

# A Prospective Study of Bacillus Calmette-Guérin Scar Formation and Tuberculin Skin Test Reactivity in Infants in Lima, Peru

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**ABSTRACT.** *Objectives.* To determine the sensitivity of the bacillus Calmette-Guérin (BCG) scar as an indicator of previous vaccination and to ascertain the tuberculin skin test (TST) response in infancy after vaccination in a community from an area hyperendemic for tuberculosis (TB).

*Methods.* In a birth cohort of healthy term infants from Lima, Peru, a single dose of BCG vaccine was administered within the first month of life. Scar formation was assessed biweekly during the first 6 months and again at 3 years after vaccination. TST response was evaluated 6 months after vaccination.

*Results.* Six months after vaccination, 99% (68) of the newborns exhibited a BCG scar (>2 mm). Scar size did not differ by sex, birth weight, age at vaccination, or nutritional status in the first 2 months. Eighty percent of the participants were found 3 years after vaccination, and all of them had a BCG scar. Mean TST reaction size 6 months after vaccination was  $2.9 \pm 0.3$  mm. No association was found between sex or age at BCG vaccination and TST size. Only 3 children had a TST >10 mm, and the 3 had a TB contact at home.

*Conclusions.* The BCG scar was a sensitive indicator of vaccination status up to 3 years after the administration of the vaccine in the first month of life. Although nearly a quarter of the children had a TST response >5 mm 6 months after vaccination, TST reactions >10 mm did not occur in the absence of exposure to a person with tuberculosis. A cutoff of 10 mm should be used for disease control purposes in people who are born in countries where TB is endemic. *Pediatrics* 2003;112:e298–e302. URL: <http://www.pediatrics.org/cgi/content/full/112/4/e298>; tuberculosis, children, Peru, BCG scar, tuberculin skin test.

ABBREVIATIONS. TB, tuberculosis; WHO, World Health Organization; BCG, bacillus Calmette-Guérin; TST, tuberculin skin test; CI, confidence interval.

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Received for publication Jan 3, 2003; accepted Jun 10, 2003.

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Tuberculosis (TB) has been declared a global emergency by the World Health Organization (WHO) and is expected to cause 30 million deaths in the coming decade.<sup>1–3</sup> Although investigations pertaining to TB vaccines are resurging,<sup>4,5</sup> immunization against TB is limited to the bacillus Calmette-Guérin (BCG) vaccine. The WHO recommends a single BCG vaccine at birth in countries with a high prevalence of active TB disease.<sup>3,6</sup>

The presence or absence of a BCG scar is often used as an indicator of previous vaccination in clinical settings as well as surveys performed by health institutions such as the Expanded Program on Immunization to assess vaccine uptake.<sup>7</sup> However, the sensitivity of the BCG scar as an index of vaccination status is still the subject of controversy.<sup>6</sup> Failure to form a scar may be related to factors such as lack of maturation of the immune system,<sup>8</sup> faulty technique, or use of a nonpotent vaccine.<sup>9</sup>

In Peru, intradermal administration of BCG is given at birth at any health facility, such as a hospital or health post. This is part of the regular immunization program following WHO guidelines.<sup>10</sup> Since 1991, the same strain of BCG has been used across the country. In 2000, the estimated incidence of active pulmonary TB in Peru was 111 cases per 100 000,<sup>11</sup> but in one area, it has been reported to be 364 per 100 000<sup>12</sup> despite 95% coverage of BCG vaccination at birth and a human immunodeficiency virus prevalence of <0.5%.<sup>11</sup> In countries with such a high TB incidence, diagnostic algorithms include the tuberculin skin test (TST) as a screening tool, particularly in children.<sup>13,14</sup>

It has been shown that the BCG may affect the TST by factors such as strain and dose,<sup>15</sup> number of vaccines administered,<sup>16,17</sup> time since vaccination,<sup>18</sup> age and nutritional status,<sup>19</sup> and method of vaccine administration.<sup>20</sup> However, the scope of these effects is unclear. Therefore, for the TST to be interpreted in the context of potential previous BCG vaccination, there is a need for a reliable indicator of vaccine status, as well as an understanding of the effect of BCG on TST size.

Reports on the percentage of children who scar after BCG vaccination vary widely.<sup>21–26</sup> This study was conducted to determine the sensitivity of scarring after BCG vaccination in a developing country hyperendemic for tuberculosis. We describe the BCG scar formation process for 6 months after vaccine

administration in a birth cohort from a shantytown in Lima, Peru. To ascertain the effect of BCG vaccine given at birth on the TST response, we also administered a TST 6 months after vaccination. For verifying whether the scar persisted throughout time, 1 additional assessment was performed 3 years after vaccination.

## METHODS

### Study Site and Population

This study was conducted in Las Pampas de San Juan de Miraflores, a periurban shantytown 25 km south of the center of Lima. It is composed of 50 communities with an estimated population of 40 000 consisting mainly of migrants of low socioeconomic status.<sup>12</sup> In the past decade, emigration rates have decreased and families in these communities are similar in socioeconomic status, housing quality, and access to public utilities.<sup>27,28</sup> This population has been described in the past.<sup>27,29,30</sup>

Between September 1998 and February 1999, 122 women who were in their last trimester of pregnancy and attending local health posts in this shantytown were invited to participate in this study. Informed written consent was obtained from the mother at this time. Six neonates who were premature and presented serious neonatal problems requiring prolonged medical treatment or hospitalization were excluded from the study. An additional 16 newborns were not included because their mothers declined participation.

Of the 100 term neonates recruited, 18 were not located for follow-up. An additional 5 did not complete the initial 6-month follow-up, and 4 were lost to follow-up before receiving the TST. Another 4 participants were excluded because they were vaccinated after the first month of life. A total of 69 children were included in the final analyses.

### Vaccination and Follow-up

Nurses from the Peruvian Ministry of Health injected 0.1 mL of the BCG vaccine (Pasteur-Mérieux-Connaught, Lyon, France) intradermally into the deltoid region of the right arm of the neonates. During 6 months after vaccination, study nurses performed biweekly in-home assessments to reassess scar presence. Scar size was measured in both transverse and vertical planes, and the average of these was recorded in millimeters. For determining persistence of scars, an in-home visit was performed 3 years after vaccination by a nurse who was unaware of vaccination status. A "visible scar" was defined as a scar measuring 2 or more millimeters. Up to 7 additional site visits were attempted for participants who were not found during routine visits. A total of 14 infants had moved out of the community. No significant difference in age, sex, and scar size was found between the children who were found 3 years later PV ( $n = 55$ ) and those who were not found ( $n = 14$ ).

### Data Collection

A study nurse interviewed the mothers and completed a data sheet and questionnaire assessing demographics, birth history, socioeconomic status, and possible contacts with active TB for the infants at the time of vaccination. The child's birth weight was obtained from the medical record. Another abbreviated questionnaire was completed each month for the next 6 months to assess current health status and nutrition. Height and weight were measured.

### TSTs

Six months after vaccination, all 69 participants received a TST using 0.1 mL of purified protein derivative containing 5TU (Tubersol, Connaught Lab. Ltd, Willowdale, Ontario, Canada). The TST was administered by 2 study nurses to the volar surface of 1 of the forearms using the Mantoux technique. Indurations were measured 48 to 72 hours later using the pen method.<sup>31</sup> The average of the transverse and vertical plane measurements was recorded as the induration size in millimeters. Participants with reactions  $>10$  mm were considered positive reactors and were referred to local health officials for complete TB evaluation and consideration of isoniazid prophylaxis.

## Statistical Analysis

For determining the average scar size over time, measurements were classified on the basis of the time of BCG vaccination in 1-week periods. We defined 2 phases in the scar development, formation and stabilization (Fig 1). For predicting mean scar size, the formation phase was fitted using a cubic polynomial regression with days after BCG vaccination as the independent variable. The stabilization phase was fitted using a linear regression model. The random effects component was used to take into account that there were repeated measurements of the same individual.<sup>32</sup>

Nutritional status indicators during the first 6 months, height-for-age and weight-for-height, were calculated according to the WHO reference population using EPINUT from Epi-Info 6.04.<sup>33</sup> Degrees of stunting were classified according to height-for-age  $z$  score as mild ( $z$  score  $<-1$ ) or moderate to severe ( $z$  score  $<-2$ ). Wasting was defined as a weight-for-height  $z$  score of  $<-2$ .

The effect of age at vaccination, sex, and birth weight on BCG scar size and TST reaction size was examined using  $\chi^2$ ,  $t$  tests, and Pearson correlation coefficient. All tests were calculated in a 2-tailed manner, and confidence intervals (CIs) were set at 95%. SPSS (SPSS, Inc, Chicago, IL) and STATA 7.0 (Stata Corp, College Station, TX) were used for data analyses.

## Ethical Approval

This study was approved by the Ethical Review Boards of Johns Hopkins University Bloomberg School of Public Health and Asociación Benéfica PRISMA (Peruvian NGO), both of which have US Federal Wide Assurance approval.

## RESULTS

### Participant Population

Of the 69 newborns analyzed in the study, 56% (38) were male. Mean birth weight was  $3.3 \pm 0.4$  kg. Three children (4.5%) were classified as low birth weight ( $<2.50$  kg). Median age at BCG vaccination was 5 days, with values ranging from 0 to 29 days.

### Scar Formation and Scar Size

Sixty-eight children exhibited a visible BCG scar ( $>2$  mm) within the first 6 months after vaccination, representing a scar failure rate of 1.4%. The child who did not form a scar during this period was a girl of normal birth weight (3.0 kg) who was vaccinated at 20 days of age. Thirty-one children (45%) were vaccinated within the first 48 hours after birth. There was no significant difference in scar size between these infants and the rest of the group ( $P = .248$ ).

After analyzing scar size by weekly periods, we identified 2 stages in the scar formation process: the formation phase and the stabilization phase (Fig 1). The formation phase, during which the scar size increased steadily, lasted on average 7.5 weeks after vaccination (Fig 2). The subsequent stabilization phase continued throughout the first 6 months of life.

Fifty-five (80%) of the participants were found 3 years after vaccination for scar presence assessment. All had a scar at this time, including the girl who initially did not form a scar. This infant showed scar presence at weeks 19 and 21 with a final scar size of 2 mm at 3 years after vaccination. Scars 3 years after vaccination were approximately 1.7 mm larger than at 6 months after vaccination (95% CI: 1.4–2.1 mm; Fig 3).

### Nutritional Status and BCG Scar

No correlation was found between nutritional status within the first 2 months after birth and BCG scar

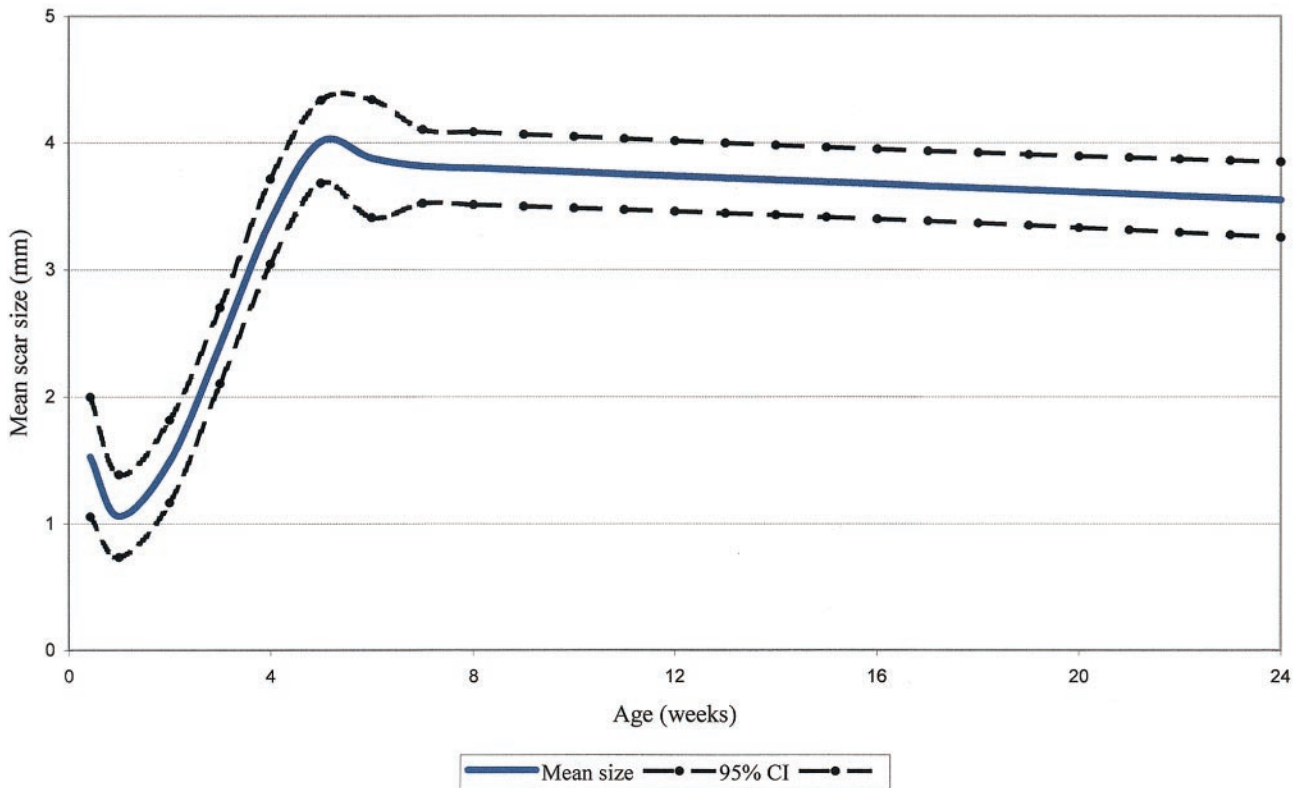


Fig 1. Mean BCG scar size during the first 6 months in infants from a hyperendemic area for tuberculosis in Lima, Peru, vaccinated during the first month of life.



Fig 2. BCG scar at 7.5 weeks after vaccination at birth.



Fig 3. BCG scar at 6 months after vaccination at birth.

size: weight-for-height ( $r = 0.151, P = .233$ ), height-for-age ( $r = -0.050, P = .694$ ; Table 1).

#### TSTs

Mean TST reaction size 6 months after vaccination was  $2.9 \pm 0.3$  mm; 3 (4%) children had a positive reaction ( $>10$  mm), and 20% had reaction sizes be-

tween 5 and 9 mm (Fig 4). There was no association between sex and TST size ( $P = .475$ ;  $t$  test). No significant correlation was found between the infants who were vaccinated within 48 hours after birth and those who were vaccinated afterward and TST size. The 3 positive reactors had a history of contact with

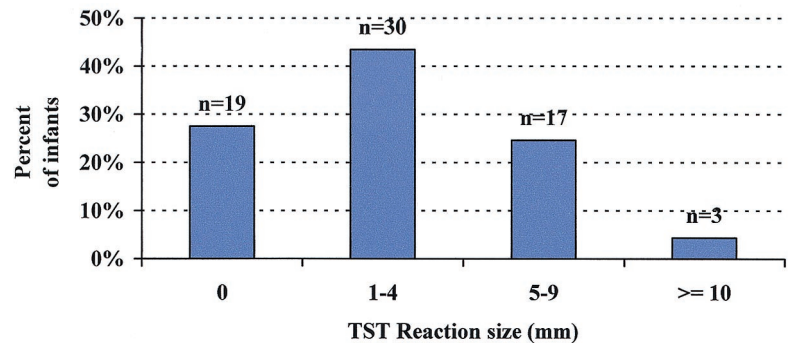


**TABLE 1.** Effects of Birth Weight, Sex, Known TB Contact, TST Positivity (10 mm and 5 mm cutoff), and Nutritional Status on BCG Scar Size During the First 6 Months After Vaccination, TST Reaction, and BCG Scar Size 3 Years After Vaccination

Characteristic	N = 69	Mean Scar Size at 6 Months PV (P Value) <sup>β</sup>	Mean TST Size at 6 Months PV (P Value) <sup>β</sup>	N = 55	Mean Scar Size at 3 Years PV (P Value) <sup>β</sup>
Birth weight*		(.861)	(.993)		(.168)
Normal	64	3.4 ± 0.1	3.0 ± 0.3	3	5.2 ± 1.6
Low	3	3.3 ± .01	3.0 ± 0.2	50	3.8 ± 1.5
Sex		(.220)	(.475)		(.854)
Male	38	3.6 ± 0.1	3.2 ± 0.3	31	5.1 ± 1.9
Female	30	3.2 ± 0.1	2.6 ± 0.3	24	5.0 ± 1.3
Age at vaccination		(.113)	(.870)		(.093)
≤48 h	31	3.7 ± 0.1	3.0 ± 0.3	26	5.5 ± 1.6
3–29 d	38	3.2 ± 0.1	2.9 ± 0.3	29	4.8 ± 1.6
TB contact†		(.004)	(.191)		(.300)
Yes	21	4.0 ± 0.1	3.6 ± 0.4	16	5.5 ± 1.8
No	48	3.2 ± 0.09	2.6 ± 0.3	39	5.0 ± 1.6
TST reaction		(<.001)	(<.001)		(.438)
≥10 mm	3	5.7 ± 0.2	10.3 ± 0.05	2	6.0 ± 0.1
0–9 mm	66	3.3 ± 0.1	2.6 ± 0.3	53	5.1 ± 1.6

PV indicates postvaccination.

**Fig 4.** TST reaction size 6 months after vaccination among infants who were vaccinated with BCG at birth.



a person with active TB at home ( $P < .001$ ,  $t$  test). They also had significantly larger BCG scars when compared with the TST-negative group ( $P < .001$ ,  $t$  test). These 3 children had chest radiographs taken as part of their subsequent medical evaluation, all of which were normal.

## DISCUSSION

In our study, the BCG scar in healthy term infants was a sensitive indicator of vaccination status when administered within the first month of life. Scar presence persisted 3 years after vaccination. When adolescents from this same community with vaccination records were examined for BCG scar presence, a similar scar failure rate was found (0.8%; CI: 0.02%–4.18%, E.M.S., unpublished observations). These results are similar to other studies showing low scar failure rates in South Africa,<sup>34</sup> Sri Lanka,<sup>35</sup> and India.<sup>36,37</sup> In other studies, reported scar-failure rates ranged from 8% to 16% when children were immunized soon after at birth.<sup>21–26</sup>

Similar to our findings, another study observed that the BCG scar is formed within the first 6 months after vaccination.<sup>38</sup> This study did not, however, document the progression of scar formation. We observed that the defining stages in the scar formation process took place particularly during the first 8 weeks, after which the scar stabilized. High repeatability of BCG scar measurements with increasing time after vaccination has also been reported before, signifying that the character of the scar stabilizes over time.<sup>21,38</sup>

Another aspect that underscores the importance of assessing BCG scar presence is its relation to TST reactivity. Our results demonstrate that a TST applied after BCG vaccination usually produces a reaction of <10 mm. This is consistent with studies that show an association between TST reactions 5 to 9 mm and the presence of a BCG scar.<sup>13,39,40</sup> We also found an association between TSTs >10 mm and contact with a person with active TB. This suggests that TST positivity of >10 mm among these infants was associated with TB exposure rather than with other factors (eg, age, nutritional status, time since vaccination). These 3 infants also had significantly larger scars than the rest of the group. This finding may suggest that other than having had previous exposure to *Mycobacterium tuberculosis*, these children might have been hyperresponders to mycobacterial antigen.

In the United States, TB cases among the foreign-born accounted for the majority of the cases (51%) in 2002.<sup>41</sup> Considering that TB disease and latent infection rates differ among countries,<sup>41,42</sup> we recommend that in foreign-born individuals with evidence of BCG at birth, only a TST reaction >10 mm should be considered evidence of previous *M tuberculosis* exposure.

Despite WHO efforts to standardize BCG vaccination by stabilization and lyophilization,<sup>25</sup> considerable microbiologic and genetic differences still exist among BCG strains.<sup>43</sup> Also notable are the discrepancies in numbers and proportions of viable and dead organisms according to dose.<sup>44</sup> These differ-

ences could account for numerous variations in reactivity and immunogenicity.

Finally, we found no association between nutritional status and scar formation. Although children with serious neonatal problems, including severe malnutrition, were excluded from our study, many of the participants were stunted at some point during the observation period. However, this did not impair scar formation.

## CONCLUSIONS

Our results show that infants who were vaccinated within the first month of life nearly always formed a scar. Thus, a BCG scar was a sensitive marker of vaccination status. Because nearly a quarter of the children had a TST response of >5 mm 6 months after vaccination, a cutoff of 10 mm should be used for disease control purposes.

## ACKNOWLEDGMENTS

Funding for this project was provided by the National Institutes of Health ITREID Training Grant TW00910, the USAID TB Research Grant HRN-A-0096-90006-00, the Training Grant T35 AI07646-01, and the anonymous RG-ER Fund for the advancement of research in tropical medicine.

We thank Dr Carlton Evans and Dr Daniel E. Roth for comments and support.

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DOI: 10.1542/peds.112.4.e298

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