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Influence of sex, age & nontuberculous infection at intake on the efficacy of BCG: re-analysis of 15-year data from a double-blind randomized control trial in south India

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***Background & objectives*: To estimate the efficacy of BCG in preventing tuberculosis over a 15-year period, and also to assess the impact of infection with nontuberculous environmental mycobacteria in a rural community in Chingleput district in Tamil Nadu in south India. We re-analysed the 15-year follow up data of a large randomized trial conducted earlier.**

***Methods*: A double-blind randomized control trial was initiated in 1968, in which over 100,000 uninfected subjects with a normal radiograph were allocated to placebo, BCG in low dose (0.01 mg) or BCG in high dose (1.0 mg); two widely used strains of BCG were employed, each in one half of the vaccinated subjects. Sensitivity to purified protein derivative (PPD-B) was also determined. The study population was followed for 15 yr by radiographic surveys of the total population once every 2.5 yr, selective case finding in suspects once in 10 months, and investigation of those reporting voluntarily with chest symptoms.**

***Results*: Coverage by radiography was of the order of 80 per cent throughout, while coverage by sputum examination of suspects was usually 90 per cent or above. The annual incidence of culture-positive tuberculosis (irrespective of smear) was estimated to be 55 per 100,000, and neither strain of BCG had any effect. The failure to protect was seen in both males and females, and in children and adults. However, in a subset of over 40,000 subjects who were also nonreactors to PPD-B, BCG had a low level of protection, *i.e.*, 32 per cent (95% CI=3-52%), 29 per cent with the Danish strain and 34 per cent with the French strain.**

***Interpretation & conclusion*: Our findings reaffirm that BCG was of little value in preventing sputum-positive cases of pulmonary tuberculosis.**

Key words BCG efficacy - community trial - 15-year follow up - India

*This report was prepared by Dr S. Radhakrishna, former Director, Institute for Research in Medical Statistics, Madras Chapter (ICMR) who had the primary responsibility for analysis and writing of this manuscript. Dr Thomas R. Frieden, Commissioner of Health, Department of Health and Mental Hygiene, New York, participated in design of the analysis and writing of the manuscript. Mr R. Subramani, Assistant Director, Tuberculosis Research Centre, Chennai was responsible for data management and data output.

Based on a 15-year follow up of subjects in a large randomized control trial in south India, we had previously reported that BCG did not offer any protection against adult forms of bacillary pulmonary tuberculosis[1]. This finding applied not only to the community at large, but also to the subset of subjects who had no tuberculous infection at intake. We re-examined the data in initially uninfected subjects (induration of <8 mm to PPD-S) using better statistical tools, and with special focus on children and those that did not have a protective infection with other environmental mycobacteria at intake. We also identified successfully a subset of uninfected subjects in those with a borderline induration to purified protein derivative (PPD-S), *i.e.*, 8-11 mm, and extended the evaluation of BCG to the totality of uninfected subjects. This report presents the findings of this re-analysis.

Material & Methods

A double-blind randomized controlled trial was initiated in 1968 in a large rural community in Chingleput district in south India, to assess the protective efficacy of BCG vaccination, employing two strains of BCG. One of these was the Danish strain 1331 that has been used in India since 1966 and in the British trial in 1950-1952 where it was found to be highly effective, and the other was the widely used French strain 1173 P 2. In this trial, all individuals in the community who were aged ≥1 yr were tested with 3 international units (IU) of PPD-S, and 10 units of PPD-B derived from *Mycobacterium intracellulare*. PPD-S was supplied by the Antigen Production Laboratory, Centers for Disease Control & Prevention (CDC), Atlanta, Georgia, USA, during the first 6 months of the intake, and by the Statens Serum Institut, Copenhagen, for the next two years. The Antigen Production Laboratory, CDC Atlanta, USA, supplied PPD-B. All subjects aged ≥10 yr had a radiograph taken, and if it was abnormal, two sputum specimens were examined by culture on Lowenstein-Jensen (LJ) medium. All persons aged ≥1 month, irrespective of their tuberculin sensitivity status, were randomly allocated to a placebo, a low dose (0.01 mg) or a high dose of BCG (0.1 mg), with half (selected at random) receiving the Danish strain and the other half the French strain. Intensive efforts were made to detect all new cases of tuberculosis occurring over the next 15 yr, by means of total population surveys (radiography followed by sputum examination of those eligible) once in every 2.5 yr, selective case-finding (once every 10 months) among those with an abnormal radiograph or chest symptoms, and by setting up a permanent diagnostic service to investigate those reporting spontaneously with chest symptoms. The details have been reported earlier[1,2].

Those with an induration of 0-7 mm to PPD-S at intake (*i.e.*, no evidence of tuberculous infection) and a normal radiograph constituted the primary population for study. Employing dual testing with PPD-S and PPD-B, it was concluded that only 2.7 per cent of the persons with 8-11 mm to PPD-S (*i.e.*, those in whom the induration to PPD-S exceeded the induration to PPD-B by at least 2 mm) had, in all probability, a tuberculous infection. The rest were regarded as uninfected at intake, and have been included in a supplementary analysis of BCG efficacy.

For each 2.5 year period in each of the 3 series, the population at risk was stratified by sex and age (0-4, 5-9, 10-14, 15-24, 25-34, 35-44, 45-54 and ≥55 yr), and deaths and culture-positive cases were estimated in each of the 16 (2 x 8) subgroups. These estimates were then pooled to obtain the death rate and the culture-positive incidence rate for the period. Employing the estimates for the 6 periods in a life-table analysis, the incidence of culture-positive tuberculosis was determined for each series over the 15-year period.

Due to operational reasons, sputum was not collected from 20 per cent of eligible persons in the first resurvey (at 2.5 yr), from 9 per cent in the second resurvey (at 5 yr), and from ≤5 per cent in the subsequent resurveys. The losses were practically the same in the 3 separate series (placebo, BCG low dose, BCG high dose). The usual practice is to ignore the potential yield from these, or presume that the proportion of positive cultures would be the same as in those examined. Instead, we estimated the probable number of positive culture results by using the relationship between radiographic status at the time and the culture result from those who had both examinations. The likelihood of a positive culture

was significantly affected by the age at the time, and also by sex in adults aged ≥15 yr. Among persons with no sputum examination, the number in each radiographic category was multiplied by the appropriate sex and age-specific probability (of the culture being positive) to estimate the number of 'missed' culture-positive cases; this was then added to the observed number of cases to obtain a consolidated total from which the incidence of tuberculosis in all persons with a radiographic examination was determined. Details and the probabilities have already been reported[3].

Statistical methods: Cox's proportionate hazard model[4] was employed to assess the statistical significance of the adjusted relative risk for BCG, after allowing for age and sex.

Results

Study population: The eligible population for this study, *i.e.*, subjects with an induration of 0-7 mm to PPD-S at intake and a normal radiograph, was 109863. This number decreased substantially over the next 15 yr (Table I), mainly due to migration (27%); other causes were death (5.5%) and development of tuberculosis (0.4%). The number of persons allocated was 36404 to placebo, 36459 to BCG low dose and 37000 to BCG high dose. The numbers in subsequent periods also were similar in the three series (Table I), as the losses due to migration were of the same order (placebo 27.0%, low dose 26.6%, high dose 26.9%) as also deaths (placebo 6.3%, low dose 6.5%, high dose 6.2%). Further analysis showed that the losses were similar in the three series in every period (migration 4-7% in the first 10 yr, 10% during 10-12.5 yr, and 14% in 12.5-15 yr; deaths 1.7% in 0-2.5 yr, 1.1-1.3% in subsequent periods). Also, the distributions by age and sex of study population were similar in the three series in every period.

Radiographic and sputum coverages: The coverage by radiography was 83 per cent in the first three periods, and 81, 79 and 77 per cent in the subsequent three periods. The coverage by sputum was 80 per cent in the first period, 91 per cent in the second period and 95-96 per cent in subsequent periods. The findings were very similar in the three series in every period.

Incidence of tuberculosis: The average annual incidence of culture-positive tuberculosis (whether smear-positive or -negative) over the 15-year period was virtually the same in the placebo, low dose and high dose series, 54, 55 and 56 per 100,000, respectively (Table II). The similarity was seen in both males and females. In children of all ages, the incidence appeared to be lower in the BCG series (than in the placebo series) but in adults of all ages it was higher; but none of the contrasts was statistically significant. The incidence in the BCG series was higher in the first 5 yr, but lower in subsequent 10 yr; again, none of the contrasts was significant. The opposing directions of the differences and the consistent nonsignificance of the contrasts suggested that there was, in reality, no BCG effect, and that the observed differences were due to chance.

Findings related to strain (Danish or French): There was no difference between the French and Danish Strains of BCG, the annual incidence of tuberculosis (per 100,000) being 56 with Danish low dose, 54 with French low dose, 58 with Danish high dose and 54 with French dose (and 54 with placebo).

Effect of nontuberculous mycobacterial infection at intake: Considering first the placebo series (to avoid confounding due to BCG), the incidence was 61 per 100,000 in 23158 subjects with a nontuberculous mycobacterial infection at intake compared with 42 per 100,000 in 13246 without such an infection (Relative risk, RR = 1.45). However, the former group was considerably older, the proportions aged ≥25 yr being 20 and 3 per cent, respectively, and those aged 10-24 yr being 42 and 10 per cent. When allowance was made for this difference, the adjusted relative risk was significantly less than 1 (RR = 0.63; P=0.02), indicating a protective effect of 37 per cent (95% CI = 8 to 57%) from the nontuberculous infection. In contrast, in the BCG series, the adjusted RR for subjects with a nontuberculous infection was 0.92, close to 1 (P= 0.6).

Efficacy of BCG in subjects with and without a nontuberculous protective infection: At intake, there were 13246 placebo and 27096 BCG subjects who had neither a tuberculous nor a protective nontuberculous infection. Amongst these, the annual

Table I. Numbers in present assessment of BCG vaccine*

Period (yr)	Total	Placebo		BCG low dose		BCG high dose	
		No.	(%)	No.	(%)	No.	(%)
0 - 2.5	109863	36404	33.1	36459	33.2	37000	33.7
2.5-5	101487	33630	33.1	33638	33.1	34219	33.7
5-7.5	96238	31867	33.1	31940	33.2	32431	33.7
7.5-10	90826	30077	33.1	30114	33.2	30635	33.7
10-12.5	83339	27613	33.1	27655	33.2	28071	33.7
12.5-15	73923	24441	33.1	24570	33.2	24912	33.7

*Those with an induration of 0-7 mm to PPD-S at intake and a normal radiograph

Table II. Annual incidence of tuberculosis, by sex, age and BCG vaccination status (over a period of 15 years)

		N (all 3 series)	Annual incidence (per 100,000) of tuberculosis*			
			Placebo	BCG series		
				Both doses	Low dose	High dose
	Total	109863	54	56	55	56
Sex	Male	50715	70	74	72	76
	Female	59148	38	37	38	36
Age at intake (yr)	0-4	30710	20	16	17	15
	5-9	30271	43	33	33	33
	0-9	60981	30	24	24	23
	10-14	20186	85	80	70	90
	15-24	13571	96	124	116	132
	25-44	10776	83	102	109	95
	≥45	4349	91	104	128	80
Period (yr)	0-2.5	109863	12	21	22	19
	2.5-5	101487	20	44	52	36
	5-7.5	96238	44	33	26	40
	7.5-10	90826	77	56	57	56
	10-12.5	83339	84	72	79	65
	12.5-15	73923	85	108	94	122

*Annual incidence of culture-positive tuberculosis, irrespective of smear.
None of the contrasts between the BCG and placebo series was significant

incidence of tuberculosis was 42 per 100,000 in placebo and 30 per 100,000 in the BCG series (Table III), a protective effect of 29 per cent (adjusted RR = 0.71, *P*=0.05). Multivariate analysis, allowing for the effect of age and sex, yielded an efficacy estimate of 32 per cent (95% CI=3 to 52%, *P*=0.03), 23 per cent for the low dose (95% CI= -15 to 49%, *P* = 0.2) and 40 per cent for the high dose (95% CI = 8 to 61%, *P* =0.02). In a subset of children aged less than 15 yr, the corresponding multivariate estimate of protection was 36 per cent (*P*=0.03), while in those aged less than 10 yr it was 26 per cent (*P*=0.18).

In subjects with a protective nontuberculous infection, there was no evidence of BCG protective effect (Table III); indeed, the suggestion was of a deleterious effect (incidences of 61and 71 per 100,000 in placebo and BCG series), but this was not statistically significant (adjusted RR=1.17, *P*=0.14). In children aged less than 10 yr and those aged less than 15 yr, there was no significant effect.

Inclusion of subset of uninfected subjects from those with of 8-11 mm induration to PPD-S: Among persons with an induration of 8-11 mm to PPD-S, 16780 were classified as uninfected. Including these, the total uninfected became 126643 (42105 placebo, 84538 BCG). Similar analyses were undertaken and yielded the same conclusions, namely, no protection from BCG, the incidence being 60 per 100,000 in both

Table III. Efficacy of BCG in persons with no tuberculous infection at intake, with and without infection with nontuberculous mycobacteria

	Age (yr)	Number of persons				Annual incidence of tuberculosis*				Protection** from BCG (%)			P	Multivariate estimate	P
		Placebo	Low BCG	High BCG	BCG (low+ high)	Placebo	Low BCG	High BCG	BCG (low+ high)	Low BCG	High BCG	BCG (low+ high)			
Infected with neither *M. tuberculosis* nor	All	13246	13315	13781	27096	42	33	27	30	21	37	29	0.05	32	0.03
nontuberculous	0-9	11537	11562	11866	23428	28	25	18	21	13	39	26	0.18	26	0.18
mycobacteria[+]	0-14	12616	12638	13003	25641	36	26	21	24	28	43	35	0.03	36	0.03
Not infected with	All	23158	23144	23219	46363	61	68	74	71	-17	-21	-19	0.10	-17	0.14
M. tuberculosis but infected with	0-9	8807	9063	8506	17569	33	24	31	27	32	11	22	0.32	22	0.31
nontuberculous mycobacteria[++]	0-14	14466	14302	14142	28444	46	40	53	47	14	-11	1	0.93	3	0.86

*Annual incidence of culture-positive (irrespective of smear) tuberculosis per 100,000 over 15 years of follow up
**Estimate of protection, together with its statistical significance, was determined by Cox regression analysis
[+]S = 0-7, B<10; [++]S = 0-7, B>10

BCG and placebo series; in the subset of 40538 subjects with no protective nontuberculous infection (13303 placebo, 27235 BCG), BCG reduced the incidence by 27 per cent (P=0.06), from 42 to 30 per 100,000; the multivariate estimate of protection was 32 per cent (P=0.04).

Discussion

In the 15-year follow up of the BCG trial[1], the incidence of tuberculosis was computed using the person-years approach, which is better suited for a steady incidence situation. However, the incidence of tuberculosis is known to have decreased significantly in the community during the study period[3]. We therefore computed the incidence and death rate in each period on the available subjects after stratifying by age and sex, and combined these in a life table analysis to obtain incidence rates of tuberculosis. Further, rather than make arbitrary assumptions about the missed yield from losses in sputum coverage (no cases or same proportion as in examined subjects), we utilized the strong association between the radiographic result at the time and culture result to predict the outcome. Such an approach had been employed in the analysis of data from a national sample survey in the Philippines[5], and by us in earlier publications[3,6]. On account of these modifications, more realistic estimates of incidence have become available for the comparisons between the BCG and placebo series. In the earlier publication of the 15-year follow up[1], 560 culture-positive cases were observed in 1128696 person-years, *i.e.*, an annual incidence of 50 per 100,000. The missed cases due to failure to examine sputum was estimated to be 46, and adding these resulted in 606 new cases or an incidence of 54 per 100,000, which differed only slightly from the estimate of 55 per 100,000 in the current study.

This study reaffirmed strongly that BCG did not offer any protection against adult forms of bacillary pulmonary tuberculosis in the initially uninfected. This conclusion applies to both males and females, as well as adults of all ages and children. The conclusion with respect to children was of special interest, as an earlier report[1] had suggested that over the 15-year period, there was a low level of protection in those aged <10 yr. That inference may be amended for the following reasons. First, the incidence in the BCG series was double than that in the placebo series in the first 5 yr. Secondly, there were over 20,000 children in each of the three series, and post facto computations suggested that the sample size was large enough to detect 40 per cent overall protection with 90 per cent Power; in the event, the estimated protection was 26 per cent (95% CI= -15 to 53%), and statistically not significant (P= 0.2). The simplest interpretation would be that BCG offered no benefit to children in preventing bacillary forms of tuberculosis.

One of the reasons advanced for the failure of BCG was the influence of environmental

mycobacteria that are highly prevalent in tropical countries and which are reported to confer a certain amount of protection[7,8], constituting a kind of 'natural' vaccination that resembles vaccination with BCG. The magnitude of protection (based on univariate analysis) was reported to be 60 per cent over a 10-year period in the United Kingdom[9], and 58 per cent over 3 yr in south India[10]. This hypothesis received support from our study, as the prevalence of nontuberculous infection was about 40 per cent and the protective effect (based on multivariate analysis allowing for the effects of age and sex) was 37 per cent. Next, in the subset of subjects who did not have this initial nontuberculous infection, there was evidence that BCG offered some protection, 23 per cent with the low dose, 38 per cent with the high dose and 32 per cent for both doses combined. The gradient with dose was suggestive and the protection was just significant (P=0.04). Even so, the findings may not have much public health importance in this community, as this subset constituted less than 40 per cent of the uninfected population and the protection was only 37 per cent.

It is thus concluded that BCG has had little effect on preventing bacillary forms of tuberculosis, the incidence of which must be brought down substantially for tuberculosis control. The latter needs an efficient diagnostic service and effective treatment programme, as in the revised national tuberculosis control programme (RNTCP) with the DOTS strategy.

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