

Non-specific effects of vaccines are important

The non-specific effects of measles vaccine have been a main focus of research at the BHP since the very beginning even though it took 15 years to formulate the idea that vaccines may have other than the targeted effects, presumably due to some form of immune stimulation. The high-titre measles vaccine (HTMV) was protective against measles but associated with two fold increased mortality for girls. Hence, such non-specific immune stimulatory effects can be very important for child survival. However, the research community did not pursue these observations, but instead searched for a new and better measles vaccines. However, if a licensed vaccine could have such

dramatic effect it can probably happen again unless we understand the specific immunological mechanisms. We have therefore aimed to document the importance of non-specific effects not only for measles vaccine but for all of the routine vaccines.

Ideally one could conduct randomized clinical trial measuring the extent of these non-specific effects beyond the targeted disease prevention of the vaccine. However, that is rarely possible because withholding recommended vaccines would be unethical. We have tried several different approaches to document the importance of non-specific effects as shown in the following chapters.

First, we have measured the effect in observational studies. This is inherently difficult because there are strong selection biases in who gets vaccinated first – see Chapter 14. Still in some observational situations we may approach natu-



ral experiments with limited selection bias as when a new vaccine is introduced (45,65), a vaccine is missing during a certain period (46), or a war interrupts vaccination services (8). We have pursued such natural experiments. Furthermore, we have exploited the fact that different vaccines apparently give very different estimates of their impact on survival. That would not happen if selection biases were the main cause of the estimates.

Second, the HTMV incident suggested a marked sex-differential effect of this vaccine (12). In the pre-vaccination era in West Africa, there was no important sex-difference in post-neonatal child mortality (Figure). Hence, if vaccines have strong sex-differential effects on mortality it suggests that non-specific immune stimulatory effects are important for child survival. As it turns out, all routine vaccines have sex-differential effects suggesting that boys and girls might in fact benefit from different vaccines or different vaccination schedules.

Third, we have also conducted randomised clinical trials to document non-specific effects of vaccines as evidence in several chapters. This has been possible in some situation because the

vaccine was not normally used (BCG to low-birth weight children, BCG revaccination), not used in that age group (measles vaccination at 4 months of age), or because several different strains are in common use (strains of measles vaccine). It should also be possible to test variations in current practice which are not the main recommendations but occurring very often; for example, administering BCG and DTP simultaneously or measles vaccine and DTP simultaneously (Chapter 21). Conducting

randomised trials, we have found major non-specific effects. However, they have not always been the ones we had expected. The immunological interactions have been more important than initially envisioned. Testing, for example, early measles vaccine, we changed the sequence of vaccinations and that has fundamentally changed the impact on survival because more children received DTP after measles vaccine



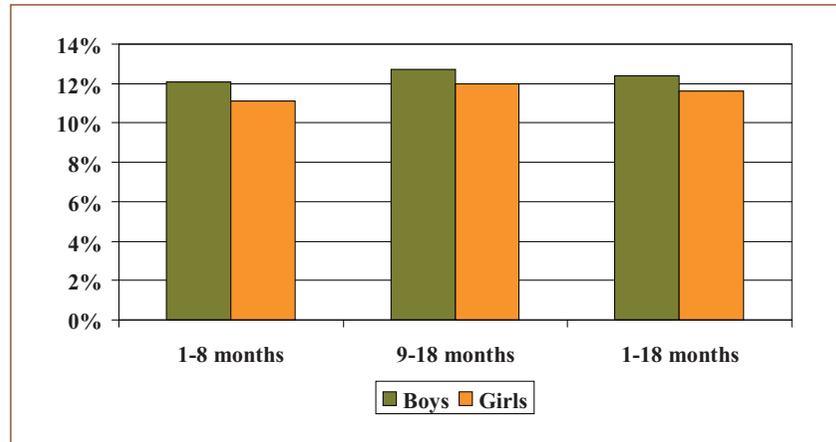


Figure. Annual mortality rates by sex prior to the introduction of vaccines. Bissau, 1978-1980

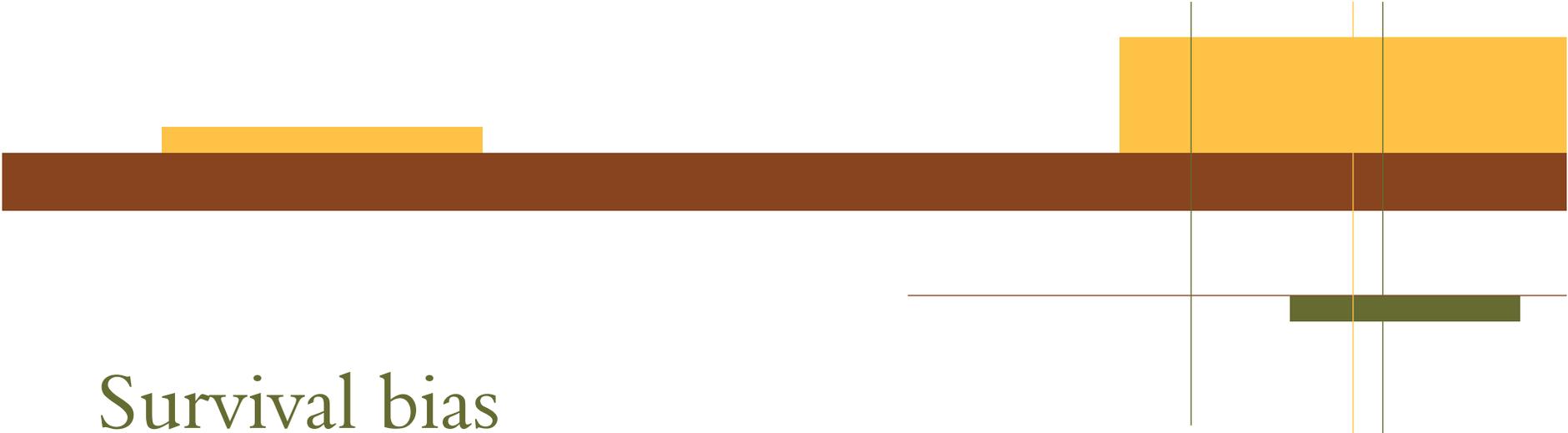
(Chapter 18). We have also experienced several times that supplementation with micronutrients amplified the immunological effects (Chapter 25).

Randomised trials will be continued but it is becoming clear that it will be increasingly difficult to test the impact on survival because once we intervene and provide services to the community we are also reducing mortality. We need to measure the impact on indicator infections and immunological parameters which are associated with survival and which will indicate whether the immunological profile induced by a vaccine is beneficial or not. This is becoming urgent as more and more new vaccines are being introduced.

Non-specific effects are very important for child survival in low-income countries. From what we know now, live vaccines are beneficial – at

least in individuals without immunodeficiency – whereas inactivated vaccines may have negative effects on other than the targeted diseases. Both beneficial and negative effects are strongest for girls. Effects may change fundamentally when vaccines are combined or the sequence inverted. Furthermore, vaccines interact with other forms of immunomodulators like micronutrients and season.

These observations question many assumptions underlying the current program of interventions for children in low-income countries. Taking these observations into consideration in planning the intervention programs may have major impact on child survival.



Survival bias

Background

Most of the observations and hypotheses on non-specific effects (NSE) of vaccines have emerged from observational studies at the Bandim Health Project (BHP). Recently, observational studies from other research groups have appeared to be focusing on the putative negative effect of DTP. The studies from BHP and elsewhere have estimated the impact of DTP to vary between mortality rate ratios of 0.2 (positive NSE) and 3.2 (negative NSE) for DTP-vaccinated compared with DTP-unvaccinated children, see Figure 1. For last five years we have discussed whether methodological differences are involved in this inconsistency (73, 74, 79, 93, 98, 99, 153, 154, 201). One bias has played a central role in this discussion: *survival bias*.

Data collection

Many of the observational studies in which child mortality has been related to vaccination status were conducted in situations where mothers keep vaccination cards on which all vaccinations received are recorded. Typically, a cross-sectional population survey is first conducted by which vaccination cards are examined to record the vaccination status of all children. After an interval of perhaps 3 or 6 months, or longer, a follow-up visit is conducted, and changes in vaccination status are ascertained by examining vaccination cards again. Deaths since the last visit are recorded. Several follow-up visits may be made. Analysis is then conducted to investigate whether there is any association between vaccination status and mortality. A number of potential sources of bias, including survival bias, arise in the interpretation of such observational data.

Survival bias

In many African societies the vaccination card from a child who died will be destroyed. For such children, no record will be collected of any vaccinations received between the date of the last visit when the child was alive and until its death. This will not be the case for children who survive, if their vaccination card is available. The vaccination status of surviving children will thus be more frequently updated between visits simply because they survived until next visit. The follow-up time from the new vaccination to next visit for these surviving children is *immortal* time in the statistical analysis – that is, it would not have been collected had the child died – and will thus bias the survival among vaccinated to the better. This bias has

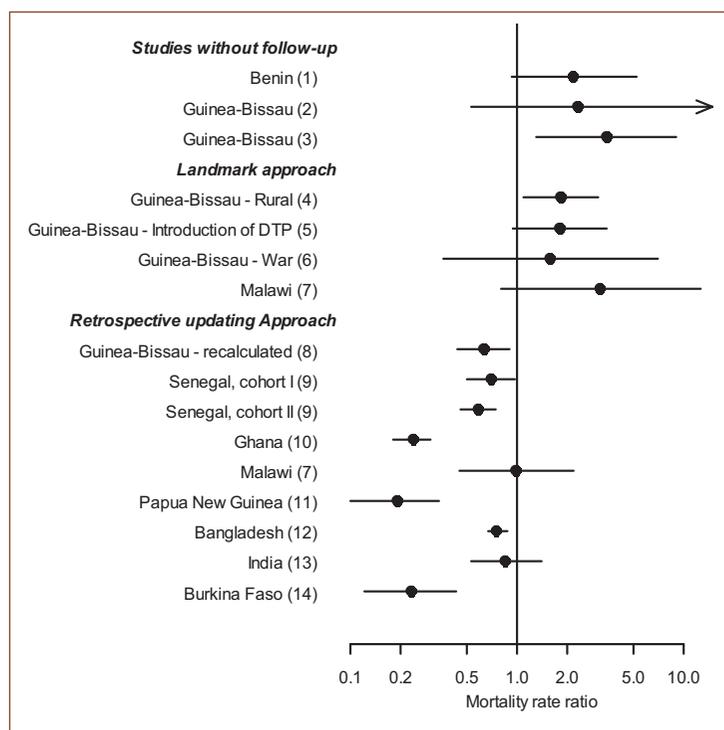


Figure 1. Mortality rate ratios for DTP-vaccinated children compared to DTP-unvaccinated children. The numbers in brackets refer to sources, see (154).

not always been taken into consideration in the statistical analyses. Two main approaches of statistical analysis of this type of data have been used.

Retrospective updating approach

In the retrospective updating approach vaccination status is a time-varying variable changing from unvaccinated to vaccinated, on the *exact date of vaccination*. This approach will introduce survival bias if information is missing on vaccinations given since latest visit for children who died.

Landmark approach

In the landmark approach vaccination status may also be a time-varying variable but only changes from unvaccinated to vaccinated, on a *date of visit*. In a survival analysis the vaccination status is thus fixed or constant between two visits. Survival bias is thus avoided but an attenuation bias is introduced (see below). The BHP has primarily used the landmark approach.

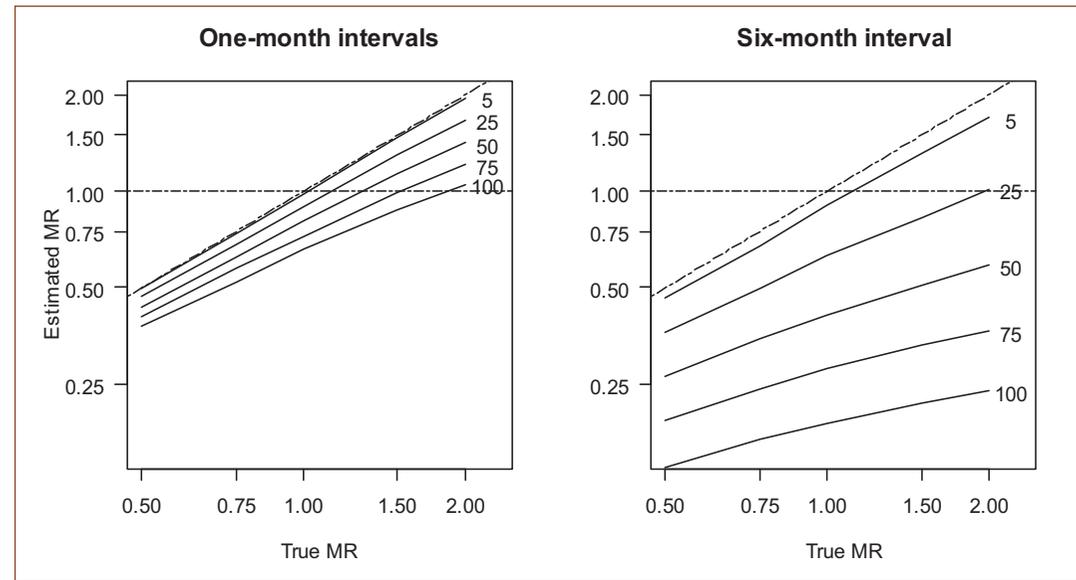
Since the relative impact of these methodological differences was not clear, we conducted computer simulations using a simple vaccination model with varying assumptions on length between visits, true mortality ratio (MR), vaccination coverage, and number of dead children missing vaccine information between visits (“card destroyed”) (153). To assess and compare the impact of the two approaches in a real life example we furthermore, using the retrospective updating approach re-analysed, a cohort from rural Guinea-Bissau which had missing vaccination data depending on the survival of the children (153).

Results from simulations

In Figure 2 it can be seen that for the retrospective updating approach, the estimated effect (MR) was on average lower than the true value, i.e. biased towards zero. The extent of survival bias will depend on the proportion of misclassified (un)vaccinated deaths, and this will depend on the interval between data collection visits and the proportion of children for whom *post-mortem* information is not obtained. In contrast, irrespective of the direction of the true effect, the landmark approach will on average for any combination of simulation parameters lead to conservative estimates of the true effect (bias towards the null hypothesis of a MR=1). The attenuation bias from the landmark approach decreases with shorter intervals and lower vaccination coverage.

Results from re-analysis of BHP data

The data were from the routine collection in rural study areas of BHP. Briefly, children were followed from an initial home visit at 0-6 months of age to a second visit six months later, and only vaccinations given before the initial visit were used to determine vaccination status in the analysis. We only had information on vaccinations given between visits for 3 of the 222 children who died between visits, thus substantial survival bias is present. The Table shows how differently the two approaches allocate follow-up time in the vaccination groups but only the three



deaths with new vaccine information have changed vaccination status. The consequence of this reallocation is a total change in the mortality ratio between DTP-vaccinated and DTP-unvaccinated children which resembles the divergent effects of DTP described above (Figure 1).

Future Perspectives

The demonstrated potentially high impact of survival bias on the analysis of NSE on survival from observational studies has recently moved the discussion about NSE much forward. In April 2008 an international workshop on NSE of vaccines was held in London from which 3

Figure 2. Retrospective updating approach. Results from computer simulation study showing effect of interval between visits, mortality ratios (MR), and percentage of dead children whose vaccination status could not be updated. Each full line with number corresponds to percentage of dead children whose vaccination status could not be updated. Dotted lines represent the identity line and MR=1.

Table. Distribution of deaths and person years according to vaccination status and analytical approach (93).

Vaccination status		Landmark			Retrospective updating		
		Deaths	PYRS*	Rate**	Deaths	PYRS	Rate
No BCG	DTP 1 dose	2	9.0	222	2	16.3	123
No BCG	DTP 2 doses	0	4.5	0	0	3.5	0
No BCG	DTP 3 doses	0	1.4	0	0	4.2	0
BCG	No DTP	33	537.6	61	33	334.4	99
BCG	DTP 1 dose	59	595.5	99	60	679.5	88
BCG	DTP 2 doses	21	266.6	79	20	443.4	45
BCG	DTP 3 doses	12	119.6	100	15	425.0	35
Vaccinated		127	1534.2	83	130	1906.3	68
Unvaccinated		95	875.1	109	92	503.0	183
All		222	2409.3	92	222	2409.3	92
Mortality ratios							
DTP 1 vs. DTP-unvaccinated		1.84 (1.10-3.10)			0.68 (0.44-1.04)		
DTP 2 vs. DTP-unvaccinated		1.38 (0.73-2.61)			0.26 (0.15-0.47)		
DTP 3 vs. DTP-unvaccinated					0.16 (0.08-0.32)		

* Person years of follow-up
 ** Mortality rate per 1000 person years.

papers on respective methodology, data collection, and possible randomised clinical trials of NSE of vaccines are to be published.

References on methodological problems:
 73, 74, 79, 93, 98, 99, 153, 154, 201

BCG at birth: Non-specific beneficial effects

Background

When the Swedish physician Näslund introduced BCG in the northern part of Sweden (Norrbotten) in the late 1920s – shortly after the discovery of BCG - he was surprised that mortality was 2-3-fold lower among the BCG-vaccinated children than among the unvaccinated children (Figure 1). The effect was difficult to explain as it was strongest during the first year of life whereas TB mostly killed older children. Näslund went as far as suggesting (in French) that “One could be tempted to find the explanation for this much lower mortality among vaccinated children in the idea that BCG provokes a non-specific immunity” (41). Many other physicians at the time noted spectacular effects on child survival when BCG was introduced in the 1920s and 1930s (125). However, the focus was on prevention of TB and Näslund’s

hypothesis was never formally tested. Still it has remained common knowledge that BCG is a strong immune stimulant in animal studies and laboratory work.

Our analyses of the impact of routine vaccinations on child survival clearly suggested that BCG was associated with a strong beneficial effect. In all the studies comparing several vaccines, BCG and measles vaccine were associated with a beneficial effect whereas DTP had a negative effect (Figure 2). On the assumption that the effect should be strongest among children who had reacted to BCG, we tested whether having a BCG scar or a positive PPD reaction was associated with an improvement in subsequent survival. In all the studies we did indeed find such an association and the effect was much stronger for girls, strengthening the possibility that this was a non-specific immunological effect (7,87,90,133).

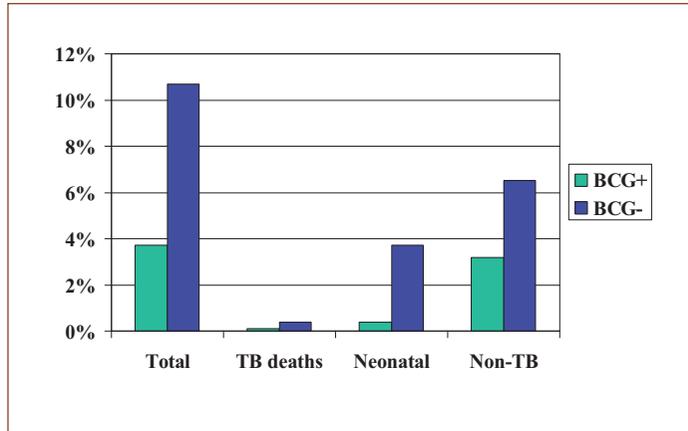


Figure 1. Introduction of BCG in Norrbotten, Sweden, 1927-31: Mortality at 0-4 years of age. 20,000 children

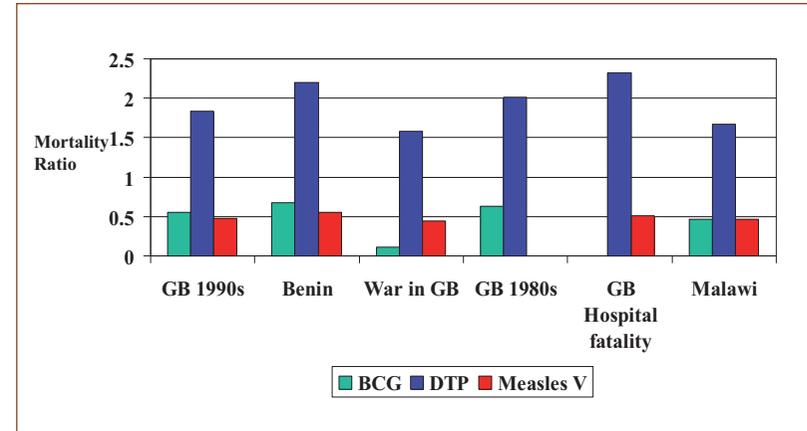


Figure 2. The mortality ratio associated with BCG, DTP and measles vaccine in several community studies from Guinea-Bissau (GB), Benin and Malawi

Still observational studies hold little persuasive power and limited policy implications. We were therefore interested in examining whether BCG had a beneficial non-specific effect in a randomised trial. For logistic reasons, BCG is recommended to be administered at birth in low-income countries and it would be unethical to withhold vaccine from some children. However, many countries, including Guinea-Bissau, have a special policy for low-birth weight (LBW) children who are assumed to be premature and recommended to wait with vaccination until they have reached normal birth weight. In an observational study we had found that BCG was considerably delayed for LBW children and that BCG at birth also to LBW children seemed beneficial (58). We therefore initiated a randomized trial of BCG at birth to LBW children in Bissau.

Results

The majority of births in Bissau are delivered at the maternity of the national hospital in Bissau city. We recruited all LBW children from Bissau city before they were discharged from the hospital. In order to follow these children who mostly were residents outside of the Bandim study area, we had to drive them home to secure proper identification of the residence. LBW children coming to the health centres in the study area for vaccination, after having been born in the home, were also offered enrolment. However, 90% of the LBW children were enrolled at the national hospital.

Between November 2004 and January 2008, we recruited 2,240 LBW children. The median age at BCG vaccination among those randomised to receive BCG at birth was 2 days; among controls

the median age was 49 days, reflecting that many will only get their BCG at 6 weeks of age when they also get DTP and OPV. Follow-up is still ongoing. However, in the first month of life before the subsequent DTP and OPV vaccines were delivered, BCG had a major impact on child survival, reducing mortality by 44% (mortality rate ratio (MRR)=0.56 (0.35-0.89)). This effect was slightly stronger for girls (MRR=0.47 (0.22-0.99)).

Public health implications

There are many implications if this observation can be verified. First, BCG should be administered at birth also to LBW children. Second, maternity wards should vaccinate with BCG before discharging the children. This would not have happened in Bissau unless we had organised and paid for the service as part of the present project. Third, though BCG is recommended at birth for normal-birth-weight children there are often marked delays in BCG vaccination, particularly in rural areas. A bottle of BCG vaccine contains 20 infant doses. It is common practice not to open a bottle for a single child but to require that there should be several children to be vaccinated. Many health centres have therefore only BCG vaccination once a week or once a month. The practice clearly delays BCG vaccination. Fourth, BCG is not a very good vaccine against adult TB, particularly in low-income countries. Numerous groups are therefore trying to develop a new TB vaccine. Replacing BCG with a new TB vaccine could be disastrous if the

new vaccine does not have the same beneficial immune stimulatory effects for children (124).

Future perspectives

As Näslund suggested BCG does indeed provoke non-specific immunity. It would be essential to find out precisely what the immunological mechanisms are (163). It is likely that one study is not enough to persuade policy makers, and more studies from other communities would therefore be desirable. We are hoping to develop a network of INDEPTH sites which will be capable of testing the effect of improving BCG delivery in communities with high neonatal mortality.

References to BCG:
7,58,68,87,88,90,115,124,
125,133,163



Oral polio vaccine at birth



Background

Oral polio vaccine (OPV) is currently recommended at birth as well as in 3 doses together with diphtheria-tetanus-pertussis (DTP)/Pentavalent vaccine (DTP+Hepatitis B+Haemophilus Influenzae type B) at 6, 10, and 14 weeks of age. This policy to provide OPV at birth was introduced 20 years ago to increase the coverage for OPV. OPV at birth may be associated with higher seroconversion rates. However, the effect of OPV at birth on the immune response to other vaccines and on overall child mortality was never studied.

We recently experienced two periods in 2004 and again in 2007 during which OPV was lacking in our study area in Guinea-Bissau, West Africa. Hence, some children did not get the recommended OPV together with BCG at birth. In 2004, we were following all infants as a part

of a large vitamin A trial; 962 children did not receive OPV at birth. We studied the effect of not receiving OPV at birth on the mortality and the immune response to BCG vaccine given at birth.

Results

Not receiving OPV with BCG at birth was associated with a significantly lower mortality in boys, but not in girls (Figure). Boys had a 2.5 fold increased mortality if they had received OPV at birth. We bled a subgroup of the children at 1½ months of age. Receiving OPV at birth seemed to have interfered with the immune response to the simultaneously administered BCG vaccine; OPV at birth was associated with significantly dampened *ex vivo* cytokine response to PPD (purified protein derivative of *M. tuberculosis*). Corroborating this finding, the in

vivo response to PPD (tuberculin skin test) at 2 months of age was significantly reduced among recipients of OPV at birth.

In 2007, we had a similar episode in which 99 children enrolled in a trial of low-birth-weight (LBW) children did not receive OPV at birth. Again, not receiving OPV at birth had sex-differential effects on subsequent mortality.

Public health implications

Based on these observations, receiving OPV at birth may have two negative effects; first, it may increase male mortality, and second, it may interfere with immunity against tuberculosis. In both observational studies, boys not receiving OPV and girls receiving OPV at birth had a 40% lower mortality than in the opposite situation.

OPV may be replaced with inactivated polio vaccine (IPV) in the global strategy to eradicate polio. However, this is unlikely to happen soon as IPV is much more expensive than OPV and polio is still not under control in some low-income countries including Nigeria, Somalia, Namibia, and India. If OPV at birth with BCG does have a harmful effect on boys, OPV at birth should be discontinued for boys immediately

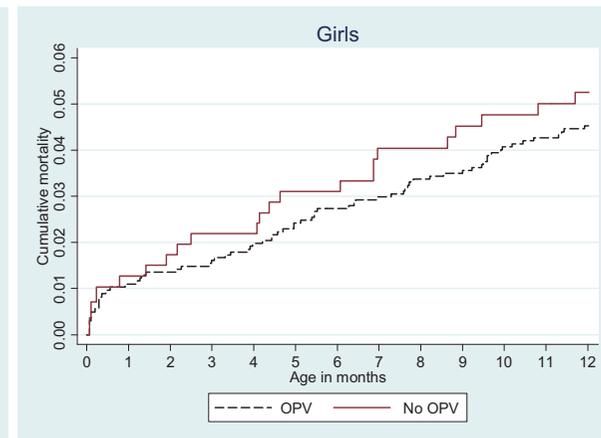
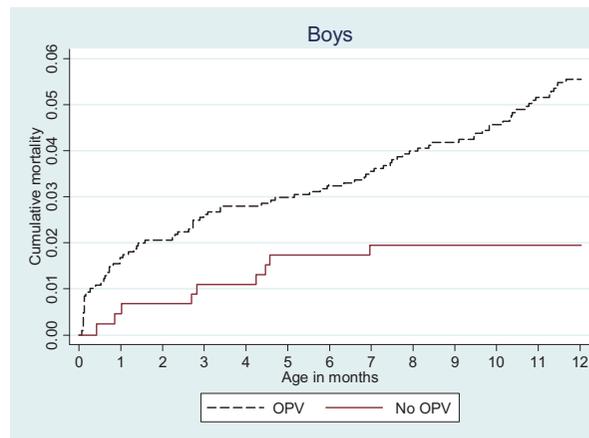
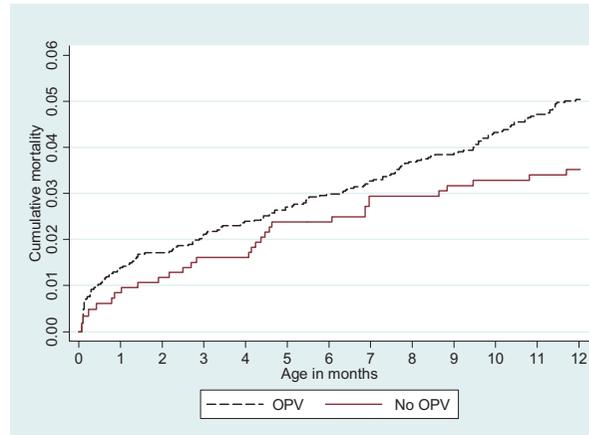
since the marginal contribution of this vaccine to polio immunity and control is limited. It is also important to document whether OPV has a non-specific beneficial effect for on girls before the vaccine is removed. If OPV has a beneficial effect on girls, OPV at birth should be continued for girls or replaced with similar immune modulation.



Future perspectives

OPV at birth is given for logistic reasons, to boost polio immunity. There have been no polio cases in Guinea-Bissau for the last 10 years.

Figure. Cumulative mortality curves as a function of receiving OPV at birth or not, overall and by sex



Hence, there is every reason to test in a randomised trial whether not receiving OPV at birth is associated with 1) decreased mortality in boys and 2) increased immunological response to BCG. Such a trial has now been initiated. The trial will contribute importantly to measuring the importance and sex-differential effects of modulation of the immune system in early life and to understanding the underlying mechanisms. The immunological data are completely novel and highlight the fact that multiple vaccines have co-regulatory effects that need to be understood at a molecular level to allow the vaccine world to design not only better vaccination schedules but also better new vaccines.

References: 223



Revaccination with BCG

Background

Many observational studies have shown that BCG vaccination is associated with lower mortality in ways that cannot be explained by prevention of tuberculosis. Children who have a BCG scar or a positive tuberculin skin test reaction have better survival, an effect particularly strong for girls. A French-Algerian study from the first part of the 20th century when BCG was used as an oral vaccine reported a 27% reduction in mortality from repeated doses of BCG administered at birth, one, and three years of age. Revaccination with BCG is not recommended by WHO since no protective effect against TB has been demonstrated. However, some countries have used revaccination among young adults.

We therefore conducted a randomised clinical trial to test whether revaccination with intradermal BCG vaccine would improve child survival.

Booster doses of DTP and OPV were administered at 18 months of age in Bissau. We randomised children to BCG revaccination at 19 months of age to reduce interference with booster DTP and planned to include 3000 children. Since animal studies had shown that BCG prevented malaria in mice we also conducted a special investigation to examine whether BCG might reduce the risk of malaria infection in 1½-2 year old children. As part of this study a large number of children were examined for malaria infection and anaemia and treated with iron if they were anaemic.

Results

The study recruited nearly 2900 children between July 2002 and April 2004 at the 3 health centres in the urban study area. The study included only children who did not have large PPD reaction; children with a large reaction were

referred to examination for tuberculosis. Enrolment was stopped prematurely in April 2004 due to a cluster of deaths in the BCG revaccination arm but we continued to follow the children to 2006. As seen in the Figure, mortality increased in the BCG arm at the end of 2003 and beginning of 2004. As a consequence there was a significant change in the mortality rate ratio (MRR) between BCG-revaccinated and control children. As a result there was no overall benefit from BCG revaccination, mortality being slightly higher among revaccinated children. Likewise there was no benefit in terms of protection against malaria infection or hospitalisation for malaria (167). However, there was a sex-differential effect; the overall hospitalisation incidence rate was significantly lower for girls than for boys among BCG revaccinated children (Incidence rate ratio (IRR)=0.66 (0.47-0.93)) whereas there was no sex-difference for controls (225).

We explored possible causes of this unexpected mortality cluster. The sudden increase in mortality coincided with a vitamin A supplementation (VAS) campaign and the treatment of a large number of anaemic children with iron in the end of November 2003 (see Figure). At the same time a measles epidemic was sweeping through the

community. During this short period mortality was nearly three-fold higher among BCG-revaccinated children (Mortality rate ratio (MRR)=2.69 (1.05-6.88)).

Micronutrients received	No vitamin A	Vitamin A+
No Iron	0.34 (0.04-3.29)	8.46 (1.06-67.7)
Iron +	Undefined P=0.070	1.91 (0.35-10.5)
All	1.29 (0.29-5.75)	4.14 (1.17-14.7)

Table 1. Mortality rate ratio (MRR) for BCG+ revaccination versus No BCG revaccination according to micronutrients received

We had monitored who received VAS during the campaign and we had administered iron ourselves. Hence, we could examine whether the increase among BCG-revaccinated children was related to the supplementation with micronutrients (Table 1). The negative effect of BCG revaccination was particularly strong among the large group of children who had received VAS during the campaign. Iron may also have had a negative effect though this was not statistically signifi-

cant. If anything the effect was opposite among children who had received neither VAS nor iron (226).

We also examined whether mortality during the whole trial depended on the vaccination status at enrolment. As seen in Table 2, BCG revaccination was associated with a significant 3-fold reduction in mortality among the children who - as planned - had received booster DTP prior to BCG revaccination. However, 60% of the children had not received all vaccines prior to enrolment and they were told to return to receive the missing doses of DTP, OPV or measles vaccine. Most did return, and among these children BCG revaccination was associated with significantly increased mortality. As a consequence the overall effect of BCG revaccination was slightly negative. The negative effect of VAS among BCG-revaccinated children was only found among children missing vaccines at enrolment and who did receive DTP or measles vaccine or both after enrolment (226).

Public health implications

BCG revaccination did have the hypothesised beneficial effect among the children who as

	Mortality rate/100 pyrs (deaths/pyrs)		Mortality rate ratio BCG/no BCG
	BCG revaccination	No BCG revaccination	
	N=1412	1419	
Booster DTP before enrolment	0.4 (5/1393)	1.0 (14/1406)	0.36 (0.13-0.99)
No Booster DTP before enrolment	1.8 (37/2012)	1.0 (21/2022)	1.80 (1.06-3.08)
All	1.2 (42/3405)	1.0 (35/3428)	1.20 (0.77-1.89)

planned had received a booster dose of DTP prior to enrolment in the BCG trial. However, the trial also suggests that BCG vaccination in a situation with several other immune stimuli (VAS, iron, DTP, measles vaccine) may have a negative effect presumably due to some form of overload and misdirection of the immune response in response to infections. BCG revaccination could presumably be used to reduce mortality in settings in which other interventions were controlled.

However, there may be little coordination between intervention programmes being offered to children in low-income countries. It is therefore quite likely that children may receive a delayed BCG vaccination in the age group in which VAS is usually administered. One study from Bangladesh in which a large proportion of the children had received BCG and DTP with measles vaccine after 9 months of age did in fact report a significant two-fold increase in mortality associated with BCG vaccination.

Table 2. Mortality rate and mortality rate ratios by BCG revaccination status, sex and sequence of vaccinations.

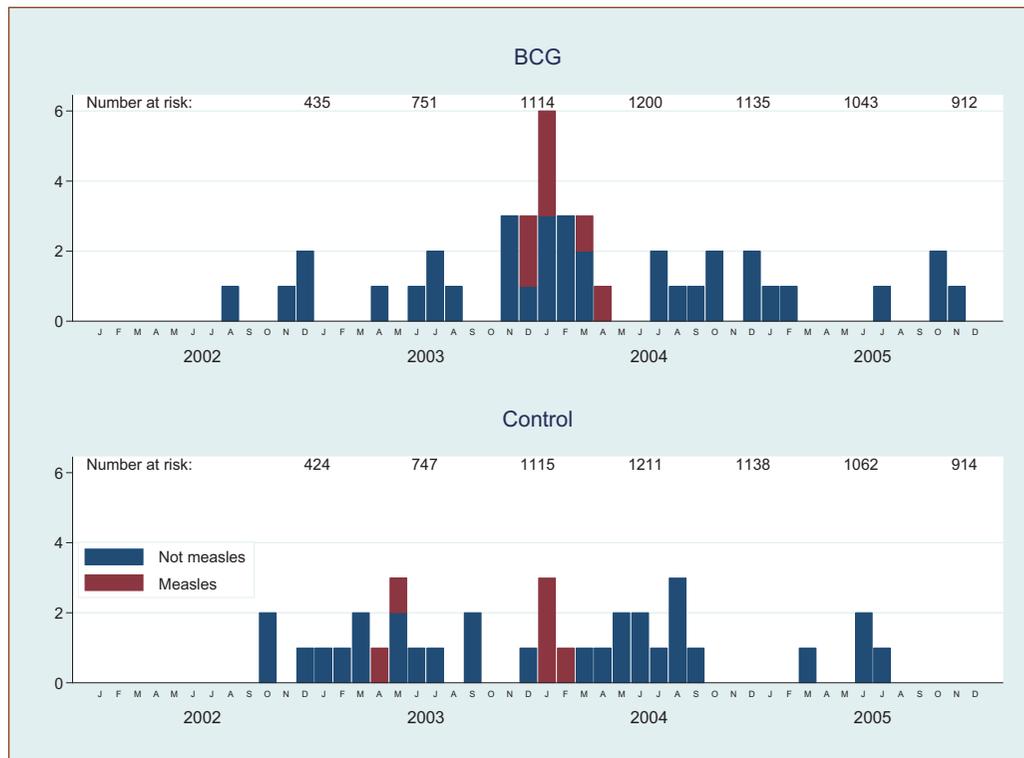


Figure. The number of deaths by month in the BCG revaccinated and the control group from July 2002 to May 2006

Future perspectives: understanding interactions

The study confirmed that non-specific effects of vaccines are important; the differential effects cannot be related to protection against tuberculosis. The study also suggested that non-specific effects may be difficult to control due to unanticipated interactions with a large number of other immune stimuli like vaccines and micro-

nutrients. To get a better understanding of the basis for these interactions will be the next challenge. This and further epidemiological studies will be needed to convince the donors and policy makers that we do need to test the likely interaction between our common interventions in childhood. Though interventions have a beneficial effect when studied in a controlled situation they may in fact have a totally different effect in a real life situation with administration in sequence with many other interventions. Though we believe that our interventions are good and efficient, they may actually have a negative effect under certain circumstances.

References on BCG revaccination: 167,225,226

High-titre measles vaccine: The sequence of vaccinations

Background

The incident of high-titre measles vaccine (HTMV) was the key event in defining the research agenda on non-specific effects of vaccines. HTMV was tested in Guinea-Bissau in the 1980s and found to be protective against measles infection. In 1989, WHO recommended HTMV to be administered at 6 months of age. However, at the same time it became clear that HTMV was associated with increased female mortality in Guinea-Bissau, an observation that was subsequently confirmed in Senegal and Haiti. WHO withdrew the vaccine in 1992.

This incident clearly showed that non-specific events are important. HTMV was fully protective against measles infection. Nonetheless the vaccine was associated with a two-fold higher female mortality but no difference for boys. So not only did vaccines have non-specific effects

these effects were also sex-differential. These effects were major. In the West African meta-analysis, HTMV was associated with a 35% higher mortality between 4 months and 5 years of age.

It has been a challenge to understand how this could happen. If it happens once it could happen again. When the vaccine was withdrawn the common assumption was that we had come too close to the natural disease and that new measles vaccines were needed to protect against measles before 9 months of age. However, that made no sense because why would that be a problem only for girls? When we detected that DTP and other inactivated vaccines were associated with increased female mortality, it offered a completely different explanation of the HTMV incident.



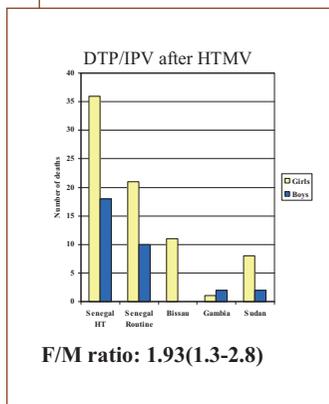
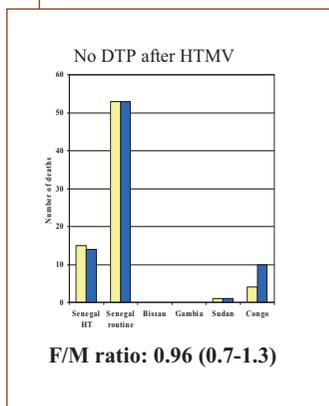


Figure. The number of female and male deaths in all studies of HTMV depending on whether DTP/IPV had been administered after HTMV

Results

In the West African studies, HTMV had been administered at 4-5 months of age and most children had received DTP or inactivated polio vaccine (IPV) after measles vaccine. We therefore tested in all the West African studies whether this change of sequence was the real cause of increased female mortality. Indeed, excess female mortality was only found among the group of children who did not receive DTP/IPV after measles vaccine, the female-male mortality rate ratio (MRR) being 1.93 (Figure). Among the small group of children who did not come back for vaccination at 9 months of age there was no difference, the female-male MRR being 0.96.

There are only two other studies of HTMV from Sudan and Congo which have collected information on mortality after vaccination. We reanalyzed these studies (119). They were also consistent with the new hypothesis. In Sudan girls had also had significantly higher mortality than boys among the HTMV recipients. In Sudan, vaccine information had not been collected after enrolment but vaccination had been registered at enrolment, and it was known that most children had received missing doses of DTP and OPV after enrolment. There was a clear association between the number of doses of DTP missing at enrolment (and presumably given after enrolment) and the female-male MRR after reception of HTMV. In contrast, in Congo the children did not receive DTP after HTMV; most children received HTMV after DTP3 or together with DTP3. The female-male MRR was 0.40 in Congo (119).

In contrast to the common belief that HTMV had a deleterious effect, HTMV per se may have been associated with low mortality. The annual mortality between 6 and 36 months of age was only 1.0% in Congo among children who had received HTMV as the most recent vaccination. In Sudan, HTMV recipients had significantly lower mortality than randomised controls receiving meningococcal vaccine as a “placebo” in the interval before the controls also received measles vaccines (119).

These studies suggested that the sequence of vaccinations was important, the most recent vaccination determining the immunological profile. For girls, it was problematic if the most recent vaccination was DTP and not measles vaccine. Hence, DTP vaccination status at enrolment could determine subsequent mortality in a measles vaccination trial if missing doses of DTP were administered after enrolment. As indicated in the Table, this did turn out to be the case in the three measles vaccine trials from which we had data. After receiving measles vaccination, children missing one or more doses of DTP (DTP0-2) had significantly higher mortality than those who had already received DTP3, but only among girls. For boys this difference did not matter.

Public health implications

These data indicate that the real problem was not HTMV but DTP and the change in sequence of vaccination. We removed a good vaccine for

Table. Mortality rate after enrolment in measles vaccination trials in relation to the DTP vaccination status at enrolment

Study	Girls			Boys		
	DTP0-2	DTP3	MR (DTP0-2/DTP3)	DTP0-2	DTP3	MR (DTP0-2/DTP3)
Guinea-Bissau (166)	7.5%	3.8%	1.97 (1.04-3.72)	6.4%	6.0%	1.06 (0.60-1.90)
Sudan (119)	6.0%	2.8%	2.16 (0.27-17.3)	1.4%	1.9%	0.71 (0.06-7.87)
Congo (119)	10.0%	2.8%	3.54 (0.71-17.5)	10.6%	5.1%	2.06 (0.46-9.22)
Total			2.10 (1.19-3.72)			1.13 (0.67-1.90)

the wrong reason. Previous studies of vaccines have not taken the interaction and sequence with other vaccines into consideration. However, the data of administering DTP after measles vaccine (Table) or after BCG (Chapter 20) suggest that these effects may be very important for the outcome in vaccination trials.

The reinterpretation of HTMV constitutes the strongest argument for the hypothesis that DTP has negative effects on girls. It is impossible to generate a totally unexpected hypothesis from unconnected studies and find it consistent with all existing data unless a causal biological process is at work. An implication of this hypothesis is that the same may happen again once new measles vaccines are going to be tested in the first months of life.

Future perspectives

These observations also question the current practice of administering missing vaccines whenever a child comes to a health centre. This may be particularly important because it is current donor policy to measure the performance of the vaccination program by the coverage for DTP3. Therefore, many children are getting DTP administered with measles vaccine or after measles vaccine. All indications are that such practices are associated with increased mortality (Chapter 21). It would seem necessary to test the practice of administering DTP with measles vaccine or after measles vaccine in randomized clinical trials.

References on HTMV and sequence of vaccinations: 10,12,20,119

Early measles vaccination: Targeted and non-targeted effects

Background

The current policy of providing measles vaccine at 9 months of age in low-income countries was defined by the World Health Organization 25 years ago. The policy was built on studies of seroconversion after measles vaccination at different ages. To interpret the data five assumptions were used: children with antibodies were fully protected; children with no measurable antibodies were fully susceptible; measles in previously vaccinated and unvaccinated children was equally severe; it did not matter whether they got measles in infancy or later; and seeing measles in vaccinated children, so-called “vaccine failures”, would lead to lack of confidence in the vaccination program. Based on these assumptions it was decided to vaccinate at 9 months rather than at 7 or 8 months of age. These assumptions were wrong and had the impact on infant survival been assessed in a

randomised trial, it would probably have been better to vaccinate at 6 or 7 months of age. “Vaccine failure” cases have lower case fatality and early vaccination would have moved measles infection to an older age group with lower case fatality. Furthermore, measles vaccination has non-specific beneficial effects which would prevent deaths as soon as measles vaccine was given irrespective of measles infection (1,3,8,84,107). However, the effect on infant survival of measles vaccine at age 9 months compared with measles vaccine at an earlier age was never tested.

There are further reasons to believe that measles vaccination before 9 months of age would reduce mortality more than measles vaccine at age 9 months. First, an increasing proportion of mothers have not had natural measles infection but have been immunised in childhood. Such mothers have lower measles antibody levels and

transfer lower levels to their offspring. Second, many studies have shown that DTP vaccine is associated with higher mortality for girls (12). Hence, it might be beneficial particularly for girls to be vaccinated early with measles vaccine and thereby reduce the time of exposure to DTP as the most recent vaccine (8).

For all these reasons, we have tried different strategies to reduce the age at measles vaccination including high-titre measles vaccination (HTMV) and two-dose schedules at 6 and 9 months of age (12,32). As described in the chapter on HTMV both of these strategies ran into problems with the sequence of vaccinations, due to a negative effect of DTP being administered after measles vaccine. In the most recent trial, we have therefore included children only if they had already received DTP3 and therefore should receive no further DTP after measles vaccination.

Since we have maintained very high measles vaccination coverage in the study area for many years we should have had no measles epidemic. However, coverage has declined in the rest of Bissau and many older children have moved to the study area. An epidemic of more than 2000 measles cases flared up soon after the new measles vaccination trial had started in 2003. This provided an unexpected opportunity for testing the effect of early measles vaccination.

Results

Between August 2003 and May 2004, 1333 children were randomised at the three local health centres in the study area, one third receiving standard Edmonston-Zagreb measles vac-



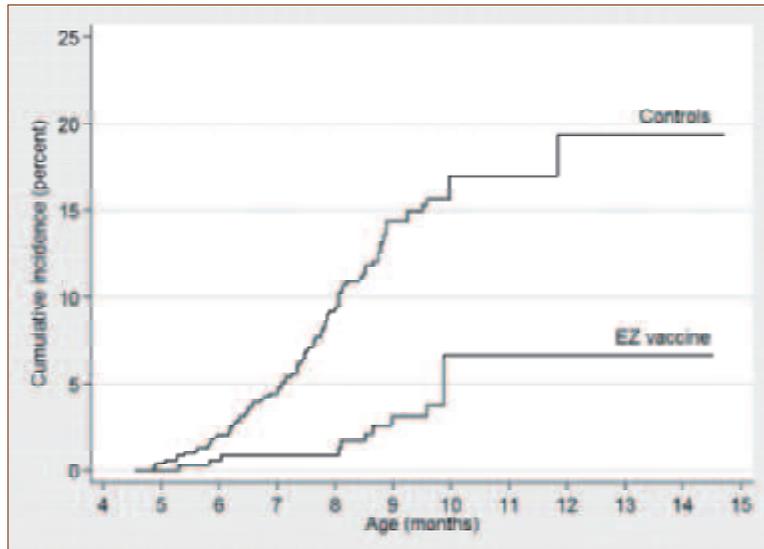


Figure. The cumulative incidence of measles in the early EZ measles vaccine and control groups

cine at 4½ months of age and the rest receiving nothing apart from medical consultation and treatment. All children were invited back at 9 months of age to receive their normal

measles vaccination or their second dose of vaccine (214).

Surprisingly only 28% of the children who had measles antibody tested at 4½ months of age had protective antibody levels, the proportion being significantly lower among girls than among boys (RR=0.55 (0.34-0.91)). Consistently with the low level of maternal antibodies, the measles incidence was very high during the epidemic. Before 9 months of age, 19% of the unvaccinated children caught measles. In spite of the fact that we offered clinical treatment to all children including at hospital, the case fatality was still as high as 8%.

Measles vaccination at 4½ months of age turned out to be highly efficacious in the interval between 4½ and 9 months of age in which the children would not normally be protected. The efficacy against serologically confirmed or definite clinical infection was as high as 94% (Figure). Early measles vaccination prevented completely measles hospitalisation and measles death. Though not significant, overall mortality was lower in the early measles vaccination group (MRR=0.18 (0.02-1.36)). Long-term follow-up is still ongoing in the present study so the final result is not yet known. However, the indication is that early measles vaccination may help reduce hospitalisations and non-measles deaths among girls.

Public health implications

In spite of many years of measles vaccination, measles is still potentially a very severe infection in this urban community. The situation has

been aggravated by declining maternal antibody levels. The majority of children are losing maternal antibody levels before 6 months of age. When the current vaccination programme was formulated 25 years ago most children only lost protection between 6 and 9 months of age. Now there is a second group of mothers who has not had natural infection and their offspring may therefore be losing protection already at 3 to 5 months of age. The challenge is therefore to find a policy which may protect both groups of children. One could fear that early measles vaccination would lead to lower antibody levels and therefore a higher risk of measles infection later in life. If that is not the case, the present study suggests that a two-dose strategy at 4 and 9 months might be a good option in countries which have had high measles vaccination coverage for a long time. Already at this stage it seems clear that EZ measles vaccine can be used as early as 4 months of age during outbreaks or in situations with a high concentration of infants.

Future perspectives

If the present beneficial effects on also non-measles deaths of early measles vaccination persist, early measles vaccination should be tested in other populations, particularly for the non-specific beneficial effects. It would be interesting to assess the impact in a situation with higher maternal antibody levels.

Following the problems with HTMV, major donors, including EU, WHO, Gates and NIH, have invested in new measles vaccines which should be able to immunise children early in life. Such new vaccines should be tested for both targeted and non-targeted effects against EZ vaccine which has a proven efficacy in this age range.

References on measles vaccine:

1,3,8,84,97,107,214

Sex-differential and non-specific effects of inactivated vaccines



Background

Several observational studies have suggested that DTP may also have non-specific effects, but in contrast to BCG and measles vaccine the effects of DTP are negative. In medical culture, observational studies have little impact. For these observations to have policy implications, it would be desirable to conduct randomised studies to test a possible negative effect of DTP. However, it is unethical to withhold a vaccine which is already recommended. Hence, the hypothesis that DTP has a negative effect on survival can only be tested indirectly, by making logical deductions and testing these. The more deductions can be shown to be consistent, the more likely it is that the underlying hypothesis reflects a causal process. This process has generated several linked observations which do suggest that DTP has an unwanted effect on girls. It should be noted that most observations have been done in situations with herd immunity to

pertussis, and hence a survival benefit from being protected against pertussis has not been evident.

Results

First, in all community studies analysing the impact of several vaccines on survival, BCG and measles vaccine have been associated with a beneficial effect whereas DTP has had a negative effect (Chapter 15).

Second, the effect of DTP has been worse for girls than for boys. This is illustrated by the female-male mortality rate ratio (MRR) in all available studies (Figure 1) (44, 45, 81, 119, 126, 134, 179). This pattern is also different for BCG and measles vaccine which are both associated with lower female mortality. Most studies have focused on mortality but a series of morbidity studies have also indicated that the relative risk

for girls and boys changes depending on the most recent vaccine, incidence being higher for girls than boys while DTP is the most recent vaccine (120,129).

Third, if DTP is in fact associated with increased female mortality one would expect that female mortality is increased over male mortality in the age group in which DTP is the predominant vaccine in communities with high vaccination coverage. Female-male mortality has not previously been analysed in this way but in all the studies we have analysed the pattern has been consistent, as illustrated by the two studies from The Gambia and Malawi (Figure 2). After an initial lower mortality for girls, female mortality increases over male mortality around 3-4 months of age, i.e. the age group in which DTP predominates. Around 9 to 12 months of age, i.e. the age group in which measles vaccine predominates, female mortality declines steeply becoming again lower than male mortality.

Fourth, if these negative effects had to do with the vaccine being “inactivated” rather than with any of the specific antigens of DTP vaccine, one should presumably find similar patterns for hepatitis B vaccine (HBV) and inactivated polio vaccine (IPV). For both vaccines we have in fact shown that they are associated with increased female mortality. For HBV, the female-male mortality ratio in Guinea-Bissau was 2.20 (1.07-4.54) during the period in which HBV was provided to a large number of the children in Bandim (65). Through the 1980s and 1990s, we used IPV as a “control” vaccine in several trials in which meas-

les vaccine was provided early at 4-6 months of age. Until the IPV recipients received measles vaccine at 9 months of age, girls had 52% (2-128%) higher mortality than boys (166).

We have obtained and analysed data sets from several other low income countries including The Gambia (134), Senegal (44,201), Ghana, Congo (119), Sudan (119), Malawi (126) and India. All studies have been consistent with the studies from Guinea-Bissau showing sex-differential and non-specific effects of vaccines (Figures 1-2).

Public health implications

Given this consistency and the fact that DTP has been found to have a negative effect for girls when administered after measles vaccine (Chapter 18) and the negative interactions with vitamin A supplementation (Chapter 25), it is difficult to imagine that DTP should not have a negative effect on mortality for girls. DTP has mainly been examined in situations in which herd immunity has been sufficient to prevent deaths from whooping cough. It is therefore not known what would happen if DTP was removed. In the long run, it will be necessary to develop a DTP-type vaccine without negative effects. However, such a vaccine does not exist and we have therefore focused on different strategies to reduce the time of exposure to DTP as the most recent vaccine (Chapters 17 and 19).

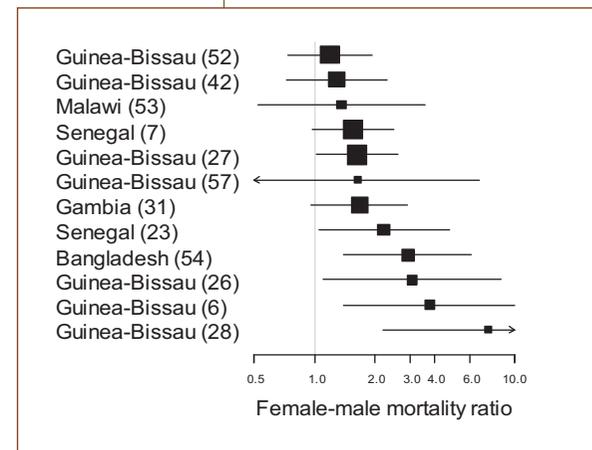


Figure 1. Female-male mortality rate ratios among DTP-vaccinated children

A number of WHO-sponsored studies has questioned our observations. These studies have often had methodological problems with survival bias (153). Furthermore, most studies have examined the effect of DTP when administered simultaneously with BCG whereas we have examined the effect of DTP administered after BCG as currently recommended by WHO (154). The effect of simultaneous BCG and DTP vaccinations is likely to be quite different from sequential administration (201).

Given the current controversy, it is still necessary to conduct further studies of the impact of DTP on overall morbidity and mortality and these should preferably be randomised clinical trials since WHO's Global Advisory Committee on Vaccine Safety has declared that it is unlikely to be persuaded by observational studies.

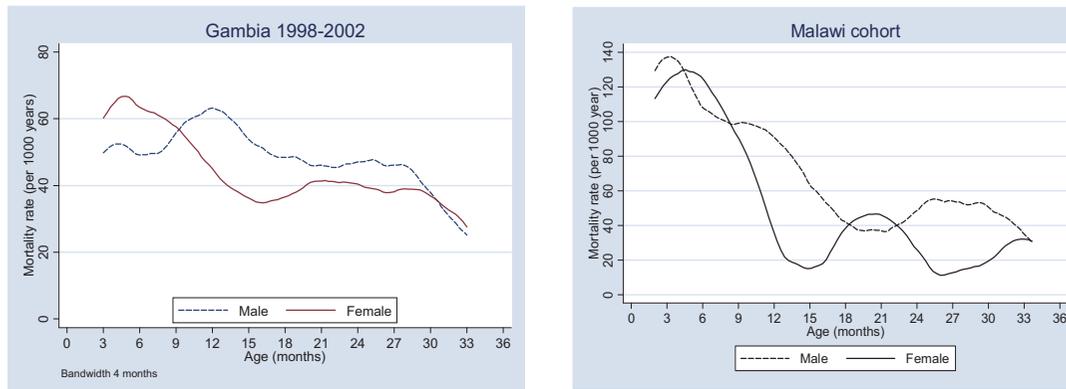
Future perspectives

We have pursued three slightly different approaches to reduce the time of exposure to DTP as most recent vaccine. As suggested by Figure 2, if the age at measles vaccination was moved forward from 9 months to 4-5 months of age this might reduce female mortality in an age group with a very high mortality. The preliminary results are supportive (Chapter 19). We have also tried to administer BCG revaccination after booster DTP to reduce the negative effect of DTP (Chapter 17). Finally, in a study which is still ongoing, we are randomising children to receive or not receive DTP booster vaccination.

Several other approaches may have to be tested in the future. There is good evidence from observational studies that the effects of combining DTP with either BCG or with measles vaccines are quite different from the effect observed when the vaccines are administered sequentially as recommended by WHO. Since such combined vaccinations are quite common, particularly in rural areas, it would be important to test their effects in randomised trials. It should also be considered to delay the initial DTP vaccination in randomised clinical trials.

References on DTP: 12,26,41,44,45,46,65,73,74,75, 79,81,93,98,99,119,120,126,129,134,149, 150,153,154,161, 166,179,201,217

Figure 2. Age and sex-specific mortality curves from Gambia and Malawi (126,134)



Out-of-sequence vaccinations

Background

The high-titre measles vaccine (HTMV) incident (Chapter 18) made it clear that the sequence of vaccinations was very important for child mortality. This made us look more systematically at the out-of-sequence vaccinations. WHO has for many years recommended a schedule of BCG and OPV at birth and then three doses of DTP and OPV at 6, 10, and 14 weeks of age, and finally measles vaccine at 9 months of age. In some countries the tradition of administering booster doses of DTP and OPV has continued even though this is no longer official WHO policy (Figure). In recent years new vaccines have been added, in particular Hib and HBV often in a penta-valent vaccine with DTP.

In practice most vaccinations may not follow this schedule. Particularly in rural areas there are many reasons why vaccinations get delayed.

If the child is sick or malnourished the mother is likely to postpone vaccination. Many health centres have only a weekly or monthly day for BCG vaccinations. As a result, in the studies from Senegal, Ghana, India, Bangladesh (154), and the Philippines (201) which have reported such data, a majority of the children received BCG together with DTP or after DTP. Likewise, delay in the administration of DTP is very common and for that reason many children receive DTP and measles vaccination simultaneously or DTP after measles vaccination. This tendency has been strengthened by the donor policy of monitoring the performance of the national vaccination programmes through the coverage for DTP3. In rural Bissau 2/3 of the doses of measles vaccine are administered with DTP. We therefore examined available data sets for indication of such changes in the sequence of vaccinations.



Receiving DTP after HTMV or after standard measles vaccine was a strong risk factor for subsequent female mortality. It has not been examined whether the combination of routine vaccinations has an impact on child survival in low-income countries. BCG and measles vaccine are associated with an overall beneficial effect on childhood survival whereas DTP is not. However, these generalisations do not permit us to predict what might happen when these vaccines are given together. We therefore examined the mortality rate and possible sex-differential effects when DTP is given simultaneously with BCG or measles vaccine.

Results

Simultaneous administration of BCG and DTP.

We have limited data on BCG and DTP combined but the indication from Bissau (44), Senegal (201) and India is that BCG and DTP combined is better for girls than for boys. For example, in a study of female-male twin pairs from Bissau and Senegal, the female twins had lower mortality as long as combined BCG and DTP were the last vaccinations, the female-male MR being 0.29 (0.02-1.03) (44). The impact on overall mortality of BCG and DTP combined or BCG after DTP may be beneficial.

Simultaneous administration of measles vaccine and DTP. So far we have been able to examine simultaneous administration of DTP and measles vaccine in 5 studies. All studies have found simultaneous DTP and measles vaccinations to be associated with higher mortality than having received measles vaccine as the most recent vaccine (Table). It should be noted that a priori the children who receive measles vaccine and DTP vaccine together may have less compliant mothers and have a higher risk of dying; however, the effect estimates seem too large to be explained merely by selection bias. All studies also suggest that combined measles vaccine and DTP may be worse for boys than for girls. One of these studies from Senegal was a randomised trial and the female-male MRR was 0.58 (0.33-1.03) (12).

The effect of combined DTP and measles vaccine might blur the beneficial effect of measles vaccine in survival analyses looking at the average effects of several vaccines without restricting the analysis to the most recent vaccination. For example, a Bangladesh study reported that the effect of measles vaccine was only 0.93 (0.65-1.34). However, when children with simultaneous DTP/BCG and measles vaccinations were excluded, the estimate of measles vaccine became 0.61 (0.44-0.85). This change implies that the

DTP/BCG and measles-vaccinated children censored in the analysis had strong excess mortality. Hence, this study supports the same trend as the one we observed in our own studies (Table).

Public health implications and future perspectives

There is little tradition for testing the interactions between different interventions. Our data suggest that it makes a difference in which order vaccines have been administered. This possibility has not been taken into consideration in the current immunisation programme which recommends that missing doses of vaccines should be administered whenever there is a possibility.

It would seem necessary to test the effect of these unplanned combinations of both BCG and DTP and of DTP and measles vaccine in randomised trials.

References on out-of-sequence vaccinations: 12,44,119,126,134,150,154, 166,201,217

Country	No. children	Study	MRR (MV+DTP versus MV)
Congo (119)	485	EZ trial at 6 to 36 months	5.38 (1.37-21.2)
Malawi (126)	751	Routine vaccination	5.27 (1.11-25.0)
Guinea-Bissau (150)	403	Hospital case fatality for children aged 6-17 months	1.87 (1.06-3.31)
Guinea-Bissau (217)	201	Campaign with vitamin A in which some children received MV and some MV and DTP; children aged 6-17 months	Undefined; p=0.015
Gambia (134)		10% (4/41) of dead children aged 9-17 mo had received DTP=MV compared with 2% (43/2539) in community	5.59 (2.10-14.8)

Table. Mortality ratio for children receiving measles vaccine (MV) and DTP simultaneously compared with having received MV alone as the most recent vaccine

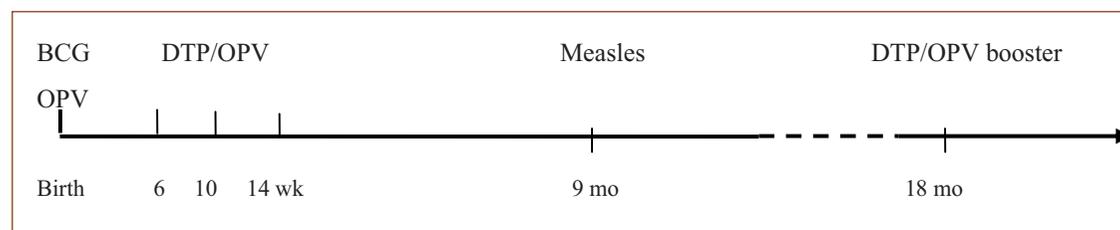


Figure: Vaccination schedule in Guinea-Bissau

Non-specific effects of smallpox vaccine in Guinea-Bissau

Background

Smallpox vaccination was introduced in 1800 and was associated with marked reductions in mortality in the industrialising countries. The last case of smallpox occurred in 1977 and in 1980 the World Health Organization recommended stopping smallpox vaccinations. No assessment was made of the health impact of discontinuing vaccination. Smallpox vaccination is associated with a strong immune stimulation and could have important non-specific effects as observed for other live vaccines such as BCG and measles vaccines.

In order to study a possible non-specific effect of smallpox vaccine, we needed access to vaccination status before 1980. In Guinea-Bissau this was possible through vaccination scar surveys. In several studies we investigate a possible non-specific effect of smallpox vaccine, some 30

years after its administration – an idea that may be over-stretching the imagination of many readers. There are, however, a number of case-control studies from high-income countries suggesting that smallpox vaccination protected against diverse chronic conditions and cancers. In a European study of people with malignant melanoma, it improved survival to have been smallpox vaccinated several years prior to diagnosis.

Results

Evaluation of smallpox vaccine scars as proxy for smallpox vaccination

We located most of the registration books of the vaccination programme in Bissau for the period 1964–1980. Sixty-nine persons with documented vaccination and still living in the study area had been visited at home to examine presence and size of smallpox vaccine scars in connection

with one of our surveys. Ninety percent (62/69) had had a smallpox vaccine scar identified, the median diameter being 16.5mm. The seven adults without a smallpox vaccine scar were revisited and five had a small scar that had not been detected. Among 1,076 teenagers who were too young to have received smallpox vaccine and whose scars were therefore due to BCG, the median diameter was 5mm (135). Smallpox scar was thus considered a good proxy for smallpox vaccination with at least 90% sensitivity. Since it was not possible to clearly distinguish between large BCG and small smallpox vaccination scars, results were also presented as any scar (smallpox or BCG vaccination) versus no scar in addition to smallpox scar versus no scar.

Adult mortality

From January 1998 to January 1999, with pauses during periods of war, field workers assessed vaccination scars for 1893 adults above 25 years of age in Bandim 1 and 2. Only individuals above 25 years of age were eligible for inclusion, since smallpox vaccination in Bissau ceased in 1980. Survival was assessed in a survey conducted in June–July 2002. Comparing individuals with a smallpox vaccine scar but no BCG scar with individuals without any scar (n=873/1373), the mortality ratio (MR) adjusted for age was 0.60 (0.40–0.91) during the non-war periods (Figure 1). The effect of smallpox vaccination may have been stronger for women (MR = 0.51) than men. The effect of smallpox vaccine appeared to be similar among individuals with a BCG

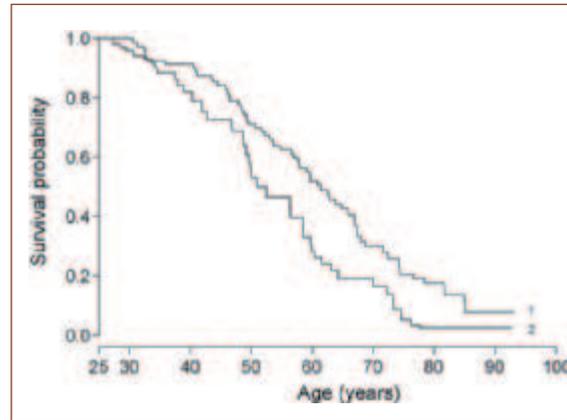


Figure 1. Kaplan–Meier survival curves for 873 people with a vaccinia scar and no BCG vaccination (1) and for 500 individuals without any scar in Bissau (2)

scar, and the effect of having any type of scar on mortality was 0.61 (0.41–0.89)(135).

It was possible to test our findings from the Bissau scar survey within a case-control study of HIV-2 infection in Caio, rural Guinea-Bissau. In 2003, 367 individuals born before 1974 (and therefore possibly smallpox-vaccinated) were examined for vaccine scars by a physician. Of these, 141 were only HIV-2-infected, 23 were only HIV-1-infected, 29 were dually infected, and 174 were uninfected. Survival was assessed in 2006 and over the three years of follow-up, 13% (47/367) had died. Individuals with a smallpox vaccine scar had lower mortality than individuals without any scar. The MR was 0.22 (0.08–0.61) adjusting for age, sex, village and HIV status. The MR for women was 0.19 (0.06–0.57) and 0.40 (0.04–3.74) for men. The tendency was the same comparing individuals with any scar to individuals without any scar, the MR being 0.25 (0.10–0.62). The association with better

survival was found for both HIV-negative and HIV-2 infected individuals (151).

HIV-infection

When analysing the Bissau scar-survey, there was an unexpected association between smallpox vaccination scars and HIV-2 infection. Persons with a smallpox scar were more likely to be HIV-2-infected compared with individuals with no vaccination scar, the prevalence ratio (PR) being 2.45 (1.06–5.65). We were able to confirm this finding within the Caio study; the prevalence of HIV-2 infection was 52% (131/251) among people with a smallpox vaccination scar but only 34% (39/116) among those without. The HIV-2 prevalence ratio for individuals with a smallpox vaccination scar was 2.08 (1.14–3.78) compared with individuals without any scar. The epidemiology of HIV-2 is consistent with a period of more intense transmission in the 1950s or 1960s (183). The smallpox vaccination campaigns could have contributed to the transmission of blood-borne infections like HIV-2, presumably due to insufficient sterilization of the knife or needle used for vaccination.

Since HIV-1 was not present in Guinea-Bissau in the 1970s, vaccination with smallpox has not contributed to the spread of HIV-1. However, considering the marked effects of smallpox vaccination scars on mortality, we turned our attention to a possible association of smallpox vaccination with HIV-1. The most recent survey of HIV-infection in Bandim was conducted in 394

randomly selected houses between 2005 and 2006. The study was designed to examine the prevalence of HIV and included individuals aged 15 years or older living; 2082 individuals born before 1974 were included and had a blood-sample collected. Among those whose HIV-1 status was determined, we also managed to determine smallpox vaccination scar status in 1472 of these individuals. There was a statistically significantly reduced risk of having HIV-1 among women with a smallpox scar compared with women without a scar. A similar tendency was observed for men (Table 1).

This is the first time smallpox scar status has been found to be associated with a lower risk of having HIV-1. The hypothesis was generated by our previous findings of reduced mortality and morbidity among smallpox vaccinated individuals as well as findings on non-specific effects of other live vaccines. The underlying mechanism is thought to be an immunological general response to the vaccines that is not only specific to the targeted disease but also modifies the capacity of the immune-system more generally.

Public health implications

With the growing evidence for non-specific effects of vaccines, examining such effects of smallpox vaccine seems relevant considering its potency as an immune stimulator. We may gain important insight into the long-term non-speci-

Males (N=598)			Females (N=874)		
Scar status	% HIV-1 pos. (N/Ntotal)	OR (95 % CI)	Scar status	% HIV-1 pos. (N/Ntotal)	OR (95 % CI)
No scar	5.7 (7/122)	1	No scar	8.1 (17/209)	1
smallpox scar	3.4 (14/417)	0.53 (0.20-1.40)	smallpox scar	4.6 (26/560)	0.44 (0.22-0.90)
BCG or other scar	0.0 (0/59)	-	BCG or other scar	8.6 (9/105)	0.66 (0.27-1.62)

Table 1. Percent HIV-1 positives and OR of having HIV-1 controlled for relevant explanatory variables (birth year, ethnicity, number of children and fieldworker). (Preliminary results).

fic effects of live vaccines. Due to the unacceptably high rate of side-effects, it is however not likely that smallpox vaccine will be reintroduced in low-income countries.

Future perspectives

The studies in Bissau have generated large cohorts of individuals who have been assessed for smallpox vaccine and BCG scarring. These cohorts will be followed to study association with other health outcomes as, for example, cholera infection during the current epidemic in Bissau. The data from Bissau also suggested a very strong association with arm-circumference, smallpox vaccinated individuals having a much larger circumference. Larger arm-circumference is a strong predictor of subsequent survival. This association as well as immunological stu-

dies of the associations between smallpox vaccine and various infectious and chronic diseases are warranted. Modified vaccinia Ankara (MVA) is used in experiments as a vector for other antigens and it would be interesting to study its possible non-specific effects.

References on smallpox vaccination: 135,151

Non-specific effects of smallpox vaccine and BCG: Taking the observations to Denmark

Background

Smallpox vaccination (vaccinia) was discontinued in the late 1970s. In several European countries, compulsory BCG vaccination practise was discontinued at the same time. A number of case-control studies from high-income countries suggest that smallpox vaccination protect against diverse chronic conditions and cancers. In a European study of people with malignant melanoma, smallpox vaccination reduced the risk of developing malignant melanoma and smallpox-vaccination improved survival among malignant melanoma patients who had been smallpox-vaccinated several years prior to diagnosis. Considering these observations and our findings from Guinea-Bissau, we wanted to study whether smallpox vaccine and BCG also had non-specific effects in a European setting. In Denmark, we could obtain information about smallpox and BCG vaccination before 1980 as

well as status of other vaccines through inspection of the school-health records.

Results

Information on smallpox vaccination was obtained from the Copenhagen School Health Records Registry (CSHRR). Persons born 1965 to 1976 were studied, since selection to vaccinated and unvaccinated would be more random during a time when a vaccination practise was ceasing than when vaccination was compulsory and a small group refused or had contraindications against vaccination (Figure). As expected, BCG coverage declined in a similar way as smallpox vaccination, just a few years later.

Atopy, allergic rhinitis and asthma From an ecological perspective, the termination of smallpox vaccination in high-income countries coinci-

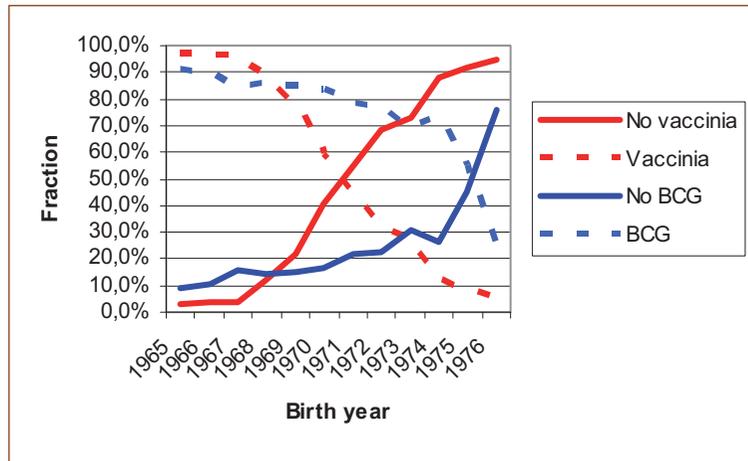


Figure. Fraction of subjects vaccinated with smallpox vaccine and BCG according to year of birth.

ded with an increased incidence of asthma. We examined the occurrence of atopy, allergic rhinitis, and asthma among Danish women within a national birth cohort study. Among the 1960 women for whom sera were available, 552 (28%) were classified as atopic; among the 1927 women with information on allergic rhinitis and asthma, 263 (14%) had allergic rhinitis, and 165 (9%) were cases of asthma. Overall, smallpox vaccination was not associated with risk of atopy or allergic rhinitis compared to unvaccinated women. However, smallpox vaccination was associated with an OR of asthma of 0.55 (0.30 to 1.00) adjusting for birth cohort, sibship size, age of the women’s mother at birth, and social class. Hence, women who had received smallpox vaccination were less likely to have asthma, an association previously not described (9).

Infectious disease hospitalisations

Through linking of the CSHRR to the Danish registry of hospitalisations we were able to determine infectious disease hospitalisation (N=765) for the 2039 individuals for whom we had determined vaccination status. Preliminary analysis shows that BCG is associated with

a lower risk of all-cause infectious disease hospitalization among women and a tendency towards smallpox-vaccinated subjects having a lower risk of all-cause infectious disease hospitalisation than subjects not vaccinated with these vaccines (Table). Smallpox-vaccinated subjects were less likely to have skin infections and BCG-vaccinated subjects less likely to be hospitalised for sexually transmitted infections (STI) than unvaccinated individuals. These observations are being pursued with studies of specific STIs including HIV-1. The preliminary indications are that BCG protects women against HIV-1 infection (hazard ratio (HR) 0.30 (0.12-0.77)) whereas the effect for smallpox vaccine was smaller (HR=0.81 (0.24-2.73)).



Future perspectives

The work on smallpox and BCG vaccination in a Danish context is in the early phase. The cohort of individuals with information on vaccination status in the 1970s will be enlarged and linkage

with disease registers will be pursued to detect other associations between vaccination status and health.

References on smallpox vaccination: 9

Table. All infectious disease hospitalisation and the subgroup of skin infections and sexually transmitted infections in relation to smallpox and BCG vaccination status. Preliminary analysis in a Cox regression model with repeated events, controlling for sex and the other vaccine

Number of hospitalizations		All infections	Skin infections	Sexually transmitted inf.
		(765/2039)	(100/2039)	(77/2039)
Smallpox vaccination				
Women:	Non-smallpox	1 (ref)	1 (ref)	1 (ref)
	Smallpox	0.85 (0.64-1.12)	0.51 (0.21-1.20)	1.10 (0.56-2.17)
Men:	Non-smallpox	1 (ref)	1 (ref)	1 (ref)
	Smallpox	0.78 (0.58-1.06)	0.45 (0.20-1.04)	1.33 (0.42-4.27)
Total	Non-smallpox	1 (ref)	1 (ref)	1 (ref)
	Smallpox	0.82 (0.65-1.02)	0.48 (0.25-0.94)	1.16 (0.62-2.19)
BCG vaccination				
Women:	Non-BCG	1 (ref)	1 (ref)	1 (ref)
	BCG	0.76 (0.59-0.99)	0.93 (0.41-2.09)	0.35 (0.19-0.67)
Men:	Non-BCG	1 (ref)	1 (ref)	1 (ref)
	BCG	1.06 (0.78-1.44)	1.36 (0.61-3.01)	0.38 (0.12-1.18)
Total	Non BCG	1 (ref)	1 (ref)	1 (ref)
	BCG	0.87 (0.71-1.07)	1.11 (0.65-1.92)	0.36 (0.21-0.63)