Author's response to reviews

Title: Early BCG vaccine to low-birth-weight infants and the effects on growth in the first year of life: A randomised controlled trial

Authors:

Sofie Blering-Sørensen (s.biering@bandim.org)
Andreas Andersen (aae@ssi.dk)
Henrik Ravn (hjr@ssi.dk)
Ivan Monterio (ivan130978@gmail.com)
Peter Aaby (p.aaby@bandim.org)
Christine S Benn (cb@ssi.dk)

Version: 2
Date: 8 December 2014

Author's response to reviews: see over
Dear Editor

We thank the reviewers for their help with improving the paper. Please find below our point-to-point response to the reviewers’ comments.

Kind regards on behalf of all authors,
Sofie Biering-Sørensen
Reviewer 1

I suggest the authors not to mention anything to do with policy of BCG use as they have done (page 13) in the discussion because the aim of using BCG (from a policy point of view) has nothing to do with growth of children.

Answer: The mentioning of BCG policy has now been removed from the discussion.

Major comments

1) Infants born at hospital versus home could have major social economic differences that would affect the primary end point (growth) of this study. Authors should stratify the analysis based on place of birth, i.e hospital versus home.

Answer: The study is a randomised trial and socio-economic differences should therefore be equally distributed among the two groups at baseline. In the paper on the mortality, there is an equal distribution between the two groups of children born/recruited at the hospital or at the health centres[1]. Hence, there is no need to control or stratify the analyses for place of birth.

2) In general, infants’ growth is largely dependent on nutritional status. Did the authors assess the nutritional intake of the children? Related to the nutritional intake question, did the authors assess the breast-feeding practices? The factors (breast feeding and nutritional intake) are key co-variates that need to be controlled for in reporting growth among infants. Other important co-variate would be the frequency of morbidity events.

Answer: We have not measured nutritional intake. However, at 2, 6 and 12 months, we asked the mother if the child was still breast-feed. We have furthermore recorded number of consultations and admissions to hospital as a marker for morbidity events. However, the present study is a randomised trial and when analysing the direct effect of BCG on growth (Figure 1), these factors should not be taken into account since we expect them to be equally distributed at baseline between the two intervention groups (see Additional file 1- presented in paper). If the morbidity events and breast-feeding practices were different at 2 months in the two randomisation groups it might be due to the effects of BCG and by controlling for these factors we might be eliminating a potential effect of BCG on growth.
Minor comments

1) Supplementary Table 1: The “MUAC*/cm (Mean, SD)” reported at all time points (2, 6 and 12 months) has similar values, yet the P-values are different. Why?

Answer: All values for baseline MUAC in the control and intervention group among children measured at 2, 6 and 12 months appear the same in Additional file 1. However, the p-value for baseline MUAC among children measured at 2 months is different from the p-values for children measured at 6 and 12 months. If more decimals for the mean and SD had been shown the difference in baseline MUAC among children seen at 2 months would have been larger than the difference at 6 and 12 months (Table 2) and hereby explained the different p-values. However, none of the p-values are significant and there is no indication that there should be any baseline differences between the two randomisation groups among children measured at either 2, 6 or 12 months.

Table 2: Baseline differences between the intervention and control groups for children measured at 2, 6 and 12 months

<table>
<thead>
<tr>
<th></th>
<th>Late BCG</th>
<th>Early BCG</th>
<th>P-value for difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children seen at 2 months visit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline MUAC/cm (Mean, SD)</td>
<td>7.7813 (0.8688)</td>
<td>7.7587 (0.8637)</td>
<td>p=0.57</td>
</tr>
<tr>
<td><strong>Children seen at 6 months visit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baseline MUAC /cm (Mean, SD)</td>
<td>7.7934 (0.8670)</td>
<td>7.7886 (0.8661)</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Children seen at 12 months visit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline MUAC /cm (Mean, SD)</td>
<td>7.7999 (0.8581)</td>
<td>7.7996 (0.8414)</td>
<td>p=0.90</td>
</tr>
</tbody>
</table>
Reviewer 2

Major comments

1) This study is complicated by the fact that 1717 of the infants, a rather high proportion of those recruited, were additionally randomised to receive Vitamin A with Vitamin E, or Vitamin E alone. The text on page 8 suggests that as early analysis had not identified an interaction between BCG and Vitamin A supplementation (VAS), the VAS status was disregarded in the analysis here; there is certainly no mention of VAS in the Results. However the second sentence of the Results section of the Abstract states that BCG had beneficial effects when given with, but not given without, VAS. Either there is an effect of VAS which must be taken into consideration, or not and this must be clarified.

Answer: As stated in the methods section, we found no interaction between BCG and Vitamin A supplementation (VAS) on mortality. However, as reported in a previous paper investigating the effect of VAS on growth, in the present study we did find a tendency towards an interaction between BCG and VAS for some anthropometric outcomes. However as these interactions have already been reported in a previous paper, we do not find it appropriate to report them again. The above-mentioned sentence about VAS in the abstract has therefore been deleted. In the methods section page 10, we have added the following to clarify the interaction between VAS and BCG. “For weight and head circumference BCG tended to be beneficial when given with VAS but not when given without VAS (interaction between “early BCG” and VAS: weight p=0.06; head circumference p=0.06). However, since the interactions were insignificant we present the results for the combined groups.”

2) The LBW children randomised to receive BCG early received it either at birth or at the first contact with a health centre after birth. Information should be included to clarify the spread of age at vaccination in both the early and late BCG groups.

Answer: We have added information about the median age (10-90 percentile) of BCG vaccination among BCG vaccinated children at the 2, 6 and 12 months visit to Additional file 1. Furthermore, in the result section page 9, we have added the following sentences “In the intervention group, the median age of BCG vaccination was 2 days (10th-90th percentile: 1-10 days) (Additional file 1). In the control group, 58% had received a BCG vaccine at the 2 months visit[1] and the median age of vaccination was 47 days (20-57 days)."
(Additional file 1). At 12 months, 81% of the children in the control group had received BCG[1] and the median age of vaccination was 49 days (22-99) (Additional file 1).

3) **One potential flaw in the design of the study is that it seems that the early BCG group received BCG Danish while the late BCG group were vaccinated at local health centres. Which BCG vaccine or vaccines did the late BCG group receive? If this was a different strain of vaccine there should be some discussion about whether this might have affected the results.**

**Answer:** The reviewer is correct in observing that different strains of BCG might have different non-specific effects. We do not know what strain of BCG the control group received. However, from observing BCG vaccinations at the health centres, we believe most BCG vaccines used in the control group came from the Russian strain (used by the Serum Institute of India). There is a possibility that different strains of BCG might have different non-specific effects; however, this has not yet been examined in randomised trials in infants.

We have now added the following sentences to the methods section page 6 “We obtained information about date of BCG vaccination in the control group from the health card which has no information about the strain of BCG used.” In the discussion section page 12 we have added the following paragraph “The children in the intervention group received the Danish strain of BCG (SSI, Denmark). Information on BCG strain was not available in the control group. Some immunological studies have suggested that the Danish BCG strain may produce stronger beneficial non-specific effects compared to other strains of BCG (17). Hence, the comparison of growth between early BCG and control groups may have been biased by the intervention group receiving a BCG strain with stronger non-specific effects.”

4) **When the purpose of the paper is to compare the effects of the early BCG and late BCG vaccination, it is unclear why in Figure 2 only a single line is plotted for both groups together?**

**Answer:** It is very good suggestion by the reviewer to stratify Figure 2 by randomisation group. However as also shown in Figure 3, there are no major differences in growth among the two randomisation groups. The lines for the two randomisation groups would therefore be almost the same and it would be difficult to disentangle the two lines from one another. We have now added a sentence to the figure 2 legend “There is no effect of BCG on the
overall estimates why the lines for the two randomization groups could not be drawn separately. “

5) In Figure 3, it does appear that there are significant negative effects of early BCG on some growth parameters for boys, but these are not considered further?

**Answer:** In figure 3, there are no significant negative effects of BCG in boys. However, there are significant interactions between BCG and sex, with a tendency towards a positive effect in girls and a negative effect in boys. Significant effects of BCG are marked with *, and significant interactions between sex and BCG are marked with #. However, these signs might look too much alike and hereby confuse the reader. We have therefore chosen to use & to mark significant interactions between sex and BCG.

6) It might help readers to know whether Guinea-Bissau is unusual in delaying giving BCG to LBW infants or if this is common in other countries?

**Answer:** We have added the following sentence to the Background section page 3: “However, according to local policy in Guinea-Bissau and other Sub-Saharan countries low-birth-weight (<2,500 gr.; LBW) infants only receive BCG when they have gained weight.”

**Minor essential reviews**

1) Page 3, Background line 3, suggest rewording to “vaccine given to low...”

**Answer:** The suggestion has been included in the paper

2) Page 3, Conclusion, line 1, suggest rewording to “recommended to be given to LBW...”

**Answer:** The suggestion has been included in the paper

3) Page 5, Study design, line 2, delete “the frames”.

**Answer:** The suggestion has been included in the paper

4) Page 5, Study design, line 10, suggest rewording to “consent was given, ....”

**Answer:** The suggestion has been included in the paper
5) Page 6, 3 lines from bottom, suggest rewording to “If children were briefly absent at the time of the home visit, an attempt was made to revisit them shortly afterwards.”

   **Answer:** The suggestion has been included in the paper

6) Page 9, Results, line 7, suggest rewording to “examined were travelling..”

   **Answer:** The suggestion has been included in the paper

7) Page 13, line 6, suggest rewording to “The weight-stratified analysis lends some support to the latter interpretation.”

   **Answer:** The suggestion has been included in the paper

**Discretionary Revisions.**

If would be useful to note that similar proportions of both the early and late BCG groups received oral polio, as this information is only included in the supplementary files.

**Answer:** A sentence has now been added to the result section page 9 “The proportion of children who received OPV at birth were also comparable in the two randomisation groups.”