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References


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Sex-differential non-specific effects of BCG and DTP in Cebu, The Philippines

From PETER AABY,* CHRISTINE STABELL BENN, JENS NIELSEN and HENRIK RAVN

In their recent paper Chan and colleagues1 studied the potential non-specific effects (NSE) of DTP in The Philippines. It is encouraging that sex-differential NSE are studied and different ways to examine these effects are being explored. Very few studies have collected post-mortem vaccination information2 and the present analysis is, therefore, particularly interesting because the team succeeded in collecting vaccination information after death from 99% of dead children. Interestingly, the study provided support for the importance of sex-differential NSE, which we have emphasized in previous publications.2–7 However, the study emphasized several key messages which contrast with observations we have made in Africa.2–9 As discussed by Chan et al. these contrasts could be due to regional differences in morbidity patterns. However, both the Cebu and our data do not suggest major differences in NSE for major morbidity categories like diarrhoea, lower respiratory infections or malaria.1,3 Hence, it seems worthwhile to explore whether the contrasts may be due to differences in methodology or vaccination policy.

Comparing sequential vaccinations: which age range?

The Chan et al. analysis reported that DTP was associated with 57% (CI 12–79%) lower mortality among BCG-vaccinated children controlling for relevant background factors.1 There was no indication that this difference was due to prevention of whooping cough1 and the difference is, therefore, presumably a non-specific beneficial effect of DTP compared with BCG.1 In Cebu 97% of the children received DTP1 and BCG would, therefore, only be the predominant vaccine in the first 2–3 months of life. Hence, with age, BCG-vaccinated children who were not yet DTP vaccinated would represent an increasingly selected and frail subgroup of children too weak to be vaccinated. This pattern is illustrated by the fact that, whereas the child mortality rate
Sex-differential effects of vaccines

The Cebu study reported that the female–male MR was 0.19 (0.04–0.86) for BCG-vaccinated children and 0.76 (0.52–1.12) for DTP-vaccinated children, suggesting a sex-differential effect of the two vaccines (interaction term, $P=0.08$). The beneficial effect of DTP was much stronger for boys [MR = 0.32 (0.14–0.73)] than for girls [MR = 0.86 (0.18–4.23)]. Therefore, it is concluded that although the protective effect was not as strong for females, there were no harmful effects associated with DTP vaccination for females. On the other hand, the increase in the female–male MR for DTP-vaccinated children resulted from a dramatic reduction in mortality among males following DTP vaccination rather than from an increase in mortality among females. As in Cebu, we have previously reported that BCG as last vaccination is associated with a stronger beneficial effect for girls than boys, giving rise to a low female-to-male MR after BCG vaccination.2,4 The major contrast between Cebu and the African data is, therefore, that DTP-vaccinated girls had slightly lower mortality than boys whereas we have usually found that DTP-vaccinated girls to have higher mortality than the boys.2–7 Differences in vaccination policy may have been important for this contrast.

In our studies most children received BCG first and then DTP, these DTP vaccinations being associated with increased female mortality.2–5 In Cebu, however, 63% of the children had received BCG and DTP simultaneously (Chan, personal communication). We have earlier reported that girls had lower mortality than boys, the female–male MR being 0.29 (0.02–1.03), among female–male twins who had received BCG and DTP simultaneously.4 In a study in Senegal in which essentially all children received the first dose of DTP and BCG together, the female–male MR after the first dose of DTP was 0.60 (0.36–0.97) whereas girls had higher mortality than boys after DTP2 and DTP3 vaccinations (unpublished data). Hence, the slightly lower female than male mortality among DTP-vaccinated children in Cebu could be the result of different sex-differential NSE for children who received BCG and DTP simultaneously vs BCG and DTP sequentially.

Analytical approaches in the Cebu analysis

The study compared the relative mortality estimates for DTP vaccination among BCG-vaccinated children using both a retrospective approach, which updates vaccination dates with information collected later and a landmark approach, which only updates vaccination status at the survey dates and which will provide a conservative estimate under the restrictions imposed in the present study. The authors conclude that the retrospective approach is a more robust method.1

normal declines with age, the rate for the BCG group increased steeply with age (see Figure 2). The Cebu paper extends the comparison of DTP and BCG to 30 months of age. Judged from the Kaplan-Meier cumulative mortality curves (KMC) in Figure 2,1 indeed most of the BCG deaths occur after 6 months of age. The study had 14 334 BCG-vaccinated children, but when the deaths occurred in the BCG group, no more than 200 boys and 150 girls on average were under observation. In contrast 4–6000 boys and 4–6000 girls were under observation when deaths occurred in the DTP group. Hence, the paper is a comparison of a large group of ‘normal’ children who received their DTP vaccines as recommended and a reference group of increasingly frail BCG-vaccinated children. Indeed, the mortality rate ratio (MR) between the DTP and the BCG groups is increasing with age. Hence, the beneficial effect of DTP would have been even stronger if the comparison had been continued beyond 30 months of age but would have been less if the comparison had been restricted to younger age groups. The KMC suggest no beneficial effect of DTP in the age range BCG and DTP have previously been compared below 6 or 9 months of age. Judged from Figure 3 and Table 3,1 DTP-vaccinated girls had higher mortality than BCG-vaccinated girls at least in the first 6 months after DTP vaccination, just as has been the case in our studies from Africa.

It is a general challenge in studies estimating the impact of childhood immunizations that vaccines are administered sequentially. The reference group for a specific vaccine, say DTP, will, therefore, mostly be the recipients of the previous vaccine, i.e. BCG. In most situations, healthy children are vaccinated first and by implication those remaining in the previous vaccine group, i.e. BCG. Hence, the mortality rate ratio (MR) between the DTP and the BCG groups is increasing with age. Indeed, the beneficial effect of DTP would have been even stronger if the comparison had been continued beyond 30 months of age but would have been less if the comparison had been restricted to younger age groups. The KMC suggest no beneficial effect of DTP in the age range BCG and DTP have previously been compared below 6 or 9 months of age. Judged from Figure 3 and Table 3,1 DTP-vaccinated girls had higher mortality than BCG-vaccinated girls at least in the first 6 months after DTP vaccination, just as has been the case in our studies from Africa.

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In situations in which nearly all children dying have vaccination data collected post-mortem, the retrospective method will indeed provide a better estimate. However, this would not be possible in most areas in which NSE of vaccination are likely to be important for child survival because mothers tend to throw the card away when a child has died. Hence, the best method may depend on the completeness of the data, which can be collected.

Out of the 14,334 children and 139 deaths included in the retrospective analysis, 4,103 children and 42 deaths were not included in the landmark approach. Children who would enter the retrospective but not the landmark analysis should mainly be children who were born or moved to the area between surveys and moved or died before the next survey. In an area with low mortality (8/1000 person-years) and low out migration (Figure 1), it seems strange that 29% of the BCG-vaccinated children never entered the landmark analysis. The children excluded from the landmark analysis had a crude mortality of 6.9/1000 person-years while the children included had a crude mortality of 9/1000 person-years, i.e. 24% lower mortality in the excluded children.

The solution to this enigma may be that the retrospective Cebu study reported only followed all children from the date of BCG vaccination. Many of the children included in the cohort were BCG or DTP vaccinated prior to the baseline survey, and were thus allowed to contribute follow-up time before the baseline survey. Since the children who died prior to the initiation of the study period were excluded, this apparently implies that the time from receipt of BCG or DTP to baseline survey is risk-free survival time in the analysis. It is not possible to predict how this might have affected the MR estimates for DTP among BCG-vaccinated children as this would depend on the relative timing of BCG and DTP vaccinations. As discussed earlier the majority of the children received BCG and DTP simultaneously and this was classified as a DTP vaccination. Therefore, it is likely that most of the risk-free time would have been added to the DTP group. This procedure may have induced a special form of survival bias, pre-survey survival time being added to the analysis for those who survived to the time of the first survey. The inclusion of such risk-free survival time may have biased the MR estimates reported in the paper.

Conclusion

The main conclusions of the Cebu study that DTP vaccination is associated with non-specific beneficial effects and that the increased female–male MR of DTP-vaccinated children is associated with reduced mortality among males following DTP vaccination rather than increased mortality among female children remain to be proven.10–13 The Cebu study concluded that there is no reason to alter current vaccination policy because DTP was not associated with any harmful effect among BCG-vaccinated girls. However, we have pointed out that there may be several methodological and vaccination policy differences between our studies and the Cebu study. In order to be able to compare the results directly, the authors are kindly requested to present the following two retrospective analyses.

1. A comparison of BCG-only vs DTP according to age groups, e.g. 1–2, 3–4, 5–8, 9–17, 18–29 months of age, making it possible to compare mortality rates between DTP- and BCG-vaccinated children in the younger age groups when the BCG-only vaccinated children are not yet a frail subgroup.

2. A comparison of female and male mortality rates according to whether the last vaccination was (i) BCG-only, (ii) BCG and DTP simultaneously, (iii) DTP after initial simultaneous BCG and DTP vaccinations or (iv) DTP after an initial BCG-only vaccination. This would allow us to compare BCG-only vs children who received the BCG and DTP vaccinations as first BCG, then DTP, as most children do in our African studies.

In both analyses, the children should only be followed from the date of the first survey when they were first seen and not from the date of a previous BCG vaccination as this introduces a potential bias.

Conflict of interest: The Cebu study contradicts our previous observations.

References

6. Aaby P, Jensen H, Samb B et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus:


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**Author's Response**

From GRACE J CHAN,* LAWRENCE H MOULTON, STAN BECKER, ALVARO MUÑOZ and ROBERT E BLACK

We appreciate the close attention Aaby *et al*. have given to our work. Deciding how to conduct the analysis of observational studies requires balancing potential biases, and we recognize the choices we made are not necessarily those Aaby *et al*. would have made. Although much of their letter is of a general nature, we think it useful for us to reply to their three main comments regarding our analysis.

**Point 1: Risk-free survival time**

Aaby and colleagues raised the concern that our study contained ‘a special form of survival bias, pre-survey survival time being added to the analysis for those who survived to the time of the first survey’. They also questioned ‘whether many of the 4,103 children have contributed risk-free time to the retrospective analysis’ (Aaby letter).

To address the concern that there was risk-free survival time, we re-ran the analysis so that:

(i) the timeline starts at zero at the latest of BCG receipt and first study visit for those who entered in the baseline survey;

(ii) the timeline starts at zero at BCG receipt or end date of the baseline survey (if BCG receipt occurred before the end of the baseline survey period) for those who entered after the baseline survey.

As a result, 3668 children out of the original 14,334 children were dropped from the retrospective analysis, having contributed risk-free time. This reduced the amount of person-time, although not the number of deaths. As seen in the accompanying table, the influence of risk-free survival time on the original results was slight, and not enough to alter any conclusions (similar results obtained in non-adjusted models) (Table 1).

**Point 2: An ‘increasingly selected frail subgroup’ among those BCG vaccinated but not yet DTP vaccinated**

The second concern raised by Aaby *et al*. addresses a potential non-measurable difference between those who received DTP vaccination and those who did not (Aaby letter). They write ‘in most situations, healthy children are vaccinated first and by implication those remaining in the previous vaccination group will increasingly tend to be unhealthy children too frail or too poor to get vaccinated’. Despite the speculative nature of this argument, it points to a fundamental and well-known shortcoming of the previous studies of the non-specific effects of DTP with respect to child mortality. All studies on this subject to date have been observational, and, as summarized by the WHO Task Force on Routine Infant Vaccination and Child Survival, ‘it is impossible to be sure that unmeasured confounding variables did not influence the findings’ in these studies.1 While the universe of potential