

# **Epidemiological studies of the non-specific effects of vaccines: II - methodological issues in the design and analysis of cohort studies**

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Running title: Analysis of studies of non-specific effects of vaccines

## **Summary**

We review sources of bias which can affect non-randomized cohort studies of non-specific effects of vaccines on child mortality. Using examples from the literature on non-specific effects, we describe different sources of selection and information bias, and, where possible, outline analysis strategies to mitigate or eliminate such biases.

## **Introduction**

Much of the controversy surrounding the impact of routine immunizations on childhood survival, particularly the putative increased mortality following DTP immunization, has focused on methodological issues. This is natural: answering apparently straightforward questions about the effects of vaccinations presents tough methodological challenges, because vaccines have not been randomly allocated and the available data are often incomplete. Indeed, *all* the observational studies that have been undertaken on the topic suffer from methodological shortcomings of one kind or another.

A striking feature of the literature on the non-specific effects of vaccines on child mortality is the sheer size of these putative effects. A recent review (Aaby et al., 2007) reported highly heterogeneous results, ranging from a 5-fold reduction to a 3-fold

increase in mortality after DTP vaccine. Similarly large effects have been reported for other vaccines, notably measles vaccine and BCG (Aaby et al., 1995, Kristensen et al., 2000). Two related questions arise: first, are these apparently very strong associations genuine, or are they the result of bias? Second, what are the reasons for the heterogeneities (indeed, the contradictory results) found in different studies?

The impact of differences in data collection procedures and data analysis has, quite properly, given rise to much discussion. Our purpose is to review these methodological issues, assess their likely impact on results, and discuss ways in which they may be addressed. We first consider selection bias, and how it might be controlled. Then we discuss sources of information bias, including survival bias. Finally, we mention some further issues of analysis.

### **Selection bias**

Possibly the single most important issue facing observational studies of the impact of vaccines on child mortality is non-random allocation of vaccines, which may induce a spurious association between vaccination and mortality (Fine et al. 2009). This bias may be regarded as a selection bias (children at lower or higher risk of mortality being differentially selected for vaccination) or as a form of confounding by factors associated with both receipt of vaccination and mortality.

The statistical tools available for controlling confounding in observational studies include: specifying appropriate inclusion and exclusion criteria; allowing for measured confounders, either directly or via propensity scores; use of instrumental variables; undertaking studies in vaccinees only; comparing mortality in males and females; and within-individual analyses. We consider each of these options in turn.

#### *Inclusion and exclusion criteria*

Children included in observational studies may differ in ways that affect their chances of being vaccinated. For example, some children may be ineligible for vaccination owing to other health conditions; they may also experience higher mortality. Such children are often excluded from randomized trials. Some observational studies have been shown to be sensitively dependent on which groups are included in the analysis (Aaby et al., 2006).

To reduce selection bias from inclusion of children who are ineligible for vaccination, appropriate inclusion and exclusion criteria should be applied where possible. These criteria should ideally relate to variables measured before the age at which vaccinations are administered, so as to avoid introducing further bias.

#### *Direct adjustment for measured confounders*

This is the most commonly used method of adjustment: potential confounders are included as covariates or stratification variables in an appropriate model (for example, a Cox survival model). However, it is a puzzling feature of studies of childhood survival that such adjustments often (though not always) have little influence on the results (Vaugelade et al., 2004, Moulton et al., 2005, Aaby et al., 2006, Elguero et al., 2005). There are several possible reasons for this (other than absence of any confounding):

(a) the relevant variables have not been measured (and indeed might not even be known);

(b) the relevant variables (e.g., socioeconomic status, health status) have been represented by proxy variables with which they are only weakly correlated, and which can at best only partly control for selection bias;

(c) the model used to adjust for covariates may not be sufficiently rich (for example it may not contain relevant interactions), owing to the low number of deaths;

(d) controlling confounders via fixed covariates may be flawed, since vaccination occurs over time: some children might die before they have the opportunity to be vaccinated, and the selection that determines whether a child is vaccinated or not is a dynamic process;

(e) presence of a large bias from other causes (e.g. information bias) which masks the effect of confounders, so that adjusting for confounders has only a small effect on the results.

### *Adjustment for selection effects by propensity scores*

An alternative method of adjusting for selection effects is via propensity scores (Rosenbaum and Rubin, 1983). This approach has been used by (Lehmann et al., 2005) and (Moulton et al., 2005).

The propensity score method involves two stages. First, a new variable is calculated, measuring the propensity for a child to be vaccinated; this calculation is typically done using logistic regression of vaccination on the available covariates and their interactions. In a second stage, the association between mortality and vaccination is investigated in an analysis stratified according to the levels of the propensity score.

There has been much debate about the pros and cons of propensity score adjustments in epidemiology, compared to direct adjustments (Drake and Fisher, 1995). An advantage of propensity score methods is that they allow for a richer model to be used to adjust for selection biases, in circumstances where there are relatively few

deaths and vaccination is far from universal. However, the method might not be useful in highly vaccinated populations, since the 'high propensity' strata are likely to contain few unvaccinated individuals.

### *Time-varying propensity scores*

Propensity scores provide no solution to problems (a) and (b) listed above, but may help to mitigate (c). However, most importantly, the propensity scores so far used have all been fixed in time, and therefore also suffer from the shortcoming detailed in (d).

An improved adjustment method would be to construct dynamic propensity scores, so as to allow for mortality, censoring and changes in the propensity of vaccination with age, as well as different vaccination patterns. Thus, at each time of death, a new propensity score would be calculated, taking into account the entire history of the individuals included in the risk set at that time. This would also allow past vaccination histories to be taken into account in the calculation of propensity scores.

Such an approach has been developed for single treatments (Li et al., 2001, Lu, 2005). An application to studies of vaccination would require consideration of several types of vaccines, and perhaps allowance for distinct vaccination histories. We recommend that dynamic propensity score matching be considered in future studies. The methodological aspects of such an approach as it applies to vaccines merit further investigation.

### *Instrumental variables*

Adjustments using propensity scores rely on the confounders having been measured. Instrumental variables analyses, on the other hand, enable unmeasured confounders to be controlled (Angrist et al., 1996). In the present context, the instrumental variable must satisfy three conditions: (a) it should be associated with vaccination; (b) it should be associated with mortality only through vaccination; and (c) it should not be associated with any confounder, measured or otherwise, of the association between vaccination and mortality.

Though it is difficult to think of a suitable instrument in the present context, future studies should at least consider this method of analysis. On the rare occasions in which they occur, natural experiments may provide a type of instrumental variable. In such instances, who gets vaccinated is heavily influenced by external factors, thus reducing selection biases. Examples where such an approach has been used include situations in which vaccines were missing or available only during certain periods (Aaby et al., 2004, Garly et al., 2004), or when vaccination was interrupted owing to war (Aaby et al., 2002, Aaby et al., 2003).

### *Studies in vaccinees*

One way to avoid bias resulting from selecting children for vaccination might be to undertake studies in vaccinees. Thus, for example, mortality rates might be compared in children receiving BCG + DTP and in children receiving BCG only (Chan et al., 2007).

Although the groups being compared have different vaccination histories, it may be that confounding due to type and number of vaccines received is less serious than that resulting from a comparison between vaccinated and unvaccinated children.

Though studies of the relative impact of different vaccines in vaccinees may be important in defining the research agenda, they can suffer from problems of interpretation: for example, if mortality following BCG and DTP is greater than that following BCG only, this could be consistent with the following scenarios: (a) both BCG and DTP are associated with higher mortality (than absence of any vaccination); (b) BCG and BCG + DTP are both associated with lower mortality; (c) BCG is associated with lower mortality and BCG + DTP with higher mortality. It is not possible, without an unvaccinated group, to differentiate between these possibilities.

In some circumstances (such as near-universal vaccination) unvaccinated groups may not be available and only vaccine – vaccine comparisons can be made. Such comparisons can also be made within studies that do include unvaccinated groups. Where an unvaccinated group can be recruited, we recommend doing so.

### *Comparing mortality rates in males and females*

Some of the hypotheses about vaccination and child survival relate to possibly differential effects of a vaccine in boys and girls. This suggests a particular kind of within-vaccine group analysis, in which mortality is compared between males and females within vaccinated and unvaccinated groups. Such comparisons will control for multiplicative factors associated with vaccination provided that these apply equally to boys and girls.

### *Modelling selection bias in multi-vaccine studies*

The paradigm underlying studies in vaccinees only is that selection bias is associated with vaccination *per se*, rather than vaccination with any particular vaccine or combination of vaccines. While this concept should be scrutinised and tested, and rejected if found wanting, it does nevertheless provide a useful framework in which to interpret results in studies involving several vaccines and vaccine combinations.

In particular, selection bias and genuine vaccine-associated biological effects may produce different interaction patterns between vaccines. Suppose that two vaccines, A and B, are given within a narrow age range, and that data are available on mortality rates after vaccine A, vaccine B, both vaccines A and B, and neither vaccine (the unvaccinated group). Then if the observed effect on mortality is a spurious effect due solely to selection bias, one might expect that the effect of vaccines A, B, and A and B together would be the same, since selection bias is associated with vaccination *per se*.

An example is provided in Table 1, using data from (Moulton et al., 2005). In males, the mortality ratios associated with receipt of DTP only, BCG only, and both DTP and BCG, are of similar magnitude, ranging between 0.54 and 0.59. The fact that the three estimates are similar is suggestive of a selection effect: children who are vaccinated are selected from a subgroup in which mortality is about half that of those who are unvaccinated. In contrast, in females, the mortality ratio in children having received both vaccines is higher than the mortality ratio in children having received DTP only or BCG only: this cannot easily be explained by a selection effect, and suggests that there might be a biological interaction between the vaccines. The plausibility of such a complex gender interaction would of course require confirmation from other studies.

*[Table 1 about here]*

Evidence for a biologic effect or a selection effect of this kind may be investigated by fitting regression models with codings of the vaccine variables that reflect the hypotheses of interest (Moulton et al. 2005).

### *Self-matched analyses*

Self matched analyses control for all age-invariant confounders that enter multiplicatively, whether measured or not (Farrington and Whitaker, 2006). However, only changes in mortality by time since vaccination are estimable: for example, if DTP vaccination doubled the mortality rate at all time after vaccination, the relative mortality shortly after vaccination compared to long after vaccination would be equal to 1. Thus, the information available from such analyses addresses only tangentially the main issues of interest. Nevertheless, what information they give is largely uncontaminated by selection bias.

### *Temporal selection biases and frailty biases*

Selection effects can also affect the timing of vaccination within individuals, thus introducing further bias in mortality ratios. These biases arise when vaccination of a child is delayed owing to disease that may be associated with mortality (Fine and Chen, 1992). The effect of this is to bias the relative mortality towards zero. Such

effects are likely to be universal, but have not been widely mentioned; two exceptions are (Lehmann et al., 2005, Breiman et al., 2004).

None of the methods for controlling selection biases so far mentioned, including self-matching, will control such temporal selection biases. Ideally, one would need to control for a time-varying state of health variable, which is impractical. A simple if ad-hoc adjustment is as follows. Define a lag period  $d$ , and in a Cox survival analysis, for an individual time of death  $t$ , only count vaccinations to time  $t - d$ , thus discounting the possibly biased allocation of vaccines in the interval just preceding the death. (Note however that this approach may not be feasible for vaccinations given close to birth, such as BCG.). (Breiman et al., 2004) used a lag period of  $d = 30$  days. We recommend that, where possible, analyses of this sort are undertaken in addition to the standard analyses (with  $d = 0$ ) so as to document this possible source of bias, and that details are provided of the reclassification of vaccination status involved.

The temporal selection biases just described relate primarily to the impact of short-term illness on timing of vaccination. Frailty bias, on the other hand, results from differential vaccination patterns in children according to their underlying state of health. Frail children (that is, children with worse than average health) may tend not to be vaccinated. If this is the case, then frail children (who may be more likely to die) are likely to be selected into the unvaccinated group, and more robust children (who may be less likely to die) into the vaccinated group. The longer the comparison is extended the more biased the comparison will be. For example, a comparison of mortality in children receiving no dose of DTP with children receiving 3 doses of DTP at a given age contrasts children who are so frail that they have not yet been able to start on DTP with children who are robust enough to have received three doses.

### **Information bias**

Commonly, not all data on outcomes and vaccinations are available. Randomly missing data will generally result in non-differential misclassification, and hence will bias mortality ratios towards 1. However, if the mechanism causing the data to be missing is related in some way to the exposure or the outcome, the presence of missing data may result in differential misclassification and hence generate information biases.

We consider in turn biases arising from missing data on outcome, and missing data on exposure. Detailed consideration of the data ascertainment process in each study setting is required in order to assess the likely presence, direction and magnitude of any information biases (Fine et al 2009).

As a general rule, we recommend that studies should provide detailed numerical accounts of missing data and reasons for missingness. Such information is best provided in the form of a flow diagram, as is now the norm for reports of clinical trials, as this indicates clearly at what stages the data were missing.

### *Ascertainment of outcome*

Deaths are typically recorded in periodic follow-up surveys of the study cohort. In most studies, it is unlikely that dead/alive status will be subject to much misclassification. There may, however, be errors in the recorded date of death, particularly when there are long time intervals between surveys. However, errors in dates of death are perhaps unlikely to be associated with vaccination status. In consequence, the effect of such errors is most likely to bias mortality ratios slightly towards unity.

### *Ascertainment of vaccinations*

In some populations, vaccination records are made at the time of vaccination and held centrally by a single agency. In this case, vaccination status is documented by consulting these central records (Lehmann et al., 2005, Breiman et al., 2004) and Study 1 of (Elguero et al., 2005). Recording errors made at the time of vaccination or at time of database reconciliation are unlikely to be associated with subsequent mortality. Thus, such errors may introduce a bias in mortality ratios towards 1, and such bias might be expected to be slight.

In other situations, vaccinations are recorded on vaccination cards held by the mothers (or other guardian). In this case, vaccination status is documented by consulting vaccination cards at regular surveys (Vaugelade et al., 2004, Kristensen et al., 2000) and Study 2 of (Elguero et al., 2005).

Ascertaining vaccination status in this way generates two problems: first, how to interpret absence of the vaccination card; and second, the fact that vaccinations are ascertained retrospectively, and hence that the ascertainment process might be affected by outcomes, resulting in 'survival bias' (Jensen et al., 2007). We consider both problems in turn.

### *Missing vaccination cards*

One may distinguish two cases: (a) the mother is interviewed, and says no card has been issued; and (b) the mother cannot be interviewed, or else the card is known to have been issued, but cannot be inspected. In case (a), the child should be coded as unvaccinated, whereas in case (b), the vaccine status should be coded as 'missing'. This was the method used, for example, by (Kristensen et al., 2000).



Some surveys do not distinguish between cases (a) and (b), and 'no card' is always taken to mean 'unvaccinated'. This will necessarily introduce some misclassification. However, provided that 'missingness' is not associated with outcome, coding 'missing' as 'not vaccinated' in this way should not introduce any differential misclassification, and thus the mortality ratio should be biased towards unity.

We recommend that as much information as possible is collected on the ascertainment process, so that the likely effect of biases can be investigated. In particular, we recommend that a clear distinction is drawn between cases (a) and (b), with textual descriptions of circumstances where appropriate. It is also important, for reasons which will become apparent below, to collect information on the dates at which cards are seen.

### *Survival bias*

Survival bias arises if 'missingness' of the vaccination records (as defined in (b) in the previous paragraph) is associated with the outcome (namely, death). This can arise, for example, if dead children's cards are destroyed soon after death, as is the case in some of the studies undertaken in West African countries: (Vaugelade et al., 2004, Kristensen et al., 2000), and the second study of (Elguero et al., 2005).

This bias has been described in detail (Jensen et al., 2007). Briefly, in such circumstances, treating 'missing' as unvaccinated will differentially place dead children in the unvaccinated group, and hence will bias the mortality ratio towards zero. The magnitude of the survival bias increases as the intervals between surveys (to ascertain vaccination status) increase, as the proportion of dead children whose vaccination cards are destroyed (and who are therefore classified as unvaccinated) increases, and as vaccination rates increase.

In some circumstances, intervals between surveys are short (for example, in the study described by (Moulton et al., 2005), the surveys took place every two weeks), and hence survival bias is unlikely to play a major role. In other situations, for example those described by (Vaugelade et al., 2004, Kristensen et al., 2000), there were substantial intervals between surveys, and in such circumstances there may be substantial survival bias.

Note also that, whatever the frequency of surveys, survival bias will only be present if dead children's records are more likely to be missing than live children's records. If no records are missing, then there will be no survival bias. If the numbers of missing records is small, it is possible to undertake a sensitivity analysis, in which two separate analyses are undertaken with missing records coded alternately as unvaccinated and then as vaccinated, so as to document the impact of survival bias on the analysis. For example, the study of (Chan et al., 2007) achieved near-complete

ascertainment of vaccination records. Sensitivity analyses showed that survival bias in this study was not an issue.

### *Landmark analyses*

In some circumstances, however, there are large numbers of ‘missing’ records due, for example, to the destruction of dead children’s records (or other informative mechanism). In such circumstances, survival bias is likely to be substantial, and sensitivity analyses are unlikely to be useful, because they yield too wide a range of values of the mortality ratio. In this situation, which has commonly arisen in studies undertaken in West Africa, a landmark analysis is recommended (Jensen et al., 2007).

In a landmark analysis, vaccination status is never updated retrospectively, and can only change at visits undertaken during the subject’s lifetime. Thus, if an individual is unvaccinated at visit 1 and vaccinated at some time point between visits 1 and 2, that individual is treated as unvaccinated until visit 2 (or death, or censoring, if these also occurred between the two visits). Since vaccination status is updated at visits at which cards are seen, it is important that the dates at which cards are seen are recorded.

This approach avoids survival bias – which biases relative mortality towards zero – at the cost of introducing non-differential misclassification, which biases the mortality ratio towards 1. This may in certain circumstances be preferable, when it is required to avoid producing spuriously protective effects. The bias from the landmark analysis increases as the intervals between visits increase and as vaccination coverage increases. For further details of this analysis method, see (Jensen et al., 2007).

### *Pros and cons of retrospective updating and landmark analyses*

The major advantage of landmark analyses over analyses using retrospective updating of vaccination records is that they avoid survival bias. When survival bias is large, the landmark approach is best, as it is more robust. However, if survival bias is not large, the retrospective updating method is preferable, since it avoids two potential problems with the landmark approach: mortality ratios biased towards 1 even if no survival bias is present, and lower power.

It is instructive, where possible, to undertake both analyses on the same data, and compare the results. Table 2 compares the results obtained using different methods, based on results published in the literature.

*[Table 2 about here]*

The studies undertaken in Guinea Bissau, Malawi, Burkina Faso and the Philippines were analysed by the two methods, using the same data. Relative precisions lower than 1 indicate that the landmark method is less precise than retrospective updating. Table 2 also includes estimates obtained from two studies in the same population in Senegal. Study 1 was undertaken using centrally held vaccination records, and thus is not affected by survival bias. Study 2 was undertaken using mother-held vaccination cards and retrospective updating, and thus may be prone to some survival bias.

In most instances in Table 2, the mortality ratio estimated using the landmark method is higher than when it is estimated using retrospective updating (there are exceptions however, for BCG and measles vaccine in the Malawi study). In two cases (for DTP1 in Guinea-Bissau and Malawi), the direction of association changes, from a mortality ratio greater than 1 with the landmark analysis, to less than 1 when the data are analysed by retrospective updating. In other cases, the difference is less marked. The relative precisions are all less than 1, indicating that the landmark method gives less precise estimates than retrospective updating.

We recommend that, where possible, both landmark and retrospective updating analyses be undertaken, so as to allow for transparency in the interpretation of the results.

### *Identifying the presence of survival bias*

As suggested above, insight into the presence and magnitude of possible biases may be obtained by undertaking several analyses by different methods, and by conducting sensitivity analyses where appropriate. Some further checks have been proposed; these are reviewed below.

It is useful to look at absolute mortality in unvaccinated children in a study, in order to check that it is broadly in line with what might be expected in that population. If it is not, this might suggest (but does not demonstrate) the presence of a selection or information bias (Aaby et al., 2007). It has also been suggested that a large mortality ratio of never vaccinated to ever vaccinated might be indicative of survival bias. This measure is more problematic, since a large ratio might also be attributable to selection effects or to a true effect. A better approach may be to compare mortality ratios for different vaccines within the same study, a uniform effect of all vaccines being consistent (but not demonstrating) the presence of bias.

Where data on cause-specific mortality are available, as in (Breiman et al., 2004), it is useful to conduct separate analyses for deaths in two groups, namely (a) those which are believed unlikely *a priori* to be related to vaccination, and (b) those which cannot *a priori* be ruled out as being caused by vaccination. Since survival bias arises because

of outcome-dependent missing vaccination data, it should most likely apply equally irrespective of cause of death.

### **Some further issues**

In this final section we briefly detail a few specific issues requiring particular care in these applications.

#### *Time line of analysis*

The appropriate time line for analysis is usually age, if necessary with delayed entry to account for variations in age at entry into the dataset. This is because of the known strong relationship between age and risk of death. Vaccinations should be regarded as time-varying covariates. In consequence, it is not usually appropriate to calculate Kaplan-Meier survival curves for periods over which vaccines are administered, or to use standard logistic regression which takes no account of the time dimension. Hypotheses relating to the sequence of vaccines should be investigated, where possible, with vaccinations specified as time-varying covariates.

#### *Complex interactions*

Some of the hypotheses relating to vaccination and childhood survival specify complex interactions between vaccination histories and sex-specific mortality. These should be investigated both using different strata (for example, separate analyses in boys and girls, and in subgroups defined by contrasting vaccination histories) and formal tests of statistical interaction. However, we recommend particular caution in the interpretation of such analyses, as well as due allowance (whether formally, via Bonferroni corrections, or informally) for multiple testing when many hypotheses are investigated.

#### *Influential observations*

The study of complex interactions between vaccines and gender involves subdividing cohorts into several groups, some of which may in consequence be small, or contain few deaths. In such circumstances, results can lack robustness owing to observations whose omission or inclusion in the analysis produce widely different results. Analyses should be scrutinised for such observations, their impact assessed, and relevant data on numerators and denominators reported.

### **Concluding remarks**

We have documented the major biases that affect studies of vaccination and childhood survival, and made some recommendations about how such issues might

be tackled. We have not sought to identify every single source of bias that might conceivably arise – an impossible task – but rather, have focussed on those which have been discussed in the literature.

In concluding we make two final recommendations. First, that data descriptions and analyses should be presented as transparently as possible, and from distinct points of view. We hope that detailing the ways in which major bias can occur, as we have done, will focus attention on possible issues of contention, which can therefore be addressed in primary publications. Second, it is helpful for critiques of studies to be focused less on identifying the possible presence of bias – which, in observational studies such as those we have considered, is inevitable – and more on assessing the likely impact on substantive results and conclusions of the biases identified.

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**Table 1** Interactions between vaccines: selection and biological effects

	Males		Females	
Vaccines	Mortality ratio	95% CI	Mortality ratio	95% CI
None	1	-	1	-
DTP only	0.54	0.24 – 1.2	0.35	0.15 – 0.83
BCG only	0.57	0.33 – 0.98	0.33	0.17 – 0.62
Both DTP & BCG	0.59	0.27 – 1.3	0.83	0.43 – 1.6

Data from: (Moulton et al., 2005)

**Table 2** Mortality ratios obtained using different methods on the same data (Guinea Bissau, Malawi, Burkina Faso and Philippines) or in the same populations (Senegal)

Study and location	Vaccine	Landmark analysis	Retrospective updating	Relative precision*
Guinea-Bissau (Jensen et al., 2007)	BCG	0.55	0.29	0.44
	DTP1	1.84	0.63	0.48
Malawi (Aaby et al., 2006)	BCG	0.45	0.69	0.62
	DTP1	3.19	0.99	0.33
	MV	0.42	0.47	0.83
Burkina Faso (Vaugelade et al., 2004)	BCG	0.50	0.37	0.41
	DTP	0.24	0.23	0.21
	BCG + DTP	0.50	0.34	0.19
Philippines (Chan et al., 2007)	DTP	0.87	0.43	0.55
		Study 1	Study 2	
Senegal (Elguero et al., 2005)	BCG+DTP	0.70	0.59	NA
	MV	0.98	0.87	NA

DTP1: 1<sup>st</sup> dose DTP; MV: measles vaccine. NA: not applicable.

\* See text

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