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Neonatal BCG vaccination has no effect on recurrent wheeze in the first year of life: A randomized clinical trial

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Background: Recurrent wheeze (RW) is frequent in childhood. Studies have suggested that BCG vaccination can have nonspecific effects, reducing general nontuberculosis morbidity, including respiratory tract infections and atopic diseases. The mechanisms behind these nonspecific effects of BCG are not fully understood, but a shift from a T_H2 to a T_H1 response has been suggested as a possible explanation.

Objective: We hypothesized that BCG at birth would reduce the cumulative incidence of RW during the first year of life.

Methods: The Danish Calmette Study is a multicenter randomized trial conducted from 2012-2015 at 3 Danish hospitals. The 4262 newborns of 4184 included mothers were randomized 1:1 to BCG (SSI strain 1331) or to a no-intervention control group within 7 days of birth; siblings were randomized together as one randomization unit. Exclusion criteria were gestational age of less than 32 weeks, birth weight of less than 1000 g, known immunodeficiency, or no Danish-speaking parent. Information was collected through telephone interviews and clinical examinations at 3 and 13 months of age; data collectors were blind to randomization group. RW was defined in several ways, with the main definition being physician-diagnosed and medically treated RW up to 13 months of age.

Results: By 13 months, 211 (10.0%) of 2100 children in the BCG group and 195 (9.4%) of 2071 children in the control group had received a diagnosis of RW from a medical doctor and received antiasthma treatment (relative risk, 1.07; 95% CI, 0.89-1.28). Supplementary analyses were made, including an analysis of baseline risk factors for development of RW.

Conclusion: Neonatal BCG had no effect on the development of RW before 13 months of age. (J Allergy Clin Immunol 2017;140:1616-21.)

Key words: *BCG, vaccination, infant, recurrent wheeze, heterologous immunity, nonspecific effects*

Especially during the winter season, recurrent wheeze (RW) is frequent in young children in high-income countries, where every third child less than 6 years of age has been reported to have asthma-like symptoms during the preceding winter.[1] A task force under the European Respiratory Society recommends the division of RW into "episodic viral wheeze," which is exclusively triggered by viral airway infections, and "multiple trigger wheeze," when the wheezing is also present between episodes of airway tract infections.[2] Thus RW is closely linked to airway infections but not necessarily to atopy and allergy[2,3]; in fact, differential effects of risk factors on infant wheeze and atopic dermatitis emphasize a different etiology.[4] However, the treatment used for RW is essentially the same as the symptomatic treatment used for asthmatic children because many of the symptoms are similar.[2]

BCG vaccine is recommended to prevent tuberculosis,[5] but as shown in 2 systematic reviews,[6,7] BCG has also been suggested as a protective measure against atopy. According to the hygiene

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Abbreviations used
MANCAS: Manchester Community Asthma Study
NSE: Nonspecific effect
RCT: Randomized clinical trial
RR: Risk ratio
RW: Recurrent wheeze

hypothesis,[8] skewed immunologic stimulation might explain some of the increase in the incidence of atopic diseases observed in recent decades. BCG has been shown to cause a strong immunologic stimulation, resulting in an IFN-γ response,[9] which could counterbalance the greater T_H2 response found in atopic subjects.[10] However, the picture is much more complicated than just a T_H1 response instead of a T_H2 response, and the exact mechanism of the BCG effect on a cellular level has been the subject of immunologic research in recent years. An English questionnaire–based study published in 2007 found neonatal BCG vaccination associated with a significant reduction in asthma symptoms in children aged 6 to 11 years.[11] However, an updated systematic review from 2014 found any protective effect of BCG against asthma likely to be transient.[7]

Randomized clinical trials (RCTs)[12,13] and observational studies[14-16] have supported that BCG can have beneficial nonspecific effects (NSEs) reducing all-cause morbidity and mortality. In West Africa early BCG vaccination of low-birth-weight infants decreased neonatal mortality, mainly by reducing the incidence of neonatal sepsis, respiratory tract infections, and fever.[12]

Since the beginning of the 1980s, BCG is no longer a part of the Danish Childhood Vaccination Program, and BCG vaccination is now only recommended for specific risk groups.

The Danish Calmette Study aimed to study NSEs of neonatal BCG vaccination in a high-income country. The RCT was powered to study hospital admissions as the primary outcome and atopic dermatitis as a secondary outcome.[17]

Based on the observed effect of BCG on respiratory tract infections and the link between respiratory tract infections and RW, we hypothesized that neonatal BCG would reduce the cumulative incidence of RW in the first year of life. The aim of the present study was to determine the effect of neonatal BCG on the secondary outcome of cumulative incidence of physician-diagnosed and medically treated RW up to 13 months of age.

METHODS

The Danish Calmette Study was an RCT conducted at 3 Danish hospitals. Newborns were enrolled from October 2012 to November 2013. Exclusion criteria were gestational age of less than 32 weeks, birth weight of less than 1000 g, known immunodeficiency, or no Danish-speaking parent. Within 7 days of birth, the newborns were allocated 1:1 to BCG vaccination (SSI strain 1331) or no intervention; in case of multiple births, siblings were randomized together as one randomization unit. The randomization was stratified by maturity. Before randomization, informed consent was obtained, and a structured telephone interview was conducted to collect data on demographics and atopic predisposition. Follow-up consisted of telephone interviews and clinical examinations at 3 and 13 months of age. The methods have been described in detail elsewhere.[17]

Outcome assessment

The main outcome of the present study was the cumulative incidence of physician-diagnosed and medically treated RW until 13 months of age, according to telephone interviews at 3 and 13 months of age. In telephone interviews parents were asked whether their child had ever had RW since birth (3-month interview) or since the last interview (13-month interview). If that was the case, they were asked about use of antiasthma treatment, duration of the treatment, and whether the diagnosis had been confirmed by a medical doctor. At both 3 and 13 months of age, the parents were asked whether their child also had wheezing in periods without respiratory tract infections. At 13 months of age, they were asked whether their child had been coughing at night in periods without respiratory tract infections and whether the child had exercise-induced dyspnea.

RW is defined by recurrent episodes of wheeze, and thus at least 2 episodes of wheeze must have been observed to use the diagnosis of RW. Generally, in Denmark the clinical diagnosis of RW is used for children with 3 or more episodes of wheezing. The Danish term for RW is "asthmatic bronchitis," a term primarily used by persons who have been in contact with the health care system.

At 3 and 13 months, the children were invited for a clinical examination at the study site, where study staff evaluated the child's breathing and made an auscultation. Telephone interviews were conducted by medical doctors, nurses, midwives, and medical students. Clinical examinations were conducted by medical doctors, specially trained nurses, and medical students.

Blinding

During telephone interviews and at clinical examinations, parents were instructed not to reveal the BCG vaccination status of the child to the study staff and to cover the vaccination site on the left shoulder with a plaster before the clinical examinations, irrespective of the child's randomization group.

Definition of atopic predisposition

A child was considered to have atopic predisposition if at least 1 first-degree relative (biological parent or full sibling) currently had or previously had 1 or more of the following atopic diseases with a diagnosis from a medical doctor: food allergy, atopic dermatitis, hay fever, or asthma. RW exclusively triggered by viral airway infections was not considered an atopic disease.

Definition of RW outcome

We defined RW as physician-diagnosed and medically treated RW based on confirmative answers given by the parents to both of the following 2 questions in the telephone interview at 13 months of age: "Has a doctor said that your child has (had) RW?" and "Has the RW been treated with anti-asthmatic treatment?" All types of antiasthma treatment were included: β_2-agonists, long-acting β-agonists, inhaled glucocorticoids, systemic glucocorticoids, and leukotriene receptor antagonists.

Objective signs of RW from the clinical examination were used as a secondary outcome.

The main definition of RW proposed in the analysis plan included all cases of parent-suspected RW, all RW diagnoses given by a medical doctor, and all signs of RW found at the clinical examinations of the Danish Calmette Study, including any rhonchus or prolonged expiration heard at auscultation.

The more specific definition of RW mentioned in the analysis plan was "clinically diagnosed RW," which was RW diagnosed by a medical doctor or by the Danish Calmette Study staff. A prior publication has documented a higher specificity of diagnosis-based than symptoms-based questions regarding asthma, allergic rhinitis, and conjunctivitis.[18] Although the present study looked for RW (and not asthma), the same might apply for RW. *Post hoc*, we decided to emphasize this more specific definition and further enhanced the specificity of the diagnosis by requiring both an RW diagnosis given by a medical doctor and treatment with antiasthma medication.

For comparison, results with respect to the broadly predefined diagnosis from the analysis plan are shown in Table E1 in this article's Online Repository at www.jacionline.org.

Handling of missing data

If a child did not participate in the 3-month follow-up, the telephone interview at 13 months of age covered the time since birth. In case of no known atopic predisposition but uncertainty about 1 or 2 subquestions (mostly whether the father had had AD), the child was coded as "no atopic predisposition." A sensitivity analysis, in which children with such uncertainty were excluded, did not change the main results (data not shown).

Statistical analyses

The statistical analysis plan was deposited with the Data and Safety Monitoring Board before the study was unblinded. The primary analysis was intention to treat. The cumulative incidences of RW in the 2 randomization groups were compared by using log-binomial regression, resulting in risk ratios (RRs) with 95% CIs. This statistical model assumes full follow-up for all participants, and therefore the main analyses of the present study were based on the 4,171 children (97.9% of the randomized children), who answered the questions about RW in the telephone interview at 13 months of age.

The randomization was stratified by maturity (≥37 weeks vs <37 weeks of gestation), and thus all results were adjusted for maturity.

There were 73 pairs of twins among the RW cohort of 4,171 children; because all siblings were randomized together as one randomization unit, both twins of the same mother received the same treatment. Supplementary cluster-adjusted analyses linking outcomes in twins were made, which derived almost identical SEs as the main analyses. Thus the results reported are without cluster adjustment.

Completion rate and distribution of background factors among the children lost to follow-up in the 2 allocation groups were compared by using the χ^2 test.

Subgroup analyses

Because previous studies of NSEs of BCG have emphasized children with atopic predisposition,[19] and shown differential effects by sex[12,20] and maternal immunity,[21] it was prespecified to conduct the analyses stratified by these subgroups. Specifically, because BCG was recommended for schoolchildren in Denmark until the late 1970s/early 1980s, some mothers had been BCG vaccinated but most had not, permitting an analysis of possible effect modification by maternal BCG vaccination status. Furthermore, we prespecified analyses for possible effect modification by factors previously found to be associated with the development of atopic diseases (ethnicity, number of siblings, pets at home, smoking, breast-feeding, and institutional child care).

A study reported that timing of BCG vaccination might be important, finding a stronger protective effect on atopy among children who were BCG vaccinated in the first week of life.[22] We prespecified an analysis stratified by age at randomization (days 0-1 vs days 2-7).

Sensitivity analyses

As a supplement to the main analysis of physician-diagnosed and medically treated RW, we conducted sensitivity analyses with different definitions of RW: parent-suspected RW, physician-diagnosed RW, and medically treated RW. We also tested whether the conclusions were similar at the 3 inclusion sites.

Risk factor analysis

For the risk factor analysis, the cumulative incidences of RW in the predefined subgroups of potential effect modifiers were compared by using log-binomial regression, resulting in RRs with 95% CIs.

P values of less than .05 were considered statistically significant, and all tests were 2-sided. STATA 14 software (StataCorp, College Station, Tex) was used for the analyses.

Sample size

The Danish Calmette Study was powered to detect an effect of BCG on all-cause hospitalizations and atopic dermatitis.[17] In the RW cohort of 4171 children, we had an incidence of RW of 9.7%. Thus we would be able to detect a 25% protection against RW with a power of 80% and an α value of 5%.

Ethics

The Danish Calmette Study was approved by the Danish Data Protection Board (2009-41-4141), the Committees on Biomedical Research Ethics (H-3-2010-087), and the Danish Medicines Agency (2612-4356, EudraCT 2010-021979-85, Protocol 2009-323). The study was registered at www.ClinicalTrials.gov (NCT01694108) and supervised by Good Clinical Practice Units and an independent Data and Safety Monitoring Board. All parents provided verbal and written consent to allow their child to participate in the trial.

RESULTS

From October 2012 to November 2013, 16,521 pregnant women were identified at the 3 recruitment sites, and 4,262 newborn infants born of 4,184 women were randomized.[17] Non-Danish ethnicity (18.0% vs 21.9%) and paternal smoking during pregnancy (18.5% vs 21.9%) were more frequent in the control group; otherwise, background factors were evenly distributed.[23] Atopic predisposition was reported for 63% of the enrolled families (2,639/4,184).

Of the 4,262 included children (2,129 BCG/2,133 control group), 4,192 (98.4%) were interviewed by telephone at 13 months of age, and of these, 4,171 (97.9% of the randomized children) answered the questions regarding RW, generating the cohort for the present study. Fewer children were lost to follow-up in the BCG group (n = 29; completion rate, 98.6%) compared with the control group (n = 62; completion rate, 97.1%; $P < .001$). Among the children lost to follow-up, there were no differences in background characteristics between the 2 allocation groups (data not shown). The median age at the last telephone interview was 375 days (10% to 90% percentiles: 370-390 days) in the BCG group and 375 days (10% to 90% percentiles: 371-392 days) in the control group.

BCG and RW

At 13 months of age, 211 (10.0%) of 2100 children in the BCG group and 195 (9.4%) of 2071 children in the control group had RW, which was diagnosed by a medical doctor and treated with antiasthma medication. Thus there was no difference between the 2 randomization groups in the risk of physician-diagnosed and medically treated RW in the first year of life, with the RR being 1.07 (95% CI, 0.89-1.28; Table I). The estimate was unchanged if we adjusted for the 2 factors that were unevenly distributed among the randomization groups (data not shown).

At 13 months of age, less than 20% of the children with physician-diagnosed and medically treated RW received treatment for at least 2 months (Table I). For this and the other measures of severity of RW, there was no significant difference in the effect of BCG between the 2 randomization groups (Table I); however, the risk of wheezing without having a cold tended to be higher among BCG-vaccinated children (RR, 1.38; 95% CI, 1.00-1.92).

Subgroup analyses

With the exception of number of siblings, we found no effect modification with BCG by the prespecified potential effect modifiers (Table I). BCG tended to increase the risk of RW among children without older siblings (Table I) but had no such effect in children with older siblings.

There was little variation in the age of vaccination, and the effect of BCG did not differ significantly between children vaccinated on days 0 to 1 versus days 2 to 7 (Fig 1). Three thousand one hundred

TABLE I. Physician-diagnosed and medically treated RW in the first year of life in relation to neonatal BCG vaccination and potential effect modifiers*

Parameter analyzed	BCG group, no. with RW/total no. (%)	Control group, no. with RW/total no. (%)	RR (95% CI) for BCG effect on RW	Test of no interaction (*P* value)
Primary outcome: intention-to-treat analysis: Physician-diagnosed and medically treated RW	211/2100 (10.0%)	195/2071 (9.4%)	1.07 (0.89-1.28)	
Different measures of severity of RW				
Antiasthma treatment for ≥2 mo†	57/2100 (2.7%)	49/2071 (2.4%)	1.15 (0.79-1.67)	
Symptoms between episodes with respiratory tract infections				
Wheezing without having a cold†	83/2100 (4.0%)	59/2071 (2.8%)	1.38 (1.00-1.92)	
Coughing at night without having a cold†	89/2100 (4.2%)	98/2071 (4.7%)	0.90 (0.68-1.19)	
Exercise-induced dyspnea without having a cold†	59/2100 (2.8%)	49/2071 (2.4%)	1.19 (0.82-1.72)	
Current use of prophylactic treatment				
Inhaled steroid‡	41/2044 (2.0%)	35/1946 (1.8%)	1.11 (0.71-1.74)	
Montelukast‡	3/2044 (0.1%)	5/1946 (0.3%)	0.57 (0.14-2.39)	
RW in relation to potential effect modifiers				
GA at birth				
Premature (GA 32+0 to 36+6; n = 133)	9/68 (13.2%)	6/65 (9.2%)	1.43 (0.54-3.80)	.55
Mature (GA ≥37+0; n = 4038)	202/2032 (9.9%)	189/2006 (9.4%)	1.06 (0.87-1.27)	
Sex				
Boys (n = 2199)	132/1093 (12.1%)	126/1106 (11.4%)	1.06 (0.84-1.33)	.87
Girls (n = 1972)	79/1007 (7.8%)	69/965 (7.2%)	1.10 (0.80-1.50)	
Atopic predisposition (missing: 73)				
With atopic predisposition (n = 2630)	142/1334 (10.6%)	124/1296 (9.6%)	1.11 (0.88-1.40)	.62
Without atopic predisposition (n = 1468)	68/727 (9.4%)	69/741 (9.3%)	1.01 (0.73-1.38)	
Maternal BCG status (missing: 51)				
Mother BCG vaccinated (n = 724)	28/366 (7.7%)	23/358 (6.4%)	1.19 (0.70-2.03)	.26
Mother not BCG vaccinated (n = 3396)	178/1712 (10.4%)	170/1684 (10.1%)	1.03 (0.84-1.26)	
Ethnicity (missing: 25)				
Non-Danish ethnicity (n = 824)	39/369 (10.6%)	31/455 (6.8%)	1.56 (0.99-2.45)	.06
Danish ethnicity (n = 3322)	172/1719 (10.0%)	164/1603 (10.2%)	0.98 (0.80-1.20)	
Siblings (missing: 2)				
At least 1 older sibling (n = 1724)	109/893 (12.2%)	116/831 (14.0%)	0.87 (0.68-1.11)	.03
No older siblings (n = 2445)	102/1207 (8.5%)	79/1238 (6.4%)	1.33 (1.00-1.76)	
Pets at home				
Yes (n = 1225)	77/638 (12.1%)	74/587 (12.6%)	0.96 (0.71-1.29)	.40
No (n = 2946)	134/1462 (9.2%)	121/1484 (8.2%)	1.12 (0.89-1.42)	
Smoking status by 13 mo of age (missing: 36)				
Ever smoked since birth of child (n = 1157)	68/563 (12.1%)	63/594 (10.6%)	1.14 (0.83-1.57)	.62
Never smoked since birth of child (n = 2978)	141/1519 (9.3%)	131/1459 (9.0%)	1.03 (0.82-1.30)	
Breast-feeding (missing: 7)				
Not fully breast-fed until 3 mo (n = 1801)	102/892 (11.4%)	91/909 (10.0%)	1.14 (0.88-1.49)	.50
Fully breast-fed until 3 mo of age (n = 2363)	109/1205 (9.0%)	104/1158 (9.0%)	1.01 (0.78-1.30)	
Started institutional child care before 13 mo of follow-up (missing: 1)				
Yes (n = 3584)	198/1820 (10.9%)	179/1764 (10.1%)	1.07 (0.89-1.30)	.62
No (n = 586)	13/280 (4.6%)	16/306 (5.2%)	0.89 (0.43-1.81)	

GA, Gestational age.
*Log-binomial regression analysis of the 4171 children who answered questions regarding RW in the telephone interview at 13 months of age (97.9% of randomized children).
†Based on information from the telephone interview at 13 months of age, in which 2100 children from the BCG group and 2071 children from the control group participated.
‡Based on information from the clinical examination at 13 months of age, in which 2044 children from the BCG group and 1946 children from the control group participated.

sixty (75.8%) of 4171 of the children were randomized on days 0 or 1 and 1011 (24.2%) of 4171 on days 2 to 7.

Sensitivity analyses

The sensitivity analyses with different definitions of RW yielded similar results (see Table E2 in this article's Online Repository at www.jacionline.org). Only very few children had RW before 3 months of age. In the telephone interview at 3 months of age, the cumulative incidence of physician-diagnosed and medically diagnosed RW was 7 (0.3%) of 2117 in the BCG group and 7 (0.3%) of 2108 in the control group (RR, 0.99; 95% CI, 0.35-2.83).

The results of the broad definition of RW mentioned in the analysis plan and reported in Table E1 are comparable with the main results in Table I: with the broad definition, there was no effect of BCG on the main outcome, and with the exception of number of siblings, we found no effect modification (see Table E1).

The cumulative incidence of RW differed between the 3 different inclusion sites, with a *P* value of less than .001 in the test of no interaction (cumulative incidence of RW at site 1: 9.2% [131/1417], cumulative incidence of RW at site 2: 13.3% [161/1211], and cumulative incidence of RW at site 3: 7.4% [114/1543]). However, the effect of BCG was the same across the 3 sites, with a *P* value of .83 in the test of no interaction

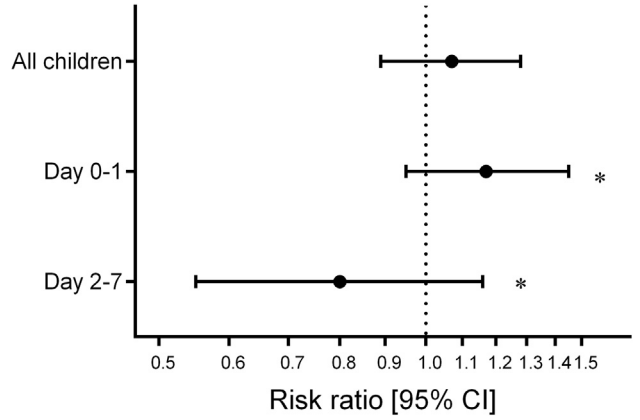

FIG 1. Physician-diagnosed and medically treated RW in first year of life in relation to age in days at randomization. Day 0 is the date of birth. Log-binomial regression analysis on the 4171 children for whom the questions regarding RW were answered in the telephone interview at 13 months of age was performed. *Test of same effect of BCG ($P = .08$).

(RR in BCG-vaccinated children at site 1: 1.06 [95% CI, 0.77-1.47], RR in BCG-vaccinated children at site 2: 0.97 [95% CI, 0.73-1.30], and RR in BCG-vaccinated children at site 3: 1.11 [95% CI, 0.78-1.59]).

Risk factor analysis

As previously shown, male sex, siblings, pets, parental smoking, and daycare attendance were associated with a higher risk of RW, and breast-feeding showed a tendency toward a lower risk of RW (see Table E3 in this article's Online Repository at www.jacionline.org). Children of mothers who were BCG vaccinated had a 31% (9% to 48%) lower risk of RW than children of BCG-unvaccinated mothers, but RW was not associated with prematurity, atopic predisposition, or ethnicity (see Table E3). In a multivariable analysis male sex, siblings, pets, parental smoking, breast-feeding, and daycare attendance were significantly associated with RW (see Table E3).

DISCUSSION

Randomization to neonatal BCG vaccination had no influence on the cumulative incidence of RW by age 13 months.

Comparison with other studies

The results of our RCT are comparable with those from a small Dutch RCT, which in 1999-2002 enrolled 121 children with atopic predisposition and randomized them to BCG or no BCG at 6 weeks of age.[19] They found no effect of BCG against asthma-related symptoms until 18 months of age. However, because of its size, the Dutch trial would only have captured very large effects.[19] In contrast, our RCT found a protective effect of BCG on the risk of atopic dermatitis in children with atopic predisposition (authors' unpublished results), and the Dutch RCT found a protective effect on the use of medication against eczema.[19] Thus, based on these 2 RCTs, the only ones conducted in high-income countries, BCG might protect children with atopic predisposition against atopic dermatitis but not against RW. The differential effect of BCG on atopic dermatitis and RW emphasizes that RW is not an atopic disease, and as previously shown, the risk factors for atopic dermatitis and RW are very different.[4]

Whether BCG has an effect on atopic asthma in older children still remains to be tested. Our choice of RW as an outcome instead of asthma was caused by the follow-up time of 13 months in our RCT.

In a systematic review from 2011 on the association between BCG and allergy (mostly in schoolchildren), the protective effect of BCG on asthma was insignificant when tested in subgroups, and there was evidence suggestive of publication bias and heterogeneity.[6]

In an updated systematic review and meta-analysis from 2014, including results from the Manchester Community Asthma Study (MANCAS) on children 6 to 11 years of age, Linehan et al[7] evaluated that the protective effect of neonatal BCG against the risk of wheeze at age 6 to 11 years initially found in MANCAS could not be found in the MANCAS cohort when the children were 13 to 17 years old. Thus the authors concluded that any protective effect of neonatal BCG vaccination on childhood asthma was likely to be transient.

The Danish Calmette Study found no overall association between BCG and the registry-based primary outcome of all-cause hospitalizations.[23] Furthermore, there was no overall effect of BCG on the secondary outcomes of hospitalization for infectious diseases (personal communication), parent-reported infectious diseases, or general practitioner visits.[24] However, the risks of hospitalization for infection and for parent-reported infectious disease were observed to be significantly decreased among BCG-vaccinated children whose mothers were also BCG vaccinated (personal communication).[24] Although RW is closely linked to respiratory tract infections, there was no indication that the effect of BCG vaccination on RW was particularly beneficial in children of BCG-vaccinated mothers, although the risk of RW was generally decreased in children of BCG-vaccinated mothers.

Because the power calculation was made for the main outcome, subgroup analyses might not have sufficient power. According to Fig 1, we cannot exclude that children vaccinated on days 2 to 7 could have had a significantly better effect of BCG vaccination against the development of RW than children vaccinated on days 0 or 1 if the sample size had been bigger. Thus timing of the intervention might be important, although our trial cannot prove this hypothesis.

The broad RW definition proposed in the analysis plan yielded a very high prevalence of RW, and some of the comments written during the telephone interviews and clinical examinations revealed the low specificity of such a broad definition. The signs and symptoms of RW are fluctuating and in milder cases difficult to distinguish from typical symptoms of having a normal cold. Thus we found it important to rely on stricter outcome criteria.

Strengths and limitations

This is the largest RCT assessing the effect of neonatal BCG on RW to date. With 98% of the included children interviewed at 13 months of age, we had a very high follow-up rate.

Our definition of RW required both a diagnosis of RW given by a medical doctor and the use of antiasthma treatment, and thus we emphasized a high specificity of the diagnosis.

A limitation was the impossibility of blinding the parents to study allocation because no other injection can mimic the typical reaction after a BCG vaccination. The study staff was blinded

with respect to the allocation status of the children, both in telephone interviews and at clinical examinations.

Because the symptoms of RW are fluctuating and some children received treatment, children typically had no signs of RW at the clinical examination, even if they were reported to have had RW.

A limitation in the analysis of the broad definition of RW based on 10 defining questions and proposed in the analysis plan (see Table E1) was the relatively high number of missing answers. However, it is reassuring that the results of the effect of BCG by using our more specific definition were similar to those obtained by using the broader definition.

Implications

We found no association between BCG vaccination at birth and the cumulative incidence of RW in the first year of life. Thus neonatal BCG vaccination cannot be used as protection against RW in a high-income country like Denmark. Moreover, the possible side effects of a BCG vaccination should always be taken into account before vaccinating an infant. In the Danish Calmette Study we have previously reported the finding of an unexpectedly high number of cases of suppurative lymphadenitis.[25] Conversely, BCG did not result in significantly more RW, and therefore when a BCG vaccination is necessary for other reasons, the child can be BCG vaccinated without concerns of RW.

Conclusion

In this large RCT from a high-income country, neonatal BCG had no effect on the cumulative incidence of RW in the first 13 months of life.

We thank the participating children and their families for their time, interest, and loyalty, and we thank the Danish Calmette Study staff for their work and commitment.

Clinical implications: Neonatal BCG vaccination had no effect on the cumulative incidence of RW in the first year of life in Denmark.

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TABLE E1. Broad definition, including any suspicion or sign, of RW in the first year of life in relation to neonatal BCG vaccination and potential effect modifiers*

Parameter analyzed	BCG group, no. with RW/total no. (%)	Control group, no. with RW/total no. (%)	RR (95% CI) for BCG effect on RW	Test of no interaction (*P* value)
Any suspicion or sign of RW, including parent-suspected RW	330/1977 (16.7%)	300/1858 (16.1%)	1.03 (0.90-1.19)	
RW in relation to potential effect modifiers				
GA at birth				
Premature (GA 32+0 to 36+6; n = 118)	14/61 (23.0%)	10/57 (17.5%)	1.31 (0.63-2.71)	.52
Mature (GA ≥37+0; n = 3717)	316/1916 (16.5%)	290/1801 (16.1%)	1.02 (0.89-1.19)	
Sex				
Boys (n = 2024)	202/1025 (19.7%)	193/999 (19.3%)	1.02 (0.86-1.22)	.72
Girls (n = 1811)	128/952 (13.4%)	107/859 (12.5%)	1.08 (0.85-1.37)	
Atopic predisposition (missing: 66)				
With atopic predisposition (n = 2424)	220/1257 (17.5%)	196/1167 (16.8%)	1.04 (0.81-1.34)	.99
Without atopic predisposition (n = 1345)	107/683 (15.7%)	100/662 (15.1%)	1.04 (0.87-1.24)	
Maternal BCG status (missing: 45)				
Mother BCG vaccinated (n = 657)	54/341 (15.8%)	38/316 (12.0%)	1.31 (0.89-1.93)	.26
Mother not BCG vaccinated (n = 3133)	271/1614 (16.8%)	259/1519 (17.1%)	0.99 (0.84-1.15)	
Ethnicity (missing: 22)				
Danish ethnicity (n = 3085)	268/1630 (16.4%)	250/1455 (17.2%)	0.96 (0.82-1.12)	.06
Non-Danish ethnicity (n = 728)	61/336 (18.2%)	48/392 (12.2%)	1.49 (1.05-2.11)	
Siblings (missing: 2)				
No older siblings (n = 2247)	171/1131 (15.1%)	131/1116 (11.7%)	1.29 (1.04-1.60)	.002
At least 1 older sibling (n = 1586)	159/846 (18.8%)	169/740 (22.8%)	0.82 (0.68-1.00)	
Pets at home				
Yes (n = 1123)	102/604 (16.9%)	96/519 (18.5%)	0.91 (0.71-1.17)	.25
No (n = 2712)	228/1373 (16.6%)	204/1339 (15.2%)	1.09 (0.92-1.30)	
Smoking status by 13 mo of age (missing: 28)				
Ever smoked since birth of child (n = 1045)	95/519 (18.3%)	95/526 (18.1%)	1.02 (0.79-1.32)	.84
Never smoked since birth of child (n = 2762)	232/1442 (16.1%)	202/1320 (15.3%)	1.05 (0.88-1.25)	
Breast-feeding				
Fully breast-fed until 3 mo of age (n = 2187)	166/1136 (14.6%)	166/1051 (15.8%)	0.92 (0.76-1.13)	.10
Not fully breast-fed until 3 mo (n = 1648)	164/841 (19.5%)	134/807 (16.6%)	1.18 (0.96-1.45)	
Started institutional child care before 13 mo of follow-up (missing: 1)				
Yes (n = 3302)	306/1717 (17.8%)	272/1585 (17.2%)	1.04 (0.90-1.21)	.59
No (n = 532)	24/260 (9.2%)	28/272 (10.3%)	0.90 (0.53-1.50)	

GA, Gestational age.

*Log-binomial regression analysis of the 3835 children (90.0% of the randomized children) who answered all 10 defining questions regarding the broad definition of RW (2 defining questions in each telephone interview and 3 at each clinical examination).

TABLE E2. Sensitivity outcomes: RW in first year of life in relation to neonatal BCG vaccination—prevalence and RR for different definitions of RW*

Parameter analyzed	BCG group, no. with RW/ total no. (%)	Control group, no. with RW/ total no. (%)	RR (95% CI)
Broad definition of RW: any suspicion or sign of RW†	330/1977 (16.7%)	300/1858 (16.1%)	1.03 (0.90-1.19)
Parent-suspected RW and/or medically diagnosed RW	316/2100 (15.0%)	290/2071 (14.0%)	1.03 (0.89-1.19)
Parent-suspected RW‡	286/2100 (13.6%)	263/2071 (12.7%)	1.08 (0.92-1.26)
Physician-diagnosed RW‡	234/2100 (11.1%)	214/2071 (10.3%)	1.08 (0.91-1.28)
Medically treated RW‡	233/2100 (11.1%)	213/2071 (10.3%)	1.08 (0.91-1.29)
RW found at the clinical examination§	73/2001 (3.6%)	70/1879 (3.7%)	0.98 (0.71-1.35)
Any RW (telephone interview/clinical examination, including parent-suspected RW)	316/2100 (15.0%)	290/2071 (14.0%)	1.03 (0.89-1.19)

*Log-binomial regression.
†The broad definition of RW is the primary outcome from the analysis plan. It includes parent-suspected RW, as well as rhonchi or prolonged expiration heard at auscultation. The lower total number in the randomization group for this outcome reflects missing answers to 1 or more subquestions. The broad definition of RW is further elaborated in Table E1.
‡Based on information given by the parents in the telephone interviews at 3 and 13 months of age.
§Based on clinical examinations at 3 and 13 months of age. Some children with telephone information concerning RW were not clinically examined.

TABLE E3. Potential risk factors for cumulative incidence of physician-diagnosed and medically treated RW in the first year of life*

	For all infants included: no. with RW/total no. (%)
Primary outcome: intention-to-treat analysis: physician-diagnosed and medically treated RW	406/4171 (9.7%)

RW in relation to potential effect modifiers	Subgroups, no. with RW/total no. (%)	RR (95% CI) for RW in subgroups, univariate analysis	RR (95% CI) for RW in subgroup, multivariable analysis, n = 4.006
GA at birth			
Premature (GA 32+0 to 36+6; n = 133)	15/133 (11.3%)	1.17 (0.72-1.89)	1.17 (0.85-1.24)
Mature (GA ≥37+0; n = 4038)	391/4038 (9.7%)	1.0	
Sex			
Boys (n = 2199)	258/2199 (11.7%)	1.56 (1.29-1.90)	1.62 (1.34-1.97)
Girls (n = 1972)	148/1972 (7.5%)	1.0	
Atopic predisposition (missing: 73)			
With atopic predisposition (n = 2630)	266/2630 (10.1%)	1.08 (0.89-1.32)	1.05 (0.87-1.28)
Without atopic predisposition (n = 1468)	137/1468 (9.3%)	1.0	
Maternal BCG status (missing: 51)			
Mother BCG vaccinated (n = 724)	51/724 (7.0%)	0.69 (0.52-0.91)	0.75 (0.56-1.00)
Mother not BCG vaccinated (n = 3396)	348/3396 (10.2%)	1.0	
Ethnicity (missing: 25)			
Non-Danish ethnicity (n = 824)	70/824 (8.5%)	0.84 (0.66-1.08)	0.92 (0.71-1.19)
Danish ethnicity (n = 3322)	336/3322 (10.1%)	1.0	
Siblings (missing: 2)			
At least 1 older sibling (n = 1724)	225/1724 (13.1%)	1.76 (1.46-2.12)	1.81 (1.50-2.18)
No older siblings (n = 2445)	181/2445 (7.4%)	1.0	
Pets at home			
Yes (n = 1225)	151/1225 (12.3%)	1.42 (1.18-1.72)	1.39 (1.15-1.68)
No (n = 2946)	255/2946 (8.7%)	1.0	
Smoking status by 13 mo of age (missing: 36)			
Ever smoked since birth of child (n = 1157)	131/1157 (11.3%)	1.24 (1.02-1.51)	1.24 (1.02-1.51)
Never smoked since birth of child (n = 2978)	272/2978 (9.1%)	1.0	
Breast-feeding (missing: 7)			
Not fully breast-fed until 3 mo (n = 1801)	193/1801 (10.7%)	1.18 (0.98-1.42)	1.24 (1.03-1.50)
Fully breast-fed until 3 mo of age (n = 2363)	213/2363 (9.0%)	1.0	
Started institutional child care before 13 mo of follow-up (missing: 1)			
Yes (n = 3584)	377/3584 (10.5%)	2.12 (1.47-3.07)	2.0 (1.38-2.90)
No (n = 586)	29/586 (4.9%)	1.0	

GA, Gestational age.

*Log-binomial regression analysis of the 4171 children who answered the questions regarding RW in the telephone interview at 13 months of age (97.9% of the randomized children).