Non-specific effects of BCG vaccination on neutrophil and lymphocyte counts of healthy neonates

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Abstract

BCG vaccination is known to reduce neonatal mortality from infections in a pathogen-agnostic manner, likely by inducing emergency granulopoiesis. In this observational study we report on whether an emergency granulopoietic response is elicited in term babies from a developed country following BCG vaccination.

We studied a cohort of neonates re-admitted to the hospital from home for feeding support. Those born >33+6 weeks of gestation who had a full blood count taken within 10 days of initial discharge from hospital were included in the study. Neonates were separated into 2 groups dependent on whether they had received BCG vaccination after birth. Clinical data including gender, weight, gestational age, method of feeding and full blood count results were retrieved retrospectively.

While lymphocyte counts increase following BCG vaccination irrespective of gender and in proportion with the time elapsed after vaccination, the increase in neutrophil counts, or emergency granulopoiesis, is only observed in boys who received BCG. Girls seem to have a higher baseline neutrophil count at around five days of life even without having received BCG immunisation. This latter increase, however, appears to be temporary as neutrophil counts decline with an increase in time elapsed from the BCG administration. The type of milk received did not seem to influence the above effects of BCG vaccination.

Our results confirm the presence of emergency granulopoiesis following BCG vaccination in a neonatal cohort from a developed country. However, this effect appears to be gender-specific and is present only in boys.

Keywords: developed country, emergency granulopoiesis, infection, severe combined immunodeficiency, TREC
Introduction

Infection is a major cause of neonatal mortality worldwide, causing around 1.4 million deaths every year. In developed countries, such as the UK, it is responsible for 7% of neonatal deaths, particularly affecting preterm neonates. Although a theoretical option for the prevention of neonatal infections is vaccination, there are no available vaccines that target the most common causes of neonatal infection. Even if there were, they are unlikely to offer immediate benefit against the targeted pathogen in the first few days after birth, when the risk of neonatal infection is highest due to the time taken for a protective immune response to develop.

There is emerging evidence that neonatal vaccination may have a non-pathogen specific impact on neonatal outcomes. In recent years, studies have found that administering the Bacillus Calmette-Guérin (BCG) vaccine reduced neonatal mortality from other infections, besides tuberculosis (TB), in a non-specific or pathogen-agnostic manner. A 2019 meta-analysis of three randomised controlled trials, with a total of 6583 babies analysed, found that neonatal mortality was significantly reduced in those who had received the BCG vaccination compared to those who had not. This protection developed within three days, much more rapidly than pathogen-specific immunity.

The causative mechanism of this effect is not well understood. It is thought to be due to the activation of the innate immune system, in particular neutrophil cells, or to the cross-reactivity of T cells. BCG vaccination triggered a rapid increase in circulating neutrophil counts in a mouse model by inducing emergency granulopoiesis. This mechanism may contribute to its protective effect in human neonates, but there is limited evidence for this, particularly in neonates born in developed countries.

Understanding the pathogen-agnostic protective effects of neonatal BCG immunisation against neonatal mortality is very relevant and timely in developed countries, as considerations for delaying BCG vaccination until after the neonatal period emerge. In this setting, introduction of neonatal TREC screening programs for severe combined immunodeficiency, raises the possibility of delaying
immunisation with live vaccines, such as BCG, until after the results of the TREC screening are available. Studies into the effect of BCG on the human neonatal immune system and its potential protective effect in relation to neonatal mortality are therefore acutely needed to either support or counter this public health strategy.

In this observational study we report on whether an emergency granulopoietic response is elicited in neonates from a developed country by investigating changes in neutrophil and lymphocyte counts following BCG vaccination.

Methods

Neonates re-admitted from home to the neonatal transitional care unit for feeding support at Birmingham Women’s Hospital between 1st January 2018 and 12th November 2020 were identified using electronic patient records. Those born >33+6 weeks of gestation who had a full blood count taken within 10 days of initial discharge from hospital were included in the study. Neonates with clinical or biochemical evidence of infection (C-reactive protein >10, positive microbiology), confirmed maternal infection (positive microbiology) or documented maternal pre-eclamptic toxaemia, endocrine or autoimmune disease were excluded. Demographics are summarized in Table 1.

Neonates were separated into 2 groups dependent on whether they had received the Bacillus Calmette-Guerin (BCG) vaccination before the initial discharge from hospital, using the Danish strain 1331 (BCG Vaccine AJV, Copenhagen, Denmark). As per national guidance, BCG immunisation was offered to all neonates whose parents or grandparents were born in a country where the annual incidence of Tuberculosis (TB) is ≥40/100,000. Clinical data including gender, weight, gestational age, method of feeding and full blood count results were retrieved retrospectively from electronic and paper clinical notes. Full blood counts
were measured according to standard diagnostic methods using a Horiba ABX Pentra Nexus DX (Montpellier, France) haematological analyser. As per Health Research Authority criteria, this study did not require ethical approval, as it was classified as a service evaluation rather than a research project.

Statistical analysis was performed using non-parametric tests, including Mann-Whitney and Kruskal-Wallis tests as well as Spearman’s correlations.

**Results**

The two groups of neonates were comparable for birthweight, gestation, gender and type of feeding. The times of discharge after delivery, readmission to hospital and blood sampling were also comparable. The median time of BCG administration was the first day of life (1 (1-1) DOL), while the median time of blood sampling following BCG administration was the fourth day (4 (4-5) days).

We first analysed cell counts between the BCG and non-BCG groups. While neutrophil and platelet counts were comparable, white blood cell and lymphocyte counts were higher in the BCG group compared to neonates who did not receive BCG (Figure 1).

Next, we studied the effect of gender on the cell counts. Interestingly, neutrophil and white blood cell counts were higher in boys who received BCG compared to those who did not, while it was comparable in girls. Lymphocyte counts showed a tendency to be higher in both boys and girls who received BCG vaccination compared to those who did not (Table 2).

While neutrophil and white blood cell counts were comparable between boys and girls within the BCG group, boys who did not receive a BCG had lower neutrophil and white blood cell counts compared to girls in this group. Lymphocyte counts were comparable between boys and girls within the BCG and non-BCG groups, respectively (Table 2).
Correlation analysis revealed a positive association between the lymphocyte count and the number of days elapsed between BCG and blood sampling both in the whole BCG group as well as within boys and girl, respectively. Furthermore, neutrophil counts showed a tendency to negatively correlate with the number of days elapsed after BCG, but only in boys (Figure 2).

Finally, we also examined the impact of the type of milk received on the cell counts. No difference was observed between feeding subgroups in the two cohorts or between BCG and non-BCG neonates receiving either breastmilk, formula milk or mixed feeding.

Discussion

Our results indicate that while lymphocyte counts increase following BCG vaccination irrespective of gender and in proportion with the time elapsed after vaccination, the increase in neutrophil counts, or emergency granulopoiesis, is only observed in boys who received BCG, as girls seem to have a higher baseline neutrophil count at around five days of life even without having received BCG immunisation. This latter increase, however, appears to be temporary as neutrophil counts decline with an increase in time elapsed from the BCG administration. The type of milk received did not seem to influence the above effects of BCG vaccination.

Brook et al. demonstrated that BCG administration was protective against neonatal polymicrobial sepsis in a mouse model, inducing the production of granulocyte colony-stimulating factor (G-CSF) within hours of administration. This in turn resulted in emergency granulopoiesis, and a marked increase in neutrophil counts, being directly and quantitatively responsible for protection from sepsis. The authors confirmed rapid granulopoiesis following BCG administration in three independent cohorts of human neonates in low-resource settings (Guinea-Bissau, The Gambia and Papua New Guinea). More specifically, BCG delayed the normal physiological contraction of the neutrophil compartment in human neonates that occurs over the first week of life. Data analysis
according to gender in these human studies was not reported. In our study, we confirm an emergency granulopoietic response in neonates in a high resource setting albeit only in males.

Earlier studies in healthy adults have identified a gender difference in response to BCG vaccination. In a recent investigation of the systemic inflammatory effects of BCG vaccination in a cohort of 303 healthy adults, Koeken et al. found that while BCG vaccination enhanced cytokine responses to ex-vivo restimulation of peripheral blood mononuclear cells with *M. tuberculosis* and *S. aureus*, it reduced circulating inflammatory proteins as measured by a targeted proteome platform. Interestingly, this effect was much stronger in men than in women.¹⁹

In our study, boys responded with a significant increase in neutrophil counts following BCG administration, while this response was not observed in girls. The baseline neutrophil count in boys on day 5 of life was lower than that in girls in the non-BCG group of neonates, and this reached equivalence following BCG vaccination. The potential clinical implications of this gender difference in the response to BCG vaccination merits further investigation.

To our knowledge, this is the first report on the changes in neutrophil and lymphocyte counts induced in neonates following BCG vaccination from a developed country. This is of importance as similar findings in low resource settings may not be directly applicable due to the different prevalence of pathogens and other healthcare associated risk factors in developed compared to developing countries. A limitation of the study is its retrospective nature. Future prospective studies with extended follow up should be considered in relation to neonatal BCG immunisation in developed countries. These should focus on providing more mechanistic insights into the epidemiological observations of the pathogen-agnostic protective effects of BCG immunisation on neonatal mortality.

With the introduction of neonatal TREC screening for severe combined immunodeficiency at birth, many countries, including the UK, consider changing the routine immunisation schedule to delay the administration of BCG to eligible neonates until after the TREC screening results are available
(possibly up to 4-8 weeks of life). While the delay of administering a live vaccine to reduce the risk of harm caused to babies with SCID is justifiable, these changes could likely have implications on the rest of the neonatal population as well. A study in rural Guinea-Bissau showed that neonatal mortality reductions associated with BCG vaccination were limited to children vaccinated within the first 4 weeks of life, with the most pronounced effect observed among those vaccinated within the first week of life.20 Similarly, in a study from Uganda, distinct patterns of T cell induction were noted when BCG vaccine was given at birth and at 6 weeks of age.21 On the contrary, CD4 and CD8 T cell responses and cytokine release after BCG immunisation at-birth and delayed to two months of age in infants born in Australia were comparable,22 suggesting that geographical and population heterogeneity may affect the BCG vaccine-induced T-cell response.

Emergency granulopoiesis may contribute to the reduction of neonatal mortality from infection following BCG vaccination described in epidemiological studies.10,11 Therefore, depending on the timing of BCG vaccination, the reduced pathogen-agnostic benefits of BCG immunisation may unintentionally negatively influence the incidence of neonatal infection. Future studies will help clarify the question of when BCG administration is most beneficial at the individual and population levels.

**Conflict of interest**

The authors have no conflict of interest.
References


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Accessed January 15, 2021


**Figure legends**

**Figure 1.** White blood cell, neutrophil, lymphocyte and platelet counts in the two study groups. Horizontal line – median, box – interquartile range, whiskers – range, * p < 0.05 vs Control

**Figure 2.** Correlation plots between various cell counts and days post BCG immunisation in neonates who received BCG. Ly – lymphocyte, Neu – neutrophil, WBC – white blood cell

**Tables**

**Table 1.** Demographics and readmission details of the neonates investigated in the study

<table>
<thead>
<tr>
<th></th>
<th>ALL NEONATES (n=99)</th>
<th>BCG GROUP (n=37)</th>
<th>NON-BCG GROUP (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIRTHWEIGHT, g</strong></td>
<td>2975 (2665-3270)</td>
<td>2910 (2565-3395)</td>
<td>3005 (2679-3255)</td>
</tr>
<tr>
<td><strong>GESTATION, weeks</strong></td>
<td>37 (36.5-39)</td>
<td>37 (37-39)</td>
<td>37 (36-39)</td>
</tr>
<tr>
<td><strong>GENDER</strong></td>
<td>Female 39 (39%)</td>
<td>Female 14 (38%)</td>
<td>Female 25 (40%)</td>
</tr>
<tr>
<td></td>
<td>Male 60 (61%)</td>
<td>Male 23 (62%)</td>
<td>Male 37 (60%)</td>
</tr>
<tr>
<td><strong>TREATMENT FOR JAUDICE</strong></td>
<td>84 (85%)</td>
<td>34 (92%)</td>
<td>50 (81%)</td>
</tr>
<tr>
<td><strong>SCREENED FOR SUSPECTED INFECTION</strong></td>
<td>25 (25%)</td>
<td>5 (14%)</td>
<td>20 (32%)</td>
</tr>
<tr>
<td><strong>&gt;10% WEIGHT LOSS</strong></td>
<td>17 (17%)</td>
<td>2 (5%)</td>
<td>15 (24%)</td>
</tr>
<tr>
<td><strong>FEEDING</strong></td>
<td>Breast 64 (65%)</td>
<td>Breast 22 (59%)</td>
<td>Breast 42 (68%)</td>
</tr>
<tr>
<td></td>
<td>Formula 19 (19%)</td>
<td>Formula 7 (19%)</td>
<td>Formula 12 (19%)</td>
</tr>
<tr>
<td></td>
<td>Mixed 16 (16%)</td>
<td>Mixed 8 (22%)</td>
<td>Mixed 8 (13%)</td>
</tr>
<tr>
<td><strong>DISCHARGE, DOL</strong></td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td><strong>READMISSION, DOL</strong></td>
<td>5 (4-5)</td>
<td>5 (4-5)</td>
<td>5 (4-5)</td>
</tr>
<tr>
<td><strong>BLOOD SAMPLING, DOL</strong></td>
<td>5 (4-6)</td>
<td>5 (5-6)</td>
<td>5 (4-6)</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR) or value (percentage). DOL – day of life

**Table 2.** Cell counts in the BCG and non-BCG groups of neonates according to gender

<table>
<thead>
<tr>
<th></th>
<th>Neutrophil (10⁹)</th>
<th>Lymphocyte (10⁹)</th>
<th>WBC (10⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>3.5 (2.3-5.3)</td>
<td>6.1 (4.3-6.8)</td>
<td>11.2 (9.5-13.6)</td>
</tr>
<tr>
<td>Boys</td>
<td>3.8 (3.1-5.3)</td>
<td>5.0 (4.1-6.0)</td>
<td>11.0 (10.1-13.5)</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>4.1 (3.5-5.5)</td>
<td>4.4 (3.4-5.9)</td>
<td>11.2 (9.4-14.3)</td>
</tr>
<tr>
<td>Boys</td>
<td>3.0 (2.1-3.5)##</td>
<td>4.5 (3.7-5.2)##</td>
<td>9.2 (8.2-11.3)##</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR). * p < 0.05 vs Girls in the same group, * p < 0.05 vs BCG group of the same gender
Fig 1
Fig 2