunoglobulin
E). Stool samples were obtained from a subset of partici-
pants in the blood sample cohort. All children included at
Kolding Hospital were invited to have a blood sample drawn
for analysis for allergic sensitisation.

6.3.5. Data collection specifically for premature children

As a safety measure we followed the premature children
(born at gestational age 32 weeks + 0 days to 36 weeks
+ 6 days) more intensively. Psychomotor development at
6, 13 and 22 months after birth was assessed by means of
ASQ.

7. Sample size consideration, power calculation

At least 20% of the child population in Denmark are
hospitalised during the first year of life [21]. Sample size
estimates were based on 95%-confidence intervals and 90%
power. To detect a 20% protection against hospitalisation
during the first 15 months of life, 3972 infants needed to
be included (1986 BCG-immunised and 1986 non-BCG-
immunised). With an incidence of 5% of eczema in the child
population below 15 months of age, it was necessary to
include 4230 infants (2115 in each group) to be able to
detect a 40% protection against eczema. We therefore aimed
at including 4300 newborns. Sub-analyses on sub-groups of
participants or less frequent events may not be adequately
powered.

8. Monitoring and rules of termination

The trial was supervised by the Good Clinical Practice Units
in the Capital Region and in the Region of Southern Denmark.
The study was monitored by an independent DSMB with three
members who evaluated the study status, patterns of adverse
reactions, and the distribution of background information and
outcomes in the two groups of randomisation. The trial was to
be terminated if BCG had a significantly negative effect
(p < 0.01) or a significantly positive effect (p < 0.001) on
physician consultations as obtained through the interviews
and/or on parental report of eczema.

9. Statistical methods

Comprehensive statistical analysis plans were developed for
the primary as well as for secondary outcomes; these plans
were deposited with the DSMB before the analyses were commenced.
Data were analysed by “intention-to-treat” and “per protocol”
and adjusted for the stratified randomisation design. The “per protocol”-analyses excluded children, who did not follow their
randomisation groups. Thus, both children randomised to BCG-
vaccination but not BCG-vaccinated, and children randomised to
no BCG-vaccination but BCG-vaccinated outside the Calmette Study were excluded from the “per protocol”-analyses. We
analysed outcomes in Cox proportional hazards model, so
hospitalisations occurring before the date of enrolment into the
trial were ignored. Children were followed until 15 months of
age, corresponding to the age of MMR immunisation (mumps,
measles and rubella) according to the Danish Childhood
Immunisation Program.

The possible effect of BCG was analysed overall from 0 to
15 months, but also analysed corresponding to the periods
when childhood vaccines are given during the first year of life:
at 3, 5 and 12 months of age. At these time points Danish
children are due to receive the pentavalent vaccine (against
diphtheria, tetanus, Bordetella pertussis, polio and Haemophilus
influenzae type B) and the pneumococcal conjugated vaccine
(vaccine against 13 strains of S. pneu). Hence, it is possible to
test if the potential BCG effect is modified by subsequent
vaccines. We hypothesise the effect of BCG immunisation to be
most pronounced in the first 3 months of life. The randomised
trials of BCG from West Africa [6,7] have shown that the
positive effects of BCG in boys occur already in the first week
of life while they occur later for girls (authors’ unpublished
data). Thus, we tested the possible effects of BCG and their
timing separately for boys and girls. P-values less than 5%
were considered statistically significant and all tests were
two-sided.

10. Registration number

The trial was approved by the Danish Data Protection Board
(J.no. 2009-41-4141), the Committees on Biomedical Research
Ethics (J.no. H-3-2010-087), and the Danish Medicines Agency
(J.no. 2612-4356. EudraCT 2010-021979-85. Protocol 2009-
323). The study was registered at www.ClinicalTrials.gov with
trial registration number NCT01694108.

11. Economy

The study was initiated and planned by the researchers and
funded by the three involved hospitals and by the Danish
National Research Foundation (DNRF108). Supplementary
funding was granted by other non-commercial foundations.
The BCG vaccine was produced by Statens Serum Institut,
Copenhagen, Denmark, and bought by the Danish Calmette
Study. The study participants were insured by the Patient
Insurance Act. There was no remuneration for participation in
the Danish Calmette Study.

12. Steering Group

The Steering Group consists of representatives from the three
inclusion sites, from Research Centre for Vitamins and Vaccines
and from Bandim Health Project. The Steering Group is
responsible for the data and blood samples collected.

13. Discussion

During 14 months in 2012–2013 a total of 4184 families
were included in the Danish Calmette study. The crude
adherences at the clinical examination at 3 and 13 months of
age were above 93%. More than 85% completed the ASQ
questionnaire at 13 months of age. Since the Danish health registers are population based, the registry-based follow-up included 100% of the study population (Fig. 1).

Collecting the main outcome data from national registries reduces the risk of bias as data were registered by people outside the study group, independent of the exposure.

If BCG vaccination at birth can reduce early childhood morbidity in high-income settings, this may have significant public health and socio-economic impact, both by improving the quality of life for the infants and their families and by reducing healthcare costs.

13.1. Trial status

The Danish Calmette Study is a large multicenter randomised clinical trial in Denmark with more than 4000 participants and an adherence at 1 year follow-up of more than 93%. The recruitment stopped November 2013 and the clinical follow-up ended January 2015. Data from Danish health registers were obtained in 2015.

13.2. Competing interests

We declare that we have no conflict of interests.
13.3. Authors’ contributions

LGS conceived the idea of the Danish Calmette Study. The Steering Group of the Danish Calmette Study (LGS, PA, CSB, GC, OP and PEK) wrote the Danish protocol of the study with statistical contributions from HR and with later additions from DLJ, LMT, TNN, JK, GTP and NMB participated substantially in the data collection and in the revision of the protocol. The drafting of this manuscript was led by LMT, with major contributions from the other authors. All authors read and approved the final manuscript.

Acknowledgements

We thank the many families for their participation and loyalty, and the Calmette study staff for their continuing commitment. We thank the DSMB-members Frank Shann, Professor of Paediatric Intensive Care Medicine, Melbourne; Per Kragh Andersen, Professor of Biostatistics, Copenhagen; and Jørn Olsen, Professor of Epidemiology, Aarhus (chair).

References


