Nonspecific (Heterologous) Protection of Neonatal BCG Vaccination Against Hospitalization Due to Respiratory Infection and Sepsis

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(See the Editorial Commentary by Iglesias and Martin on pages 1620–1.)

Background. Bacille Calmette-Guerin (BCG) vaccination has been suggested to have nonspecific beneficial effects in children from developing countries, reducing morbidity and mortality caused by unrelated pathogens.

Objective. We aimed to assess the heterologous protective effects of BCG vaccination against respiratory infection (RI) and sepsis not attributable to tuberculosis in children born in Spain.

Methods. We conducted a retrospective epidemiological study using data from the Official Spanish Registry of Hospitalizations (CMBD-HA) to identify differences in hospitalization rates (HR) in BCG-vaccinated children (Basque Country, where neonatal BCG is part of the immunization schedule and has a 100% coverage) as compared to non-BCG-vaccinated children (from the rest of Spain, where BCG is not used).

Results. A total of 464,611 hospitalization episodes from 1992 to 2011 were analyzed. The HR due to RI not attributable to tuberculosis in BCG-vaccinated children was significantly lower compared to non-BCG-vaccinated children for all age groups, with a total preventive fraction (PF) of 41.4% (95% confidence interval: 40.3–42.5; P-value <.001). According to age group, PF was 32.4% (30.9–33.9; P-value <.001) for children under 1 year old, 60.1% (58.5–61.7; P-value <.001) for children between 1 and 4 years old, 66.6% (62.8–70.2; P-value <.001) for children between 5 and 9 years old, and 69.6% (63.3–75.0; P-value <.001) for children between 10 and 14 years old. The HR due to sepsis not attributable to tuberculosis in BCG-vaccinated children under 1 year of age was also significantly lower, with a PF of 52.8% (43.8–60.7; P-value <.001).

Conclusions. BCG vaccination at birth may decrease hospitalization due to RI and sepsis not related to tuberculosis through heterologous protection.

Keywords. BCG vaccination; nonspecific effects; heterologous effects; children.

The Bacille Calmette-Guerin (BCG) vaccine is one of the most widely used childhood immunizations [1], particularly in developing countries where tuberculosis is a leading cause of human disease and death. The BCG vaccine has a documented protective effect against meningitis and miliary tuberculosis in pediatric patients [2], but in recent years the scientific community has drawn attention to its heterologous (ie, nonspecific) protective effects [3]. These include risk reductions in cancer [4], allergy [5], infections caused by different pathogens [6], and overall childhood mortality [7], as the immune nonspecific effects seem to be more marked in younger individuals [8]. Nevertheless, the incidence of tuberculosis

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has declined, and most European countries debate the convenience of removing BCG from their vaccination schedule. Subsequent studies have suggested that this may have led to increased incidence of a wide range of conditions, such as atopic dermatitis [9], asthma, acute lower viral respiratory infections (RI) [10], malignant melanoma [11], and lymphoma [12].

In 2012, Kleinnijenhuis et al. [13] provided an immunological explanation for the protective nonspecific effects of BCG when they documented that it enhances the release of monocyte-derived cytokines in response to unrelated bacterial and fungal pathogens.

BCG vaccination was part of the national immunization program (NIP) in Spain until 1982 and thus administered to every newborn immediately after birth. Since then, only one Spanish region, the Basque Country (BC), has continued to administer BCG routinely as part of the NIP, with an uptake rate of almost 95%. Considering this unique setting and the importance of clarifying the nonspecific effects of BCG, we conducted an epidemiological study to assess the hospitalization rate (HR) due to RI and sepsis not attributable to tuberculosis in children <14 years of age in the BC, as compared to the rest of Spain.

METHODS

This is a retrospective epidemiological and hospital-based surveillance study covering a period of 15 years (from 1997 to 2011) performed using a national registry database of hospitalization. We aimed to assess differences in HR due to selected pathologies between BCG-vaccinated and non-BCG-vaccinated children in Spain. In Spain we have 2 distinctly different populations with regards to BCG vaccination: one (in the BC) where BCG is administered at birth; and the other (in the rest of Spain) where BCG is not administered due to different health policies. In order to avoid possible biases related to the geographic distribution and/or environmental influence on the epidemiology of the assessed pathologies, comparisons between the BC and the neighboring regions (Cantabria, Navarra, and La Rioja) and between the BC and the nearby coastal regions (Galicia, Cantabria, and Asturias) were also performed.

Source of Data

The Official Spanish Health Service surveillance system for hospital data, known as Conjunto Mínimo de Datos (CMBD-HA, Minimum Set of Acute Hospitalizations Database) was used as the data source. This database includes personal, administrative and medical data of all patients admitted to hospitals in every administrative region of Spain, with diagnoses codified according to the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM). The CMBD-HA is publicly available, and data are made anonymous. Ethics committee approval was obtained prior to the start of the study.

Selection Criteria and Data Collection

Children aged <15 years that were admitted to any hospital of the Spanish Health Service hospital network with one or several of the following diagnosis items were considered eligible for the study: upper or lower RI and sepsis not attributable to tuberculosis, and tuberculosis disease.

Our team designed a search strategy by selecting specific disease category codes according to the ICD-9-CM, as follows: RI included common cold (ICD-9-CM code 460), acute sinusitis (ICD-9-CM code 461), acute pharyngitis (ICD-9-CM code 462), acute tonsillitis (ICD-9-CM code 463), acute laryngitis and tracheitis (ICD-9-CM code 464), acute upper respiratory infection of multiple or unspecified sites (ICD-9-CM code 465), bronchitis and bronchiolitis (ICD-9-CM code 466), viral pneumonia (ICD-9-CM code 480), pneumococcal pneumonia (ICD-9-CM code 481), other bacterial pneumonia (ICD-9-CM code 483), pneumonia caused by nonspecific microorganism (ICD-9-CM code 483 to 486) flu (ICD-9-CM code 487 and 488), empyema (ICD-9-CM code 510) and lung abscess (ICD-9-CM code 513); tuberculosis included primary tuberculosis (ICD-9-CM code 010), pulmonary tuberculosis (ICD-9-CM code 011), other respiratory tuberculosis (ICD-9-CM code 012), tuberculous meningitis (ICD-9-CM code 013), gastrointestinal tuberculosis (ICD-9-CM code 014), osteo-articular tuberculosis (ICD-9-CM code 015), genito-urinary tuberculosis (ICD-9-CM code 016), tuberculosis in other organs (ICD-9-CM code 017), and miliary tuberculosis (ICD-9-CM code 018). Sepsis not attributable to tuberculosis included sepsis (ICD-9-CM code 38). The number of admissions for each age group included only cases born after 1982, which is the year when routine administration of BCG was halted in Spain outside the BC.

Statistical Analysis

A Fisher exact test [14] was employed to study the relationship between HR for the selected diseases and the geographic location where the patients were born (BC vs the remaining regions of Spain). Preventive fractions (PF) with the corresponding 95% CI are shown in Table 1 (PF higher than 0 means that there is a lower HR rate in the BC). PF were calculated for each of the diagnosis categories considered. PF gives the percentage of cases that can be prevented if a population is exposed to an intervention (ie, neonatal BCG vaccination) compared to an unexposed population, and it was calculated as 1-odds ratio. Scenarios with <10 patients were not considered. A nominal significance level was set to P-value <.05. Considering that several pathologies and age groups were tested, a conservative approach of multiple test correction of significance level was applied using a Bonferroni correction test, dividing the significance level by the number of tests in each of the comparisons [15].
An additional confirmation analysis was performed comparing the BC with its neighboring regions only (see Table 2) and the coastal regions. This analysis aimed to control for any geographic and/or environmental influence other than BCG vaccination. The statistical analyses were performed using R version 3.0.2 [16].

RESULTS

A total of 464,611 hospitalization episodes fulfilling the inclusion criteria were retrieved and analyzed. The main results are summarized in Tables 1–4.

Analysis of BCG-vaccinated (BC) vs non-BCG-vaccinated Children (the Rest of Spain)

- Risk of hospitalization due to RI not attributable to tuberculosis (Figure 1A)

A total of 446,915 hospitalizations in children <15 years old with RI not attributable to tuberculosis were retrieved. More than 90% of admissions due to this cause occurred in children under 4 years old, and 11,028 (2.5%) of the records come from the BC.

In the BC, HR related to RI not attributable to tuberculosis were lower than in the rest of Spain, with a total PF of 41.4% (95% CI, 40.3–42.5; P-value <.001) for the studied pediatric population. According to age groups, HR showed a significant decrease for all ranges in the BC compared to the rest of Spain, with a PF of 32.4% (95% CI, 30.9–33.9; P-value <.001) for children under 1 year old; 60.1% (95% CI, 58.5–61.7; P-value <.001) for children between 1 and 4 years old; 66.6% (95% CI, 62.8–70.2; P-value <.001) for children between 5 and 9 years old; and 69.6% (95% CI, 63.3–75.0; P-value <.001) for children between 10 and 14 years old.

- Risk of hospitalization due to sepsis not attributable to tuberculosis (Figure 2A)

A total of 10,384 hospitalizations in children under 15 years old with sepsis not attributable to tuberculosis were retrieved. Over 85% of admissions due to this cause occurred in children under 4 years old, and 280 (2.7%) records were from the BC.

In the BC, HR related to sepsis not attributable to tuberculosis were significantly lower than in the rest of Spain, with a total PF of 35.7% (95% CI, 27.5–43.1; P-value <.001) for the studied pediatric population. According to age groups, HR showed a significant decrease only for children under one year old in the BC compared to the rest of Spain, with a PF of 52.8% (95% CI, 43.8–60.7; P-value <.001).

- Risk of hospitalization due to tuberculosis infection (Figure 3A)

A total of 7312 hospitalizations in children under 15 years old with tuberculosis disease were retrieved. There
Table 2. Comparison of Hospitalization Rates Between Basque Country and Frontier Regions

<table>
<thead>
<tr>
<th>Pathologies</th>
<th>1 y Old</th>
<th>2–4 y Old</th>
<th>5–9 y Old</th>
<th>10–14 y Old</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF (95% CI)</td>
<td>P Value</td>
<td>PF (95% CI)</td>
<td>P Value</td>
<td>PF (95% CI)</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>21.7% (19.5, 23.9)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.0% (33.8, 40.1)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.5% (39.0, 53.2)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>36.1% (20.7, 48.7)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.8% (–61.4, 9.6)</td>
<td>.197</td>
<td>18.7% (–90.5, 26.2)</td>
</tr>
<tr>
<td>Tuberculosis disease</td>
<td>72.1% (58.9, 81.6)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.0% (49.4, 83.1)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.2% (31.3, 74.2)</td>
</tr>
<tr>
<td>Child mortality</td>
<td>8.7% (–20.6, 30.7)</td>
<td>.536</td>
<td>2.8% (–29.6, 26.9)</td>
<td>.833</td>
<td>8.1% (–28.3, 34.0)</td>
</tr>
</tbody>
</table>

Results are expressed as preventive fraction (PF) with 95% confidence intervals (CIs) and P-values.  
<sup>a</sup> Significant with Bonferroni correction.

Table 3. Comparison of Hospitalization Rates Between Basque Country and Galicia, Asturias, and Cantabria Considered as One Region

<table>
<thead>
<tr>
<th>Pathologies</th>
<th>1 y Old</th>
<th>2–4 y Old</th>
<th>5–9 y Old</th>
<th>10–14 y Old</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF (95% CI)</td>
<td>P Value</td>
<td>PF (95% CI)</td>
<td>P Value</td>
<td>PF (95% CI)</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>44.9% (43.4, 46.2)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.7% (61.0, 64.4)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.7% (67.0, 74.0)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>79.2% (75.0, 82.8)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.8% (53.6, 70.5)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.2% (19.0, 62.4)</td>
</tr>
<tr>
<td>Tuberculosis disease</td>
<td>86.4% (80.5, 90.8)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.6% (73.4, 90.5)</td>
<td>&lt;.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.7% (50.1, 79.9)</td>
</tr>
<tr>
<td>Child mortality</td>
<td>–7.8% (–35.3, 14.4)</td>
<td>.528</td>
<td>–7.6% (–35.2, 14.7)</td>
<td>.525</td>
<td>15.6% (–9.1, 35.1)</td>
</tr>
</tbody>
</table>

Results are expressed as preventive fraction (PF) with 95% confidence intervals (CIs) and P-values.  
* Significant with Bonferroni correction.
were 81 (1.1%) records for the BC. More than 85% of admissions due to this cause occurred in children older than 1 year of age.

In the BC, HR related to tuberculosis were significantly lower than in the rest of Spain, with a total PF of 74.0% (95% CI, 67.6–79.4; P-value < .001) for the studied pediatric population. According to age groups, HR showed a significant decrease for all children older than 1 year of age in the BC compared to the rest of Spain, with a PF of 77.6% (95% CI, 68.3–84.7; P-value < .001) for children between 1 and 4 years old; 75.3% (95% CI, 60.8–85.4; P-value < .001) for children between 5 and 9 years old and 61.4% (95% CI, 42.4–75.4; P-value < .001) for children between 10 and 14 years old.

- Child mortality rate (Figure 4A)

A total of 10,707 deaths in children under 15 years old were retrieved; 417 (3.9%) of which took place in the BC. No differences in child mortality rates were observed between the BC and the rest of Spain for any age group.

Analysis of the BC vs Frontier Regions
In order to confirm the previous results and to avoid possible biases related to the geographic distribution and/or environmental influence on the epidemiology of the assessed pathologies, a comparison between the BC and the neighboring regions (Cantabria, Navarra, and La Rioja) was also performed. Results are shown in Figures 1B, 2B, 3B, and 4B.

In the BC, HR related to respiratory infection not attributable to tuberculosis (Figure 1B), and tuberculosis disease (Figure 3B) were significantly lower than in the neighboring regions, with total PF of 36.9% (95% CI, 35.2–38.5; P-value < .001), and 79.9% (95% CI, 74.2–84.5; P-value = .019), respectively, for the studied pediatric population statistical difference has been
found for sepsis and children under 1 year of age (95% CI, 33.8% 15.2, 48.3; \( P \)-value = .001). No differences in the child mortality rate were observed between the BC and the neighboring regions for any age group (Figure 4B).

This same comparison was performed between the BC and nearby coastal regions (Galicia, Cantabria, and Asturias) with similar findings (see Table 3).

**DISCUSSION**

To the best of our knowledge, the present population-based survey over a 15-year time period is the first epidemiological study to test the heterologous protective effects of neonatal BCG vaccination against nontuberculous infections in children from a developed country. In particular, our study shows that neonatal
BCG vaccination might have an additional protective effect against moderate to severe forms of RI and sepsis requiring hospitalization.

BCG vaccination was part of the national immunization in Spain until 1982, when this standard practice was withdrawn and routine BCG vaccination no longer recommended. Since then, this vaccine is only administered to all neonates in the BC, with an uptake rate of almost 95% (http://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/coberturas.htm). This situation has led to a unique scenario that allows the assessment of differential immunization practices regarding the use of BCG. It should be noted that, except for BCG, the immunization schedule is the same in all of the different administrative regions, following the Interterritorial Council recommended immunizations for Spain (https://www.msssi.gob.es/ciudadanos/proteccionSalud/infancia/vacunaciones), which includes the administration of hepatitis B, tetanus and diphtheria toxoids and acellular pertussis, haemophilus influenzae type b (Hib), inactivated-poliovirus, measles, mumps, and rubella and meningococcal C antigens. In addition, all regions have almost the same rate of vaccination coverage, ranging from 90% to 100% depending on the age / antigen [Statistical data – vaccine uptake]. (https://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/coberturas.htm). On account of this, except for BCG, the immunization schedule is the same in all of the different administrative regions, following the Interterritorial Council recommended immunizations for Spain. Most strikingly, the data show an average 40% decrease in HR due to any respiratory tract infection and a 36% decrease in sepsis HR in children of the BC compared to the rest of Spain. The epidemiological differences tend to dilute with age, more clearly in sepsis admission, suggesting a time-limited impact of this benefit of BCG vaccination. An alternative explanation for this finding may be the fact that the number of hospitalization episodes for the selected pathologies was smaller with age and, thus, the power of our study to find significant differences in older children may be lower.

Furthermore, in order to control for the possible influence of environmental or geographic factors in the results, we conducted an additional analysis of the BC data as compared to the surrounding regions and the findings were the same.

As expected, the HR due to tuberculosis disease were on average 74% lower in the BC than in the rest of Spain in general, and also, specifically, lower than in neighboring or nearby coastal regions of the BC. Most strikingly, the data show an average 40% decrease in HR due to any respiratory tract infection and a 36% decrease in sepsis HR in children of the BC compared to the rest of Spain. The epidemiological differences tend to dilute with age, more clearly in sepsis admission, suggesting a time-limited impact of this benefit of BCG vaccination. An alternative explanation for this finding may be the fact that the number of hospitalization episodes for the selected pathologies was smaller with age and, thus, the power of our study to find significant differences in older children may be lower.

Epidemiological studies and randomized trials in Africa have shown that the BCG vaccine reduces child mortality [17, 18], mainly by preventing neonatal sepsis and RI not attributable to tuberculosis [19, 20]. Our results show the same reduction in RI (irrespective of age) and sepsis (during the first year of life). However, no differences in child survival were observed. As mortality (both overall, and specifically attributable to sepsis and RI) is significantly lower in Spain (and developed countries in general) compared to Africa, there is no sufficient statistical power to detect variation in this setting. This means that a potential difference in mortality is possible but may remain undetected as the size of the effect would be small.
Since the introduction of BCG to protect against tuberculosis, physicians have increasingly reported unintended effects of this vaccine on the response of the patients to unrelated infections [21, 22]. In the last 10 years, immunological studies indicate that BCG alters the immune response to nontuberculous pathogens; for instance Ota et al described in 2002 the way in which BCG could influence the antibody and cytokines responses to human neonatal vaccination [23], and Mathurin et al observed in 2009 that CD4 T-cell mediated heterologous immunity between mycobacteria and poxviruses [24]. More recently, in 2012, Kleinnijenhuis et al demonstrated that BCG induces heterologous protection against unrelated pathogens via epigenetic reprogramming of monocytes, thus highlighting the need to assess both the specific and nonspecific effects of vaccines.

In addition, other epidemiological studies suggest that the heterologous effects are more marked in younger individuals, considering the early influence on neonatal sepsis in particular. This finding is consistent with an effect on innate immune mechanisms [25–28], as neonates and children under 1 year suffer from many deficits in innate immune function due to immaturity. It has also been described that BCG immunization at birth influences the antibody response to routine immunizations administered later in infancy.

Some considerations related to the limitations of this study should be addressed. Considering that data retrieved from the CMBD-HA are made anonymous, it is impossible to identify if the same child has been hospitalized more than once. However, we assume that any contribution of readmitted cases to the result would likely be small and similar across the Spanish regions. Lack of notification of a particular disease could represent another limitation of the database; but this factor is expected to affect all the different Spanish regions considered uniformly. Moreover, it is important to note that the CMBD is a mandatory register with an estimated coded valid discharge rate of almost 100% without significant variations within Spain (http://www.msssi.gob.es/estadEstudios/estadisticas/cmbd.htm). To counterpoint possible differences in hospitalization policies and codification practices, we selected broad related groupings and potentially overlapping codes. The number of hospital beds per capita in the BC is slightly higher than the average in Spain (3–3.5 vs 2.5–2.75 / 1000 people) which might make inpatient management more probable in BC, and thus underestimate the beneficial effects of BCG vaccination on HR. Furthermore, our study is focused in hospitalized patients, and care should be taken regarding extrapolations made on the possible impact of BCG vaccination on the global epidemiology of the analyzed entities. Hospitalization policies are not universal for the hospital system in Spain and may have varied over the study period between and within regions, which may indeed affect the results. However, the tuberculosis-related admission rate served as a positive control reinforcing the validity of our findings. Finally, genetic differences between the populations compared could be raised to explain our results. Despite the fact that the control of genetic influences is beyond the scope and design of our study, a recent study [29] using a dense genome-wide SNP array was unable to show any genetic distinctiveness in the BC compared to the rest of the Iberian populations.

In summary, the present study supports the association of neonatal BCG vaccination with a lower risk of hospitalization due to RI and sepsis not attributable to tuberculosis. Although we did not find a significant effect on child mortality, the study suggests that BCG vaccine may have significant beneficial effects on the health of pediatric patients from a developed country. Additional research is needed to define the path toward obtaining unequivocal evidence on these issues that would support future robust, evidence-based adjustments in immunization policies.

Notes

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Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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