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Neonatal BCG vaccination and infections in the first year of life: the MIS BAIR randomised controlled trial

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Summary: In an RCT in a high-income setting (with neonatal Hepatitis B vaccination), infants randomised to neonatal BCG vaccination had a small, -3.2 (95%CI -9.0 to 2.6), reduction in the risk of respiratory tract infection in the first year of life.

ABSTRACT

Background: Bacille Calmette-Guérin (BCG) vaccination has beneficial off-target effects that may include protecting against non-mycobacterial infectious diseases. We aimed to determine whether neonatal BCG vaccination reduces lower respiratory tract infections (LRTI) in infants in the MIS BAIR trial.

Methods: In this investigator-blinded trial, neonates in Australia were randomised to receive BCG-Denmark vaccination or no BCG at birth. Episodes of LRTI were determined by symptoms reported in parent-completed 3-monthly questionnaires over the first year of life. Data were analysed by intention-to-treat using binary regression. Clinicaltrials.gov (NCT01906853).

Results: From August 2013 to September 2016, 1272 neonates were randomised to the BCG vaccination (n=637) or control (n=635) group. The proportion of participants with an episode of LRTI in the first year of life among BCG-vaccinated infants was 54.8% compared to 58.0% in the control group, resulting in a risk difference of -3.2 (95% CI -9.0 to 2.6) after multiple imputation. There was no interaction observed between the primary outcome and sex, maternal BCG or the other pre-specified effect modifiers.

Conclusions: Based on the findings of this trial, there is insufficient evidence to support the use of neonatal BCG vaccination to prevent LRTI in the first year of life in high-income settings.

Keywords: infant, BCG, infection, off-target, non-specific, heterologous

INTRODUCTION

Bacille Calmette–Guérin (BCG) is a live-attenuated vaccine given to over 120 million infants worldwide annually. In addition to protecting against tuberculosis (TB) and other mycobacterial infections, BCG has beneficial off-target (heterologous, non-specific) effects [1, 2]. A recent WHO-commissioned meta-analysis concluded that BCG vaccination reduces all-cause mortality in children under 5-years of age by 30-53% [3]. This effect was most evident for infants in high-mortality settings. However, subsequent studies have reported inconsistent effects [4, 5]. The protection afforded by BCG against infant mortality has been attributed to reduced deaths from infections other than TB, particularly respiratory tract infections (RTIs) and sepsis [5].

BCG-mediated protection against infections have been reported against a range of pathogens including bacteria, viruses, fungi and protozoa in animal models [6, 7]. In adults, BCG vaccination reduced yellow fever vaccine viremia in a human infection challenge model[8] and reduced RTIs in randomised controlled trials (RCTs) [9-11]. In children, BCG-mediated protection against RTIs and sepsis has been reported in retrospective studies [12, 13]. However, recent RCTs in infants in Uganda and Demark have found inconsistent effects of neonatal BCG vaccination on infant infectious diseases [14, 15].

In high-income countries, routine BCG vaccination is being increasingly discontinued due to low TB prevalence. However, this may have detrimental impacts on overall infant and child health if BCG vaccination protects against non-mycobacterial infections. With an established safety profile and the potential to provide protection against unrelated and even novel pathogens (such as SARS-CoV-2 [16]), neonatal BCG vaccination may provide a means to reduce the impact of infectious diseases in infants. We established the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS

BAIR), a multicentre RCT to determine whether, in a low-mortality setting, BCG vaccination reduces allergy, infections and asthma. Here we report the effect of neonatal BCG vaccination on infections in the first year of life.

METHODS

Study design and participants

The protocol for the MIS BAIR RCT has been published previously [17]. Briefly, from August 2013 to September 2016, pregnant women were recruited from four hospitals in Victoria, Australia. Inclusion criteria were healthy neonates with birth weight >1500 grams; gestational age ≥32 weeks, and an English-speaking parent. The exclusion criteria were any indication or contraindication for BCG vaccination [18], serious underlying illness or medical instability, skin infection or other skin condition, need for treatment with hepatitis B immunoglobulin, multiple birth of more than twins, or an older sibling in the study.

The MIS BAIR trial has ethical and governance approval from Royal Children's Hospital HREC (No. 33025) and Mercy Health Human Research Ethics Committee (HREC, No. R12-28) with additional governance approval from Barwon Health and St John of God, Geelong, Victoria. A parent of each participant gave written or electronic consent prior to randomisation. Parent(s) were informed of their option to withdraw from the trial at any time. The trial was registered at clinicaltrials.gov (NCT01906853) and was monitored by an independent data safety and monitoring committee.

Randomisation and Masking

Neonates were randomised during the first ten days of life to BCG vaccination or no BCG vaccination in a 1:1 ratio. In the case of twins, only the first twin of each pair was randomised and included in the outcome analyses, the second twin received the same intervention. Randomisation was stratified by recruitment site, mode of delivery and plurality of birth, using random permuted blocks of various sizes using the Research Electronic Data Capture (REDCap) platform randomisation function [19]. Parents were not blinded to group allocation as BCG vaccination results in the formation of a scar at the vaccination site. Follow-up staff and researchers involved in data analysis were blinded.

Due to global shortages in BCG-Denmark supplies during 2016, this trial was granted ethical approval to continue using a single batch of BCG-Denmark vaccine beyond the manufacturer-assigned use by date. Parents were informed at consent and, from June 2016 to September 2016, BCG vaccine viability was monitored [20]. There were 116 infants randomised in this time period, 57 to the BCG vaccination group.

Procedures

Within 24 hours of randomisation, neonates allocated to BCG vaccination group were injected with a single 0.05 mL intradermal dose of BCG-Denmark vaccine (Danish Strain 1331, Statens Serum Institute, Denmark) over the left deltoid muscle by trained study staff.

Neonatal hepatitis B (HepB) vaccination is included in the routine Australian vaccination schedule [21]. Participants in MIS BAIR received HepB vaccination in the first week of life as part of routine medical care.

At the time of recruitment, randomisation, and 3, 6, 9 and 12 months post randomisation, a web-based questionnaire was administered to parents using the REDCap platform. Questionnaires included questions on whether, within the preceding 3 months, infants had episodes of illness (defined as cough, cold or runny or blocked nose, wheeze, rattly chest or difficult breathing, fever, rash, vomiting or diarrhoea, or runny eyes or conjunctivitis). For each episode of illness, parents were asked to provide information on the symptoms, duration, age at onset, medical consultations, hospital admissions, diagnosis and any treatment. Information about vaccinations, medications or supplements, other diseases or disabilities, household composition, childcare attendance and overseas travel was also collected 3-monthly. In addition, infant infections, medications, neonatal intensive care admissions, special care nursery admissions and hospital re-admissions in the perinatal period, were obtained from the birth hospital records. Records of routine scheduled vaccinations were obtained from the Australian Immunisation Register.

Outcomes

The primary outcome was any episode of lower RTI (LRTI) in the first year of life. An LRTI was defined as any episode of illness with parent-reported symptoms of wheeze, rattle or rattly chest [22]. The secondary outcomes were: any episode of illness with symptoms of (i) diarrhoea with vomiting, (ii) rash with fever, and (iii) any infection; (iv) hospitalisation for RTI; (v) hospitalisation for any infection; and rate of episodes of (vi) LRTI, (vii) upper RTI (URTI), (viii) fever, and (ix) any infection (symptoms of wheeze, rattle or rattly chest, difficulty breathing, fever, runny nose, blocked nose, cough or

diarrhoea with vomiting). As studies suggest that the beneficial off-target effects of BCG may be mitigated by subsequent non-live vaccinations [3, 23, 24], the primary and secondary outcomes were analysed both over the first year of life and from birth until their first diphtheria-tetanus-acellular pertussis (DTPa) vaccination (or two months of age, whichever occurred earliest).

Based on previous studies of BCG vaccination and infections, the following subgroup analyses were prespecified: maternal history of BCG vaccination, sex, delivery mode, season of birth and HepB vaccination at randomisation (prior and up to 24 hours post randomisation; or more than 24 hours after randomisation, or no HepB vaccination). Rate was defined as the number of events per month of exposure, and prevalence as the proportion of participants with the event in the first year of life or until their first DTPa vaccination (or two months of age, whichever occurred earliest). All outcomes and analyses were prespecified in the statistical analysis plan, which was finalised and signed prior to unblinding of the data. Statistical analyses were done by an independent statistician who was not involved in the collection or preparation of the data.

Power calculation and sample size

Based on previously reported LRTI prevalence in Australian infants [22], 27% of participants were expected to have at least one episode of LRTI in the first year of life. To have 80% power to detect an absolute reduction of 7%, a sample size of 575 neonates in each group was needed. With the expectation that complete data would be available for 80% of participants, we aimed to recruit 1428 neonates.

Statistical analysis

For the primary and binary secondary outcomes, the difference between the two groups was estimated using binary regression. Results are presented as risk difference (RD) with 95% confidence interval (CI). For secondary outcomes measured as rates (cases/months at risk), the difference between groups was estimated using Poisson regression and is presented as incidence rate ratio (IRR) with 95% CI. All analyses were intention to treat and adjusted for mode of delivery (vaginal delivery or Caesarean section). For the primary outcome, results were estimated using both multiple imputation (50 datasets, chained equation, further details in Supplementary Methods and Supplementary Tables 1-2) and complete case analysis. Secondary outcome analyses were done as complete cases. All analyses were completed using Stat v16.1 (StataCorp LP, Texas, USA).

Role of the funding source

The study funders had no role in study design, data collection, analysis, interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Across the four hospitals, 8522 pregnant women were assessed for eligibility, of which 1640 were not eligible for inclusion and 5218 families declined participation (Figure 1). Of the 1396 enrolled, 66 declined participation after birth, 33 no longer met inclusion criteria and 11 were born after cessation of randomisation. The trial ceased randomisation in September 2016 due to lack of availability of BCG-Denmark [25] and low viability of the BCG vaccine used beyond the

manufacturer's assigned expiration date [20]. In total, from August 22, 2013 to September 6, 2016, 1272 neonates were randomised, with 637 in the BCG vaccination group and 635 in the control group (no BCG vaccination). Seven participants randomised to the BCG vaccination group did not receive BCG: five refused BCG vaccination, one withdrew from the trial and one could not be vaccinated within 24 hours of randomisation. Five participants randomised to the control group received BCG outside the trial.

Baseline characteristics were similar between the two allocation groups (Table 1 and Supplementary Table 3). Routine vaccination, environmental and household factors were also similar between participants in both groups (Tables 1 and Supplementary Table 3). Of the 168 infants who did not receive HepB vaccination prior or up to 24 hours post-randomisation, 91 did not have HepB vaccination during the neonatal period. The proportion of neonates with any family history of allergic and atopic disease (atopy, asthma, eczema, hay fever or secondary allergies) was high, with a family history of hay fever being the most common at over 60% in both groups (Table 1 and Supplementary Table 3).

Overall, 84.8% of participants answered the infection questions in all 4 questionnaires. The cumulative months at risk for the year was 14,034 months over the entire cohort, 1,860 of which were prior to the participants' first DTPa vaccination. There were 5,559 infectious illness episodes among 1212 (95.3%) participants in the first year of life; of these, 393 were prior to the participants' first DTPa vaccination.

In total, 135 participants (10.6%) had missing data on the primary outcome, 78 (12.3%) in the control group and 57 (9.0%) in the BCG vaccination group. Loss to follow-up was greater in the control group than in the BCG vaccination group, 51 (8.0%) participants versus 31 (4.9%).

One-year post randomisation, 54.8% of BCG-vaccinated participants had at least 1 episode of parent-reported LRTI compared to 58.0% in the control group (Table 2), a difference of -3.2% (95% CI -9.0 to 2.6). Using complete case analysis, the LRTI proportion was 55.3% (321 participants) in BCG group and 58.3% 325 participants) in the control group (RD: -3.0%, 95%CI -8.7 to 2.8) (Table 2, Figure 2-3, Supplementary Table 4). Therefore, the number needed to treat to prevent one infant from having an episode of LRTI in the first year of life is 31.3.

The RD and IRR for BCG-vaccinated compared to no BCG vaccination participants are presented in Figure 2 and Supplementary tables 5-6. Few children had non-respiratory tract infections prior to their first DTPa vaccination (Figure 3). There was increased risk of diarrhoea with vomiting for BCG vaccinated infants compared to controls at 12 months of age (RD 5.1%; 95% CI 0.0% to 10.2%) but this was not observed prior to first DTPa (RD -0.4%; 95% CI -1.3% to 0.65). The prevalence of hospitalisations for infections was low both prior to DTPa and by 12 months of age (Figure 3). Prior to DTPa vaccination, BCG-vaccinated participants had a lower prevalence for three binary secondary outcome measures (prevalence of LRTI, diarrhoea with vomiting and rash (with fever)) and lower rates of illness for all four continuous secondary outcome measures (rate of any infection, LRTI, URTI and fever) (Figure 2). In contrast, over the 12-month period, BCG-vaccinated participants had higher prevalence for three binary (prevalence of hospitalisations for infections, hospitalisations for RTI and diarrhoea with vomiting) and three continuous (rate of any infection, URTI and fever) secondary outcome measures (Figure 2).

There was no statistical evidence of an interaction for the prespecified potential effect modifiers on the primary outcome: maternal BCG vaccination status, delivery mode, sex, season of birth or timing of HepB vaccination (Table 3). Similarly, for the BCG vaccination group, there was no interaction between the primary outcome and development of a BCG scar or timing of BCG vaccination (Table 3).

DISCUSSION

In a high-income country with routine neonatal HepB vaccination, we did not find strong evidence that neonatal BCG vaccination reduces the prevalence of LRTI in the first year of life. This finding is consistent with the Calmette trial in Denmark, which also reported no reduction in infectious illness in infants up to 13 months of age [14]. However, there was a small and consistent effect of BCG in reducing all four measures of LRTI: namely the prevalence and rate by both 12 months of age and prior to first DTPa vaccine.

There was also a consistent reduction in most other measures of infectious illnesses prior to first DTPa, and an increase after first DTPa. This is in line with previous studies reporting early protective effects of neonatal BCG vaccination that are reduced or reversed after administration of a non-live vaccine. Recent RCTs of neonatal BCG vaccination in Denmark and Uganda reported decreased RRs in BCG-vaccinated infants prior to their first DTP-containing vaccination and increased RRs after these vaccinations [14, 15]. Similarly, several studies in high-mortality settings reported reduced all-cause infant mortality after BCG vaccination, but a loss of this protective effect after DTP [3]. Potential factors that may contribute to this include immunological changes induced by non-live vaccines [24, 26] and different causes of infection and mortality in the first months of life compared

to later in infancy [27]. In our trial, the mean age at first DTPa was 48 days, which provided only a short period of time in which outcomes measured prior to first DTPa could occur. This is in contrast to other high-income countries, such as Denmark, where DTPa is scheduled from 3 months of age [14]. Therefore, our results do not exclude a potential benefit from BCG prior to non-live vaccines as the outcomes measured in the narrow time frame prior to first DTPa in our trial are at risk of type II error due to the low power of rejecting the null hypotheses.

Factors that could contribute to differences in the findings between RCTs of BCG off-target effects against infections [8-11, 14, 15] in different settings include age- and population-related differences in immune responses, susceptibility to infection (e.g. due to low nutrition or immunosuppression), maternal BCG vaccination status (in infant studies), breastfeeding (in infant studies), maternal vaccination during pregnancy, previous BCG vaccination (in adult studies), hereditary factors (e.g. family history of allergic disease), environmental and pathogen exposures, and cause of death [28-33]. Vaccine-specific differences may also influence results including strain and viability of the BCG vaccine used [34, 35]. Factors associated with trial design may also contribute to these differences, for example participants in both MIS BAIR and the Calmette trial had high rates of family history of allergic or atopic disease (83% and greater than 64% respectively) [36, 37]. This is likely due to both studies assessing the effects of BCG vaccination on allergic and atopic disease [17, 38]. Infants of allergic parents and infants who develop allergy have altered immune cell function at birth [39]. Therefore, allergic predisposition among infants in these two trials may have impacted the effects of BCG. In our trial, infants received neonatal HepB vaccination as per the Australian guidelines. Given the evidence that administration of non-live vaccines after BCG vaccination may interfere with its beneficial off-target effects [3, 23, 24], it is plausible that HepB vaccination may have influenced the findings of this trial. There are currently two clinical trials underway (NCT02444611 and NCT03246230) investigating the interactions between BCG and HepB vaccination on infant

immunity. However, the lack of an observed interaction of HepB vaccination timing in the subgroup analysis and the consistency in findings between this trial and the Calmette trial (which did not include neonatal HepB vaccination) suggest that, in this setting, the timing of HepB vaccination did not alter the effects of BCG vaccination on infant infections.

In RCTs in high-mortality settings, BCG-mediated reductions in all-cause infant mortality are associated with reductions in infectious disease deaths, particularly RTIs and sepsis [5]. Our trial assessed infant hospitalisation for respiratory and other infections as a measure of severe infectious disease rather than all-cause mortality as infant mortality in Australia is low. Similarly, we did not report sepsis as a separate outcome (recorded as part of hospitalisation for any infection) as neonatal and infant sepsis are also uncommon in Australia. We did not observe a significant effect of BCG on our hospitalisation outcome measures. However, in the period prior to DTPa vaccination, where an effect of BCG may be more likely, the number of hospitalisations was minimal, thus the trial was insufficiently powered to detect any effects of BCG vaccination.

Immunological studies from participants in the MIS BAIR trial have revealed that, in contrast to adults, neonatal BCG vaccination reduces *in vitro* cytokine responses to a range of unrelated pathogens and Toll-like receptor agonists [40, 41]. It is plausible that neonatal BCG vaccination is protective in high-mortality settings by reducing detrimental effects of an overactive immune system such as the cytokine storm associated with sepsis, rather than boosting anti-pathogen immune responses. In this scenario, it would not be expected that BCG protects against mild-moderate infectious disease.

Despite the premature cessation of recruitment, our trial had high rates of follow-up and randomised a sufficient number of participants to detect the pre-specified effect on the primary outcome. For non-hospitalisation outcome measures, reporting of symptoms rather than doctor diagnosis enabled comparison across a range of illness severities regardless of medical attendance. A number of potential modifiers of the effect of BCG on LRTI prevalence were selected based on previously reported effects on susceptibility to infection, alterations in BCG-mediated protection from infections or all-cause mortality and immunomodulatory effects of BCG. We did not detect any interaction between the effect of BCG on LRTI in the first year of life and the pre-specified effect modifiers, including history of maternal BCG vaccination, sex, delivery mode, season of birth, and HepB vaccination at randomisation [3, 14, 15, 42-45]. However, this trial was powered for the primary outcome analysis, not subgroup analyses and therefore the findings of the interaction analysis should be considered as exploratory. There was a potential effect of maternal BCG vaccination on LRTI in infants independent of neonatal BCG vaccination. However, multiple potential confounding factors (such as children under school age and smoking in the household) were also associated with maternal BCG vaccination status, thus this observation requires investigation in future studies.

An unavoidable limitation of our trial was the inability to blind parents to the participant's BCG vaccination status. This appeared to influence parent questionnaire completion rate as there was higher loss to follow-up among participants who were randomised to no BCG vaccination (Figure 1). In addition, our sample size was based on an estimated LRTI prevalence of 27% in the non-intervention group requiring a risk difference of 7% for an estimated BCG effect size of 25%. However, the proportion of LRTI cases in the control group was 58.3%. The proportion and rate of LRTI reported in this study are consistent with previous studies in infants in Australia [22, 46]. However, for an estimated BCG effect size of 25%, this higher prevalence means the sample size was

sufficient to detect a minimum risk difference of 15%. For episodes of illness, in which participants were examined by a medical professional, parent-reported symptoms in questionnaires were often inconsistent with parent-reported doctor's diagnosis. While this discrepancy may be due to inaccurate recall or understanding of the doctor's diagnosis, it also suggests inaccurate recall or differences in interpretation of the symptoms used to define the trial outcomes [47, 48]. Although randomisation should mitigate this problem, the inability to blind parents to allocation means there may have been recall bias. However, we also observed no difference for hospitalisation outcomes (i.e. illness resulting in at least overnight admission to hospital), which are likely to be unaffected by this.

In conclusion, the small effect size observed in this trial does not justify the general use of neonatal BCG vaccination to prevent LRTI in infants in high-income settings. Larger trials are needed to more accurately assess the effect of BCG prior to the receipt of non-live vaccines.

Contributors

NC was the lead investigator and responsible for study conception, design and funding acquisition. NC, BF, SD and CZ developed the final scientific protocol and ethics application and all other authors provided critical evaluation and revision. KG co-ordinated and NC, DC, PV, CM and VA were involved in implementation. NLM developed and NC, KG, LFP, VA, NC, SD, FS, RR-B, KLFr, KLFl and MS contributed to the statistical analysis plan. NLM led and KLFr, SD, LFP and KG contributed to statistical analysis. NLM drafted the manuscript, coordinated manuscript preparation and revision. All authors provided critical evaluation and revision of the manuscript.

Declaration of interests

All authors declare no completing interests.

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Table 1. Baseline characteristics of MIS BAIR participants and characteristics at 12m by randomisation group

Baseline Characteristics	Randomised assignment n (%)	
	No vaccine n=635	BCG vaccine n=637
Maternal factors		
Maternal age (yrs) at delivery (n=1271) mean (SD)	32.7 (4.7)	32.6 (4.8)
Mother education level (n=1269)		
No education / up to year 10	34 (5.4%)	41 (6.5%)
Year 12 / trade	175 (27.6%)	165 (26.0%)
University	426 (67.1%)	428 (67.5%)
Maternal birth country (region) (n=1272)		
Australia	456 (71.8%)	467 (73.3%)
East & South East Asia	48 (7.6%)	37 (5.8%)
UK or Ireland	36 (5.7%)	34 (5.3%)
Other[a]	95 (15.0%)	99 (15.5%)
Maternal smoking during pregnancy (n=1269)		
No	615 (96.9%)	611 (96.4%)
Yes	20 (3.1%)	23 (3.6%)
Maternal BCG vaccination (n=1206)		
No	442 (73.5%)	446 (73.7%)
Yes	159 (26.5%)	159 (26.3%)
Maternal vaccinations during pregnancy (n=1272)		
dTpa	107 (16.9%)	103 (16.2%)
Influenza	134 (21.1%)	134 (21.0%)
Both: dTpa and Influenza	203 (32.0%)	217 (34.1%)
Neither: dTpa or Influenza	191 (30.1%)	183 (28.7%)

Mother GBS positive during pregnancy (n=1272)		
No	527 (83.0%)	547 (85.9%)
Yes	108 (17.0%)	90 (14.1%)
Rupture of membrane > 24hrs (n=1233)		
No	577 (93.7%)	565 (91.6%)
Yes	39 (6.3%)	52 (8.4%)
Paternal factors		
Paternal age at delivery (n=1239) mean (SD)	34.5 (5.5)	34.5 (5.6)
Paternal birth country (region) (n=1255)		
Australia	462 (73.9%)	463 (73.5%)
East & South East Asia	21 (3.4%)	31 (4.9%)
United Kingdom or Ireland	40 (6.4%)	43 (6.8%)
Other[a]	102 (16.1%)	93 (14.6%)
Infant factors		
Birthweight in grams (n=1272) mean (SD)	3399 (507)	3424 (485)
Participant's sex (n=1272)		
Female	312 (49.1%)	318 (49.9%)
Male	323 (50.9%)	319 (50.1%)
Grandparent's ethnicity (n=1272)		
Caucasian (3 or 4 grandparents)	475 (74.8%)	474 (74.4%)
Asian (3 or 4 grandparents)	39 (6.1%)	43 (6.8%)
Caucasian (2 grandparents) and Asian (2 grandparents)	30 (4.7%)	32 (5.0%)
Other	91 (14.3%)	88 (13.8%)
Plurality (n=1272)		
Singleton	625 (98.4%)	626 (98.3%)
Twin	10 (1.6%)	11 (1.7%)
Gestational age (weeks as decimal) (n=1272) mean (SD)	39.2 (1.4)	39.4 (1.4)

HepB vaccination prior to or up to 24hrs post randomisation (n=1271)		
No	73 (11.5%)	95 (14.9%)
Yes	562 (88.5%)	541 (85.1%)
SCN/NICU admission >24 hrs (admission prior to randomisation) (n=1268)		
No	581 (91.8%)	593 (93.4%)
Yes	52 (8.2%)	42 (6.6%)
Age at randomisation (hrs) (n=1272) mean (SD)	47.8 (45.2)	46.0 (41.3)
Birth factors		
Mode of delivery (n=1272)		
C-section	229 (36.1%)	231 (36.3%)
Vaginal	406 (63.9%)	406 (63.7%)
Season of birth (n=1272)		
Summer	137 (21.6%)	149 (23.4%)
Autumn	185 (29.1%)	177 (27.8%)
Winter	156 (24.6%)	158 (24.8%)
Spring	157 (24.7%)	153 (24.0%)
Familial or environmental factors		
Number of household habitants (n=1272)		
1	6 (0.9%)	10 (1.6%)
2	284 (44.7%)	291 (45.7%)
3	205 (32.3%)	186 (29.2%)
≥ 4	140 (22.1%)	150 (23.6%)
Number of household habitants under school age (n=1271)		
0	372 (58.6%)	384 (60.4%)
1	216 (34.0%)	201 (31.6%)
2	43 (6.8%)	47 (7.4%)
≥ 3	4 (0.6%)	4 (0.6%)

Number of household habitants of school age (n=1271)		
0	530 (83.5%)	531 (83.5%)
1	65 (10.2%)	62 (9.7%)
2	31 (4.9%)	33 (5.2%)
≥ 3	9 (1.4%)	10 (1.6%)
Any household habitants attending scheduled daycare for ≥3 days per week (n=1259)		
No	537 (85.5%)	533 (84.5%)
Yes	91 (14.5%)	98 (15.5%)
Smokers living in the house during pregnancy (n=1269)		
No	516 (81.5%)	531 (83.5%)
Yes	117 (18.5%)	105 (16.5%)
Family history of doctor diagnosed asthma[b] (n=1269)		
No	336 (53.0%)	318 (50.1%)
Yes	298 (47.0%)	317 (49.9%)
Family history of hay fever[b] (n=1270)		
No	227 (35.7%)	207 (32.6%)
Yes	408 (64.3%)	428 (67.4%)
Participant characteristic at 12 months		
Breastfeeding cessation by (n=1175)		
no breastfeeding	16 (2.8%)	13 (2.2%)
<1 week	16 (2.8%)	17 (2.8%)
1 week - 3 months	100 (17.5%)	107 (17.8%)
>3 - 6 months	68 (11.9%)	58 (9.6%)
>6 - 9 months	60 (10.5%)	66 (11.0%)
>9 - 12 months	85 (14.8%)	84 (14.0%)
>12 months	228 (39.8%)	257 (42.7%)

Daycare attendance during first year of life (n=1242)		
No	376 (61.2%)	409 (65.1%)
Yes	238 (38.8%)	219 (34.9%)
Smokers living in the house during first year of life (n=1147)		
No	482 (85.5%)	512 (87.8%)
Yes	82 (14.5%)	71 (12.2%)
Any travel overseas in first year of life (n=1148)		
No	448 (79.3%)	443 (76.0%)
Yes	117 (20.7%)	140 (24.0%)
Age (days) at first DTPa vaccination or 2m of age[c] mean (SD)	47.9 (5.9)	47.6 (6.0)
Any sibling/household member under school age children attending non-parent care		
No	400 (69.4%)	406 (70.1%)
Yes	176 (30.6%)	173 (29.9%)

[a] Data for each 'Other' region (Africa, Continental Europe, Middle East, North America, Oceania excluding Australia, South America, South Asia) presented in Supplementary Table 3[49]

[b]present or past history in at least one first degree relative of the participant (mother, father, full or half sibling).

[c]whichever occurred first

BCG, Bacille Calmette-Guérin; CI, confidence interval; DTPa, diphtheria-tetanus-acellular pertussis (infant dose); dTpa, diphtheria-tetanus-acellular pertussis (adult/booster dose); GBS, group B streptococcus; HepB, hepatitis B; hrs, hours; m, months; NICU neonatal intensive care unit; SCN, special care nursery; SD, standard deviation; yrs, years

Table 2

Primary Outcome Any episodes of LRTI by 12 months of age	No BCG	BCG	Measure of effect	[1]Adjusted estimate of effect (95% CI)	p-value
Multiple imputation (50 imputations, n=1272)	58.0%	54.8%	RD	-3.2 (-9.0 to 2.6)	0.28
Complete case analysis (n=1137)	58.3% (325/557)	55.3% (321/580)	RD	-3.0 (-8.7 to 2.8)	0.31

[1] adjusted for stratification mode of delivery

BCG, Bacille Calmette-Guérin; CI, confidence interval; LRTI, lower respiratory tract infection; RD risk difference

Table 3. Interaction analysis of potential effect modifiers.

Primary outcome: Intention to treat subgroup analysis	No vaccine n (%)	BCG vaccine n (%)	Interaction p-value
Mother history of BCG (n=1077)			
No maternal BCG vaccination (n=794)	239/389 (61.4%)	231/405 (57.0%)	ref
Maternal BCG vaccination (n=283)	72/138 (52.2%)	73/145 (50.3%)	0.74
Sex of child (n=1137)			
Female (n=554)	142/269 (52.8%)	145/285 (50.9%)	Ref
Male (n=583)	183/288 (63.5%)	176/295 (59.7%)	0.69
Delivery mode (n=1137)			
C-section (n=416)	129/207 (62.3%)	120/209 (57.4%)	Ref
Vaginal (n=721)	196/350 (56.0%)	201/371 (54.2%)	0.61
Season of birth (n=1137)			
Summer (n=251)	61/110 (51.3%)	69/132 (52.3%)	Ref
Autumn (n=315)	93/157 (59.2%)	91/158 (57.6%)	0.80

Winter (n=286)	74/140 (52.9%)	83/146 (56.8%)	0.75
Spring (n=285)	97/141 (68.8%)	78/144 (54.2%)	0.08
HepB vaccination (n=1137)			
>24hrs post randomisation or no neonatal HepB vaccination (n=158)	38/66 (57.6%)	52/92 (56.5%)	Ref
Prior or ≤24hrs post randomisation (n=979)	287/491 (58.5%)	269/488 (55.1%)	0.78
Intervention group only for primary outcome subgroup analysis			RD (95%CI) p value
Participant has BCG scar (by 12 months of age)			
No (n=28)		12/28 (42.9%)	ref
Yes (n=496)		272/496 (54.8%)	11.4% (-7.4% to 30.3%) p=0.24
BCG vaccination was given in the first 48hrs of life			
No (n=208)		114/208 (54.8%)	ref
Yes (n=372)		207/372 (55.6%)	1.5% (-7.1% to 10.1%) p=0.73

BCG, Bacille Calmette-Guérin; CI, confidence interval; HepB, hepatitis B vaccination; hrs, hours; RD, risk difference; ref, reference

Figure Legends

Figure 1. Consort diagram for the MIS BAIR infection outcomes.

Participants considered as having answered illness questions if the initial symptom question was answered in a given questionnaire. BCG, Bacille Calmette-Guérin.

Figure 2. The effect of BCG vaccination on infectious illness[a] in the first year of life.

a) Risk Difference (95% CI) of prevalence and b) incidence rate ratio (95% CI) of rate (events/month) in infectious disease in BCG-vaccinated (n=637) compared to control (no BCG vaccination, n=635) participants within the first 12-months of life (closed symbols) and prior to first DTPa vaccination (open symbols). Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; DTPa, diphtheria-tetanus-acellular pertussis; m, months; RTI, respiratory tract infection; LRTI, lower RTI; URTI, upper RTI

Figure 3. Infectious illnesses in the first year of life and prior to DTPa.

a) Prevalence (95% CI) and b) rate (events/months exposure) (95% CI) of infectious disease in BCG vaccinated (n=637) compared to control (no BCG vaccination, n=635) participants within the first 12-months of life and prior to first DTPa vaccination. Abbreviations: BCG, Bacille Calmette-Guérin; CI, confidence interval; DTPa, diphtheria-tetanus-acellular pertussis; RTI, respiratory tract infection; LRTI, lower RTI; URTI, upper RTI

Figure 1

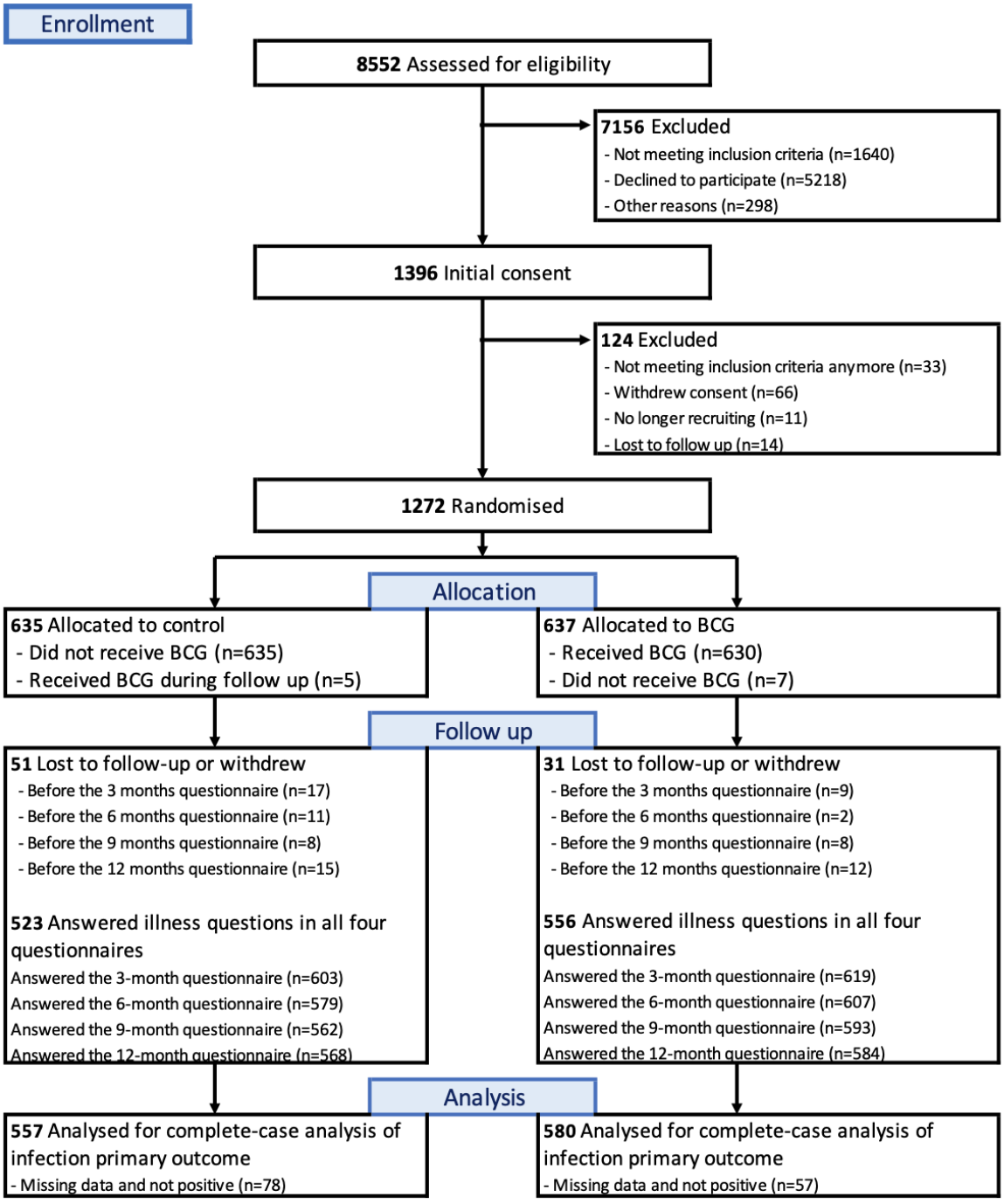

Figure 2

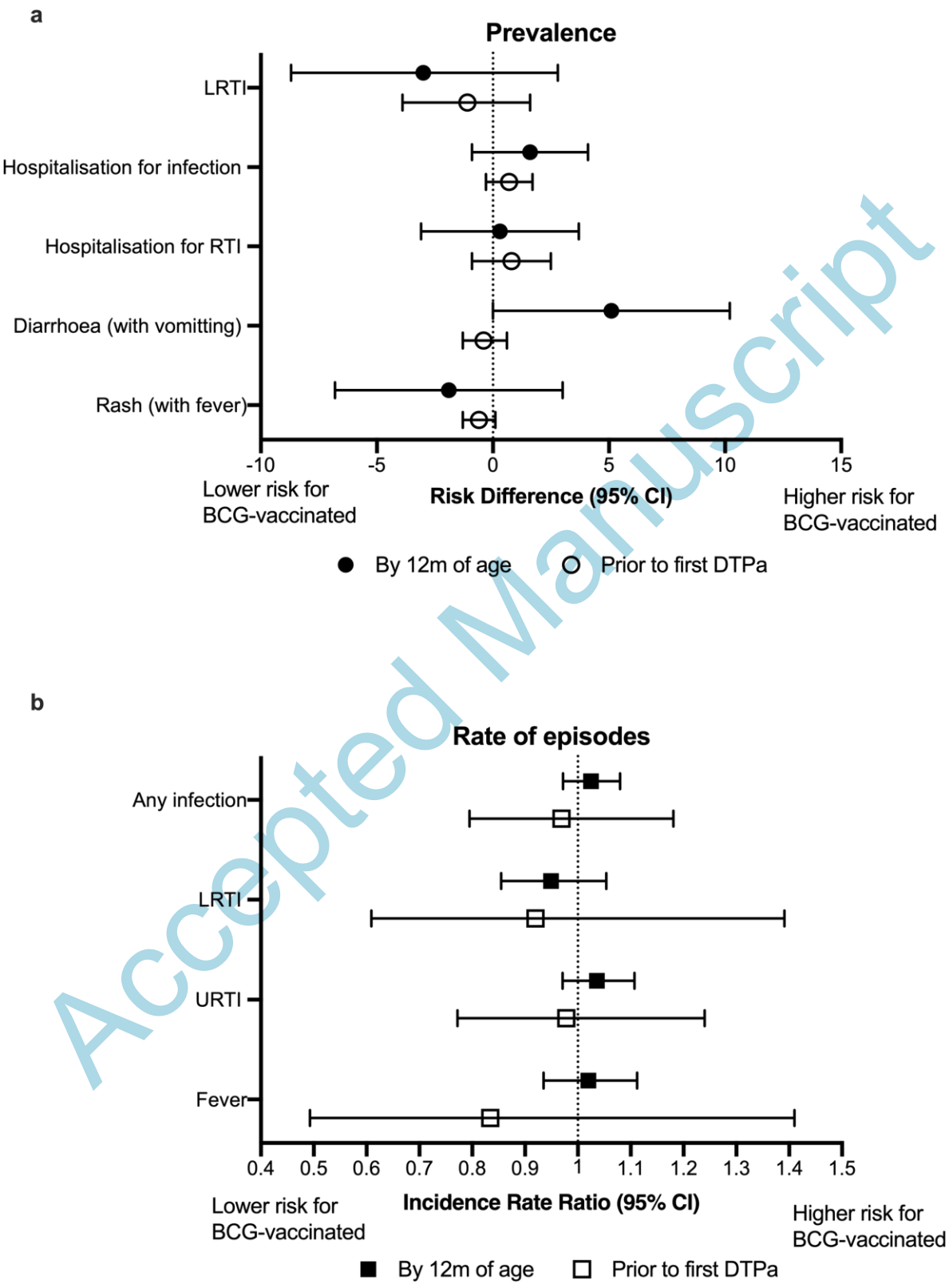

Figure 3