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Neonatal BCG protection against leprosy: a study in Manaus, Brazilian Amazon

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Summary There is clear evidence that BCG protects against leprosy, but cross-immunity with environmental mycobacteria can interfere with vaccination protection. Some have cast doubts whether BCG vaccination can offer a significant impact against leprosy in the Brazilian Amazon, which is an endemic area for leprosy and with a high prevalence of environmental mycobacteria. This study was designed to estimate the vaccine effectiveness of neonatal BCG against leprosy in Amazon region, in Brazil. This is a cohort study nested in a randomized community trial. The study had two main results. First, neonatal BCG vaccination in Brazilian Amazon elicited protection of 74% (95% CI 57–86) against all forms of leprosy cases. Second, the highest protection was observed for multibacillary cases, 93% (95% CI 71–98). It is concluded that the study provides evidence that neonatal BCG may have an important and overlooked impact on the occurrence and transmission of leprosy, maybe even more in the future when the cohort which received a high coverage of BCG reaches the age of high incidence of leprosy.

Introduction

There is clear evidence that BCG protects against leprosy, but the level of protection has ranged from 20% to 90% in different studies.[1,2] One hypothesis to explain this variation is cross-immunity with environmental mycobacteria (EM).[3,4]

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Brazil is a large country and has the second greatest number of cases of leprosy in the world detected each year.[5] The Brazilian leprosy control programme advocates the identification and treatment of all cases to control the disease, and as a complementary measure the BCG vaccination among household contacts of leprosy cases.[6] The disease has an uneven geographical distribution in the country, and the Brazilian Amazon region is an important endemic area where a high prevalence of EM is assumed.[7] Because of the hypothesis that infection with EM can interfere in the protection elicited by BCG [4], some have cast doubts on whether BCG vaccination can offer a significant impact against leprosy[8] or against tuberculosis[9] in the Brazilian Amazon. Indeed, in Manaus, the largest urban centre in Brazilian Amazon, the vaccination coverage among household contacts of leprosy cases is low.

The present study took opportunity of an ongoing community trial of BCG vaccination of school children in a population with high coverage of neonatal BCG (REVAC-BCG trial) to estimate the vaccine effectiveness of neonatal BCG against leprosy in Amazon region in Brazil.

Materials and methods

STUDY DESIGN

This is a cohort study nested in one of the study sites of the REVAC-BCG community trial. This trial aims to evaluate the vaccine effectiveness of BCG given to schoolchildren against tuberculosis and leprosy and has two study sites, the cities of Salvador and Manaus. This cohort study was conducted in Manaus, where the trial was implemented in 1998. Details of the trial have been described elsewhere.[10,11] Briefly, it was a cluster randomized without placebo community trial. The study population of this cohort study was followed up in two periods and had thus two components (see Figure 1). First, from 1989 to 1997 (before implementation of the trial) corresponded to a historical cohort study involving the trial population from both control and intervention trial arms. Second, from 1999 to August 2002, during the trial follow-up, corresponded to a prospective cohort study involving only the trial population in the control arm, as this study is concerned with the neonatal dose and most individuals of the intervention arm received two doses.

STUDY SITE

This cohort study was conducted in the city of Manaus, with about 1,500,000 inhabitants (1999 census) on the banks of the Negro River, Brazilian Amazon region, with a tropical

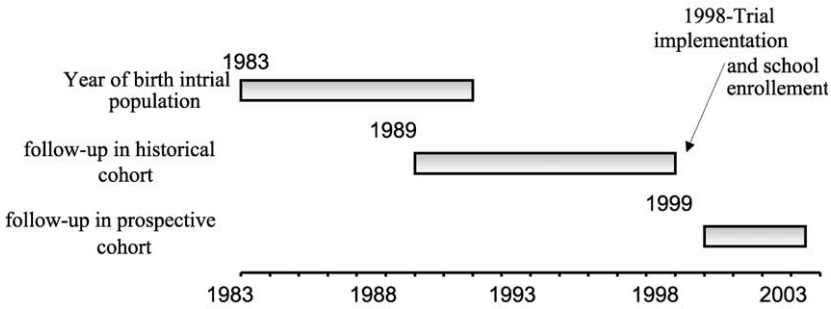

Figure 1. Historical and prospective follow-up periods.

climate and high humidity, close to the equator line (latitude 3° 7′ south, longitude 60° 130′ west). Differently from the other trial site (Salvador), Manaus has a higher incidence of leprosy with a new case detection rate (NCDR) of about 5–6 per 10,000 every year.

STUDY POPULATION

The cohort participants consisted of the schoolchildren recruited into the trial, who were born between 1983 and 1991 (aged 7–14 years at recruitment into the trial), and residing in Manaus. The trial, and this study, was restricted to children attending state schools. Only children with no BCG scar or one scar entered in this cohort study ($n = 112{,}744$ in total study population; $n = 60{,}458$ in control group). Identification and vaccination data recorded in the REVAC-BCG trial database were used in this cohort study.

VACCINATION ASCERTAINMENT

The presence of a scar compatible with BCG vaccination was used as a surrogate of BCG neonatal vaccination in the REVAC-BCG trial and in this cohort study. BCG scar reading was performed during the recruitment phase of the trial in 1998 (Manaus) by trained health workers who visited the schools, and data entered in the trial database. Validation of the scar as a marker of BCG vaccination in the trial population in Manaus is published elsewhere.[12] In summary, BCG scar reading had a sensitivity between 94% and 98%, depending on age group, when the gold standard was the agreement between parental information about past vaccination and vaccination card.

CASE ASCERTAINMENT AND CLASSIFICATION

Data on all cases regarding identification data, clinical form, and date of diagnosis of all leprosy cases in the city of Manaus are routinely entered in a computerized database of the local control programme. In this cohort study, leprosy cases diagnosed between 1989 and 1997 (historical cohort), and between 1999 and August 2002 (prospective cohort), in children eligible to the trial population, were ascertained from the local leprosy control programme. All cases reported as indeterminate, tuberculoid and BT (bordeline tuberculoid) were classified as paucibacillary (PB) cases; and LL (lepromatous lepromatous), BB (borderline borderline), BL (borderline lepromatous) as multibacillary (MB). Cases reported as dimorphous were classified as multibacillary, but those with negative baciloscopy and who received PB multi-drug therapy (MDT) regime were classified as paucibacillary.

CASE LINKAGE

The leprosy cases recorded in the database of the local leprosy control programme were linked to the records in the REVAC-BCG trial database. The matching between cases and trial study population was done based on variables present in both databases: subject's name, date of birth, sex, and mother's name.

INCIDENCE OF LEPROSY AND TUBERCULOSIS FOR GEOGRAPHICAL AREA

The city of Manaus is divided in 56 administrative areas (districts), and two categorical variables were created indicating if the schools were the students attended in 1998 were located in districts with incidence of leprosy (NCDR) and tuberculosis below or above to the incidence of the city in 1996 as a whole. These variables were taken as a proxy of the baseline risk and socio-economic status (as leprosy and tuberculosis are diseases related to poverty) of the trial study population and in the prospective cohort. However, chosen was made to not use these variables in the analysis of the historical cohort because they may not express the baseline risk during the longer period before the trial in the historical cohort, which included period until 6–7 years before 1996.

STATISTICAL PROCEDURES

Incidence of leprosy per 10,000 (NCDR) was estimated in children separately by BCG scar, current age, sex, and leprosy classification. Confidence intervals (95% CI) of crude rates and rate ratios were estimated according to an approximation to the Poisson log-likelihood for the log rate parameter.[13] If the number of cases was smaller than 30, the confidence intervals of rates were estimated according to exact confidence limits for a Poisson-distributed variable.[14] Interaction was done using the log likelihood ratio test.[13] Age was categorized in groups and was treated as time-varying variable, expressed as current age (age during the cohort follow-up) through expansion of the data-set in time scales.[13] BCG vaccine effectiveness was estimated as (1–rate ratio) × 100. Adjusted rate ratios for current age, sex, year of birth and incidence of leprosy and tuberculosis for geographical areas were obtained by using standard Poisson regression. All the statistical analysis was done using STATA version 7.0[15].

Results

In the historical cohort study, 128 leprosy cases diagnosed between 1989 and 1997 in Manaus were linked to the trial study population. One hundred and seven (107) cases were classified as paucibacillary cases (21 indeterminate, 78 tuberculoid, three dimorphous, five BT) and 21 as multibacillary cases (three LL, one BL, 17 dimorphous). The three reported dimorphous cases classified as paucibacillary had negative baciloscopy and received the paucibacillary MDT regime.

In the prospective cohort study, 53 leprosy cases diagnosed between 1999 and August 2002 were linked to the trial study population. Forty-three (43) were classified as paucibacillary cases (23 tuberculoid, nine indeterminate, 10 BT, one just reported as paucibacillary case) and 10 cases as multibacillary (one BB, four dimorphous, three BL, two LL). All these four dimorphous cases had positive baciloscopy and were treated with multibacillary MDT regime.

Among cases in the historical cohort, 35 (27·3%) had no BCG scar and 93 (72·7%) had one scar; 75 (58·6%) were females and 53 (41·4%) males. In the prospective cohort, 22 (41·5%) had no BCG scar and 31 (58·5%) had one scar; 24 (45·3%) were females and 29 (54·7%) males. These figures compare with 84·0% with BCG scar and 51·3% of females in the total trial population (84·8% and 51·4% in control group, respectively).

For the whole trial population, the prevalence of BCG scar was higher for those born in recent years: 79·8%, 85·6% and 88·9%, for those born in 1983–1986, 1987–1988, and

1989–1991, respectively. For the control group these figures were 80·9%, 86·3%, 89·5%, respectively.

Table 1 shows the crude rates according to sex, current age and incidence of leprosy and tuberculosis in geographical areas in 1998. In historical cohort, females and older individuals had higher rates than males (rate of 1·51 versus 1.13) and younger people (rate of 3·22 versus 0.28). In prospective cohort, males presented higher rates than females (rate of 2·93 versus 2.30) and older individuals yet had higher rates than younger (rate of 2·93 versus 1.91). In the prospective cohort, rates of leprosy were higher among those attending schools in 1998 located in areas with higher rates of leprosy and tuberculosis (but without statistical significance).

In the historical cohort, the rate per 10,000 for multibacillary cases were 0·22 (21/968,369 person years; 95% CI: 0·17–0·37) and for paucibacillary cases was 1·04 (107/968,369; 95% CI: 0·86–1·27). In the prospective cohort, the rates were 0·49 (10 cases/203,507 person years; 95% CI: 0·26–0·91) and 2·11 (43 cases/203,507; 95% CI: 1·57–2·84), respectively (data not shown).

Table 2 shows the crude and adjusted (current age, sex and calendar year of birth) rate ratios for leprosy in historical cohort, according to presence of BCG scar and case classification. The adjusted estimates of vaccine effectiveness were: 41% (95% CI: 12—60%) for all leprosy cases, 77% (95% CI: 45–90%) for multibacillary cases, 26% (95% CI: –16–53%) for all paucibacillary cases, and no statistically significant protection for paucibacillary cases excluding intermediate and for indeterminate cases. Except for multibacillary cases, the difference between crude and adjusted rate ratios varied between

Table 1. Rates of leprosy per 10,000 separately by sex, current age and incidence of leprosy and tuberculosis in geographical areas (prospective cohort)

Study variable	No cases	Person-years	Rate per 10,000	Rate ratio (CI 95%)
Historical cohort (n = 128)				
Sex				
Female	75	497,950	1·51	1
Male	53	470,420	1·13	0·75 (0·53–1·06)
Current age (years old)				
0–5	10	360,490	0·28	1
6–9	77	480,610	1·60	5·78 (3·00–11·16)
10–15	41	127,270	3·22	11·61 (5·82–23·18)
Prospective cohort (n = 53)				
Sex				
Female	24	104,530	2·30	1
Male	29	98,977	2·93	1·28 (0·74–2·19)
Current age (years old)				
7–9	4	20,990	1·91	1
10–14	25	100,520	2·49	1·31 (0·45–3·75)
15–18	24	82,000	2·93	1·54 (0·53–4·43)
Incidence of tuberculosis in before follow-up (1998)[a]				
'Low'	34	143,471	2·37	1
'High'	18	56,423	3·19	1·35 (0·72–2·45)
Incidence of leprosy before follow-up (1998)[b]				
'Low'	21	99,011	2·12	1
'High'	32	102,398	3·13	1·47 (0·82–2·69)

[a] Excluding 1074 individuals and one leprosy case with missing data about incidence of tuberculosis.
[b] Excluding 623 individuals (without leprosy case) with missing data about incidence of leprosy.

Table 2. Rate ratios for leprosy according to presence of BCG scar, separately by clinical forms, in historical cohort

Study variable	Neonatal BCG scar		Rate ratio (95% CI)	
	One	No		
All cases (n = 128)				
Cases (rate per 10,000)[a]	93 (1·15)	35 (2·23)	Crude estimate	0·51 (0·35–0·76)
			Poisson regression[b]	0·59 (0·40 to 0·88)
Multibacillary cases (n = 21)				
Cases (rate per 10,000)	11 (0·13)	10 (0·64)	Crude estimate	0·21 (0·09–0·50)
			Poisson regression[b]	0·23 (0·10–0·55)
Paucibacillary cases (n = 107) (including indeterminate)				
Cases (rate per 10,000)	82 (1·01)	25 (1·59)	Crude estimate	0·63 (0·41–0·99)
			Poisson regression[b]	0·74 (0·47–1·16)
Paucibacillary cases (n = 86) (excluding indeterminate)				
Cases (rate per 10,000)	65 (0·80)	21 (1·34)	Crude estimate	0·60 (0·37–0·98)
			Poisson regression[b]	0·69 (0·42–1·13)
Indeterminate cases (n = 21)				
Cases (rate per 10,000)	17 (0·21)	4 (0·26)	Crude estimate	0·82 (0·28–2·40)
			Poisson regression[2]	1·01 (0·34–3·02)

[a] Total person year of 811,554 and 156,815, for vaccinated and unvaccinated, respectively.
[b] Estimate controlled for current age, sex and year of birth (categorical variables).

13% and 18%, indicating confounding effect (taking the threshold of 10% to define confounding), attributed to the association of year of birth with presence of BCG scar and leprosy rates. There was no statistically significant interaction between presence of BCG scar and current age, year of birth and sex (data not shown).

Table 3 shows the crude and adjusted rate ratios for leprosy in prospective cohort, according to presence of BCG scar, and case classification. The adjusted estimates of vaccine

Table 3. Rate ratios for leprosy according to presence of BCG scar, by clinical forms, in prospective cohort

Study variable	Neonatal BCG scar		Rate ratio (95% CI)	
	One	No		
All cases (n = 53)				
Cases (rate per 10,000)[a]	31 (1·80)	22 (7·10)	Crude estimate	0·25 (0·15–0·44)
			Poisson regression[b]	0·25 (0·14–0·43)
Multibacillary cases (n = 10)				
Cases (rate per 10,000)	3 (0·17)	7 (2·26)	Crude estimate	0·08 (0·02–0·30)
			Poisson regression[b]	0·07 (0·02–0·29)
Paucibacillary cases (n = 43) (including indeterminate)				
Cases (rate per 10,000)	28 (1·62)	15 (4·84)	Crude estimate	0·34 (0·18–0·63)
			Poisson regression[b]	0·33 (0·17–0·62)
Paucibacillary cases (n = 34) (excluding indeterminate)				
Cases (rate per 10,000)	21 (1·22)	21 (4·19)	Crude estimate	0·29 (0·15–0·60)
			Poisson regression[b]	0·28 (0·14–0·56)
Indeterminate cases (n = 9)				
Cases (rate per 10,000)	7 (0·41)	2 (0·65)	Crude estimate	0·63 (0·13–3·03)
			Poisson regression[b]	0·69 (0·14–3·35)

[a] Total person year of 172,505 and 31,003, for vaccinated and unvaccinated, respectively.
[b] Estimate controlled for current age, sex, year of birth and incidence of leprosy and tuberculosis in geographical areas before the trial follow-up (categorical variables).

effectiveness were 75% (95% CI: 57–86%) for all leprosy cases, 93% (95% CI: 71–98%) for multibacillary cases, 67% (95% CI: 38–83%) for all paucibacillary cases, 72% (95% CI: 44–86%) for paucibacillary cases excluding intermediate cases, and no significant protection for indeterminate cases. Differently from the historical cohort, there were slight differences between crude and adjusted rate ratios, indicating no confounding effect with the study variables. Similarly to historical cohort, there was no statistically significant interaction between presence of BCG scar and current age, year of birth and sex.

Discussion

The study had three main results. First, neonatal BCG vaccination in Manaus (Brazilian Amazon) elicited protection between 41% and 74% against all forms of leprosy cases. Secondly, when the categories were considered separately, the highest protection was observed for multibacillary cases, with little or no protection for indeterminate cases. Third, this protection did not change with current age.

This study consisted of two cohorts, one historical and another prospective, which might well have brought about different results, and hence were analysed separately. First, in the prospective cohort, individuals were older than in the historical cohort. In Manaus, the rates for leprosy increase with age and become more stable among adults. Therefore, the leprosy rates were more similar across age groups in the prospective cohort and hence less associated with year of birth. This can explain why year of birth, which was also associated with presence of BCG scar in both cohorts, was a confounder in the historical cohort (because was also associated with leprosy rates) but not in the prospective study (because was not associated with leprosy rates).

Second, individuals were included into the study population when they enrolled into schools, and in the historical cohort, by the nature of the design, enrolment was after case detection. This is known as ‘late entry’ and can introduce selection bias.[13] Individuals who got disease (leprosy cases) might be less likely to be enrolled into schools because of the disease and hence more likely to be excluded from the cohort. The estimate of vaccine protection would be biased if the proportion of cases enrolled into school among those vaccinated were different from those unvaccinated. This might well happen, for instance, if BCG vaccination caused shifts in clinical forms, leading to milder cases, as suggested in previous studies.[16,17] Mild cases could be more likely to be enrolled into schools than severe cases. Consistently with this possibility, in the historical cohort the proportion of paucibacillary cases linked in the trial population (22%) was slightly higher than the proportion of multibacillary cases linked (17%) (data not shown), although this was not statistically significant. A differential enrolment would lead to a differential loss of unvaccinated cases (as these would be more likely to be multibacillary), and therefore to an underestimation of vaccine protection. This bias would not be present in the prospective cohort, as the leprosy cases were traced after the cohort was established, with no differential loss of vaccinated cases. This again is consistent with the protective effect estimated in the historical cohort being lower than the protective effect estimated in the prospective cohort. If this is the explanation for the differences in protective effect estimated in the two cohorts, then the unbiased, correct measure, is the higher protection estimated in the prospective cohort.

Third, this is an observational study, and these estimates of effect measure are valid as long as confounding can be controlled for. One important potential confounder is socio-economic status, related to vaccine uptake as well as risk of leprosy. Incidence of leprosy and tuberculosis before the follow-up in geographical areas where schools were located were used as proxy for baseline risk and socio-economic status in prospective cohort. Therefore, the estimates obtained from prospective cohort should be considered less likely to be distorted by confounding effect.

If one accepts that BCG shifts cases from the multibacillary to the paucibacilary end of the leprosy spectrum, one could postulate scenarios where BCG could increase the risk of paucibacillary cases. This was observed for indeterminate cases,[17,18] as well as a higher rate of leprosy among those vaccinated (all with tuberculoid and indeterminate forms) than among those unvaccinated (Bechelli and Quagkiato, 1953 and 1956; cited in [19]). This shift would lead to an underestimated vaccine protection for paucibacillary forms, as the incidence would increase with vaccination. This hypothesis is consistent with results in both cohorts in our study, in which the lowest vaccine effect was observed for paucibacillary cases, and the highest value was for multibacillary cases.

There may be an interaction in immune protection among different *Mycobacteria* species.[4,20] It has been suggested that the variation in BCG protection against leprosy (as well as against tuberculosis) could be caused by the presence of infection by environmental mycobacteria (EM). But studies so far have failed to disentangle the intriguing interaction between BCG and EM, which remains the subject of lively debate. For instance, the study population of the Malawi trial had a high prevalence of skin reactors for EM antigens, and prior sensitisation to fast growers EM led to reduced risk of both leprosy and tuberculosis,[20] even though BCG still protected against leprosy, but not against tuberculosis.

Most endemic areas of leprosy in Brazil are located in tropical or equatorial regions, such as Manaus, and there is some evidence that Manaus has a high prevalence of EM. First, the city is located in area with high humidity and temperature, factors favouring the growth of EM.[21] Second, a recent survey demonstrated a prevalence of infection for *Mycobacterium avium* of nearly 60% among the REVAC-BCG trial study population.[22] Third, isolates of EM have been found to be more common in sputum from tuberculosis patients in Manaus than in other areas in Brazil.[23] However, the results of our study do not support the idea that the protection offered by neonatal BCG vaccination against leprosy is substantially reduced because of the high prevalence of EM in Manaus. The findings of this study are consistent with previous studies in Brazil, in which shown that BCG vaccination confers protection against leprosy.[2,24,25]

It is worth noting that neonatal BCG in Brazil has reached coverage rates of 90% in the late 1990s (this was reflected in our data by the higher prevalence of BCG scar among those born in recent years). Therefore, most Brazilians up to the age of 15 will have at least some protection against leprosy. Perhaps more important, we found protection against the multibacillary cases, suggesting BCG must have an impact on transmission of leprosy. This impact would also depend on the proportion of the multibacillary cases in the whole population that come from schoolchildren, the vaccine coverage rate, and for how long protection lasts. In the literature, there is evidence that BCG elicits protection against multibacillary cases as well as paucibacillary.[16,26,27]

The finding that this protection did not change with age suggests that the protection lasted for 10–15 years after vaccination. It was recently reported that BCG protection against tuberculosis can last for 50–60 years after vaccination.[28] If BCG also elicits a long-lasting

protection against leprosy, neonatal BCG, which is given routinely to prevent tuberculosis, may have an important and overlooked impact on the occurrence and transmission of leprosy in the future, when the cohort which received a high coverage of BCG reaches the age of high incidence of leprosy and replaces the previous generation. However, this finding must be interpreted carefully because of the low power to assess interaction.

In conclusion, this study provides evidence that neonatal BCG may have an important and overlooked impact on the occurrence and transmission of leprosy, perhaps even more in the future when the cohort which received a high coverage of BCG reaches the age of high incidence of leprosy.

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