

Breastfeeding and improved child health: Effect or confounding

Background

Not only is breastfeeding the ideal nutrition for infants; in environments with a high pressure of infectious diseases, breastfeeding is essential for a reduction in child morbidity and mortality. However, only few randomised studies have investigated the practical implications of breastfeeding promotion, and most data originate from observational studies. Introduction of complementary food leading to complete cessation of breastfeeding is a gradual process affected by many biological, behavioural and cultural factors. Thus, data from observational studies always bear a considerable risk of being influenced by unknown confounders. In settings with excessive child mortality and a strong association between breastfeeding and mortality, it is important to know if and how improved breastfeeding practices can reduce infant and child mortality.

Results

In a randomised study including 1,721 children the intervention group received individual health education encouraging the mothers to postpone introduction of water and weaning food until the child had reached the age of 4-6 months (189). Both water and weaning is introduced early in this community. However, for both water and weaning food the introduction was significantly delayed in the intervention group. Despite mothers being responsive to the intervention by postponing water and weaning food, we did not find an improvement in child health during the 6 months of follow-up. There was no difference in diarrhoea morbidity or hospitalisation between the intervention group and the control group. Children aged 4-6 months of age, those in the intervention group had a significantly lower weight compared with the control group and though not statistically signi-





ificant, mortality was slightly higher in the intervention group, the hazard ratio (HR) being 1.86 (0.79 - 4.39). However, the sample size was too small to detect a difference in mortality.

In an observational study following 1,724 children (2), we investigated the impact of the mother's reason for weaning on subsequent mortality. Following termination of breastfeeding, 66 children

died before 36 months of age. Sixty-two % were weaned because they were "healthy". For 237 children weaned due to a new pregnancy the mortality ratio was 3.25 (1.45-7.30). Median length of spacing between an index child and a new sibling was 28 months irrespective of whether the index child survived or died before three years of age. The majority of the deaths occurred before the birth of the new sibling. Thus, confounding due to the mothers reasons for weaning may play a major role in the results from observational breastfeeding studies.

Public health implications

Promotion of breastfeeding has theoretically a great potential for reducing in morbidity and mortality in low-income countries. However, in real life it is questionable whether large scale promotion of exclusive breastfeeding will lead to any improvement in infant health in countries with a tradition for a long breastfeeding period. It would demand a great effort to change current behaviour patterns while the effect on mortality seems small. There is little reason to discourage good local practices unless there are strong data justifying such a change.

Future perspectives

It should be investigated if more appropriate approaches might help to avoid premature (< 1 year) stop of breastfeeding, for example, maintaining breastfeeding during illness or hospitalisation, or supply secure birth control methods to avoid short intervals between pregnancies. Our studies do not include infants under the age of 7 days. The fact that infant mortality is very high in the first 7 days of life makes it an important issue to investigate.

References on breastfeeding: 2, 24, 147, 189

Vitamin A and vaccines

Background

High-dose vitamin A supplementation (VAS) and vaccines are among the most important tools to reduce child mortality in low-income countries. To increase VAS coverage the World Health Organization (WHO) has recommended the integration of VAS with the Expanded Programme on Immunization (EPI). Two main strategies have been pursued. First, it is recommended to provide VAS at routine vaccination contacts after 6 months of age. Second, VAS can be provided at national immunisation days or vaccination campaigns (Table 1).

The WHO VAS policy was introduced after a number of randomised trials in the mid/late eighties and early nineties had shown that high-dose VAS for children between 6 months and 5 years of age reduces overall mortality by impressing 23-30 %. The effect was ascribed to the

prevention and treatment of vitamin A deficiency. None of the trials had linked VAS with vaccinations or studied the effect of VAS according to vaccination status. The implementation of the EPI was still in its youth, and vaccination coverage was low. Hence, the current WHO policy of providing VAS with vaccines has never been tested in randomised trials. In other words, one of the major policies to reduce child mortality has never been evaluated for its overall effect on child mortality.

In fact the effect of VAS may depend on the type of vaccine with which it is given. In 2003, we published the hypothesis that VAS amplifies the non-specific effect of vaccines, being beneficial when administered with the live BCG and measles vaccines, but potentially harmful when given with the inactivated DTP vaccine (26). Since its formulation, we have aimed to test this hypothesis in observational and randomised trials.



Results

To date we have been the only group to conduct studies with the specific aim to explore vitamin A-vaccine interactions in terms of mortality and continuously compare the evidence for our hypothesis against the evidence for the “prevention-of-deficiency”-hypothesis. Since it would be unethical to randomise children to most vaccines, we have had to be pragmatic when designing the trials. Hence, our studies have taken many different forms.

A smaller dose may be even better than a high dose

One of the observations which were contradictory according to the “prevention-of deficiency” hypothesis was made in a WHO multicenter trial. Almost 10,000 children were randomised to 25,000 IU vitamin A or placebo with the three DTP vaccines. The children who received vitamin A would receive 25,000 IU with measles vaccine at 9 months of age whereas those who had received placebo would receive 100,000 IU with measles vaccine. According to the survival curves, mortality was slightly higher among VAS compared with placebo recipients during the first 6 months of life. However, the curves crossed and mortality from 9 to 11 months of age when follow-up ended was significantly higher in the group that had received 100,000 IU with measles vaccine than in the group that had received 25,000 IU. If VAS worked only by preventing vitamin A deficiency this was an implausi-

ble finding. However, if VAS interacted with vaccines and their non-specific effects, a smaller dose of vitamin A might be better than a large dose. Hence, when a national campaign providing oral polio vaccine (OPV) and VAS to children aged 6 months-5 years was due in Guinea-Bissau in November 2002 we randomised children to the WHO-recommended dose of vitamin A or half that dose. We hypothesised that the smaller dose would be even more beneficial than the recommended dose. In brief, as hypothesised we found a tendency for a better effect of a smaller dose (109). This was due to a strongly significant beneficial effect in girls. The beneficial effect of the low dose tended to be most apparent in girls who had DTP as their most recent vaccine prior to the campaign. The finding was in line with the finding from the WHO multicenter trial – and hence both studies were incompatible with the “prevention-of-deficiency” hypothesis. The results suggest that VAS exerts its effects on mortality by other mechanisms than merely prevention and treatment of vitamin A deficiency.

VAS with BCG at birth In 2002, we initiated a large randomised trial of VAS given with BCG vaccine at birth. The trial was born from the observation that two previous trials of VAS at birth, both conducted in Asia, had found significant beneficial effects on mortality. The trial is described in more details in the chapter on neonatal vitamin A supplementation. In brief, VAS given with BCG at birth tended to be beneficial



as long as BCG vaccine was the most recent vaccine. However, the results suggested that VAS interacted negatively with subsequent DTP vaccine in girls, resulting in increased overall mortality, and increased risk of diarrhoea and measles as well as impaired vitamin A status in girls (173, 179, 205, 209).

VAS with missing vaccines In 2003, Guinea-Bissau had national immunisation days in November, providing VAS and missing vaccines to all children above 6 months of age who came to the health posts. We registered all participating children along with their treatment. Hence, we were able to test the hypothesis that VAS would be beneficial when given with the live measles vaccine, but negative when given with DTP vaccine. This proved to be the case (217). The effect of VAS differed significantly depending on the type of vaccine with which it was given (Figure 1). Receiving VAS with DTP compared with receiving only VAS was associated with significantly increased mortality. Furthermore, receiving VAS with DTP compared with not receiving neither VAS nor DTP (non-participants) was also associated with significantly increased mortality, though non-participants in such campaigns normally have higher mortality than participants. Numbers were small and it was an observational study. In particular we cannot exclude that children, who were missing DTP vaccines, had a higher risk of dying a priori compared with children who were missing measles vaccine or who did not miss any

vaccines, though control for background factors did not change the conclusions. Nonetheless the results provided support for our main hypothesis. Combining VAS with measles vaccine seemed more beneficial than combining VAS with DTP. In this study there were no sex differences, the combination of VAS and DTP seemed equally bad for boys and girls.

A reanalysis of one of the original VAS trials The latest opportunity to test our hypothesis came when we were allowed to reanalyse data from one of the original vitamin A trials. The trial, conducted in rural Ghana from 1989 to 1991, enrolled 6-90-month-old children and randomised them to VAS or placebo every 4 months for a period of 2 years. The trial was undertaken in the period when coverage with routine vaccinations was low and many children had no vaccination card. In the beginning of the trial vaccination status was assessed. The original team had not analysed data by vaccination status. The trial had shown a 19% significant mortality reduction after VAS. However, when we reanalysed the data we found that VAS only had a beneficial effect in children without a vaccination card, the mortality reduction being significant 36% in these children, but only a non-significant 5% reduction in children with a health card. This differential effect was particularly pronounced in girls. Among children with a card the effect of VAS differed significantly in boys and girls. This





was due to a significantly negative effect of VAS in girls who had received 0 to 2 doses of DTP at enrolment and were likely to receive DTP during follow-up. The reanalysis supports that VAS interacts with vaccines and the effect differs between the two sexes, in particular that the combination of VAS and DTP may have negative effects on girls.

Discussion

The initial formulation of the hypothesis of vitamin A-vaccine interactions has led to a series of studies of different designs. We conducted an observational study during the vitamin A campaign, randomised trials with VAS given with BCG at birth, and two different doses of VAS, and we reanalysed data from an old vitamin A trial from the perspective of vaccination status.

The results did not support the existing interpretation that VAS acts by preventing vitamin A deficiency, since a smaller dose seemed more beneficial than a larger dose in girls. The results on the other hand supported the hypothesis that VAS and vaccines interact. First, the effect of VAS given with DTP was different from the effect of VAS given with measles vaccine, and children who received VAS with DTP had hig-

her mortality than children who had received VAS alone or who did not receive anything. Second, VAS given with BCG at birth interacted with subsequent DTP vaccines in girls. Third, the effect of VAS depended on vaccination status, being beneficial in boys, but harmful in girls who received DTP during follow-up.

Public health implications

If VAS and vaccines interact to produce at times beneficial but at time harmful effects, it would be of outmost importance for future programmes.

Future perspectives

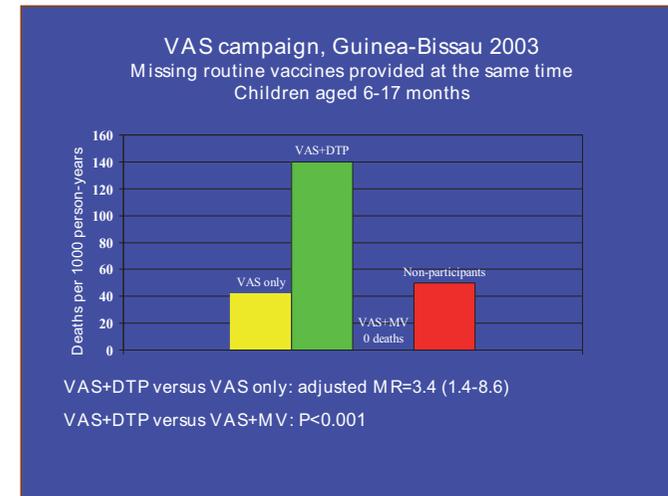
Testing VAS vaccine interactions is particularly important since the circumstances under which the original VAS trials were conducted have changed; vaccination coverage is increasing, particularly for DTP vaccine, since the national vaccination programmes are now being evaluated based on the coverage for DTP3. The final proof of VAS-vaccine interactions would have to come from randomised trials. Since it is unethi-

Table I. WHO's vitamin A policy

Target group	Immunization contact	Vitamin A dose
All mothers, irrespective of their mode of infant feeding, up to six weeks post-partum if they have not received vitamin A supplementation after delivery	BCG, OPV-0 or DTP-1 contact up to six weeks	200 000 IU
Infants 9-11 months of age	Measles vaccine contact	100 000 IU
Children 12 months and older		200 000 IU
Children 1-4 years old:	Booster doses* Special campaigns* Delayed primary immunization doses*	200 000 IU

* Avoid giving a dose too soon after a previous dose of vitamin A supplement — the *minimum* interval between doses is 1 month (exceptionally, the interval may be reduced in cases of clinical VAD).

Figure I.



cal to withhold recommended vaccines, such trials would have to be designed in special ways, for instance testing the effect of VAS or placebo with DTP versus the effect of VAS or placebo with measles vaccine, or by testing the effect in situations in which a vaccine is normally postponed, e.g. testing VAS versus placebo

in low-birth weight children who are randomised to receive the recommended delayed BCG vaccine versus in children who are randomised to receive an early BCG vaccine.

References on vitamin A supplementation and vaccines: 26,91,109,121,149,173,179,184,209, 217

Neonatal vitamin A supplementation

Background

In 2002, two trials from Asia had both found a significantly beneficial effect of VAS at birth. We hypothesised that VAS interacted with routine childhood vaccinations, being beneficial when given with the live BCG vaccine at birth or live measles vaccine at 6-9 months of age, but not when given with inactivated diphtheria-tetanus-pertussis vaccine (DTP) at 1-5 months of age (26). We aimed to investigate the effect of high-dose VAS given with BCG vaccine at birth in an African setting with high infant mortality. Since BCG vaccine is postponed for LBW infants weighing below 2500 g, we only enrolled infants weighing > 2500 g. The primary study hypothesis was that VAS would be associated with at least a 30% reduction in mortality during the first year of life. We expected the effect to be most pronounced within the first 4 months of life like it had been in the two previous trials.

Furthermore, evidence for sex-differential effects of VAS accumulating during the trial made us hypothesise that VAS would be particularly beneficial for boys.

Results

VAS, BCG, and overall mortality. Unexpectedly we found no overall effect of VAS on overall mortality (209) (Figure 1). The effect, however, was not the same in boys and girls (Figure 2). Boys if anything tended to have a beneficial effect of vitamin A throughout the first year of life. In girls the effect became negative after the first months of life (209). The finding made us speculate that perhaps vitamin A given with BCG at birth had interacted negatively with subsequent DTP vaccines in girls. A post hoc analysis revealed that indeed there was a tendency for a beneficial effect of VAS as long as BCG vaccine

was the last vaccine to be received, but once DTP vaccine was received there was a significantly negative effect of having received VAS at birth in girls.

VAS, BCG, and morbidity. Within the trial we conducted a subgroup study of the effect on rotavirus infection and diarrhoea of VAS with BCG at birth (205). Unexpectedly we also experienced a measles epidemic which gave us the chance to study the effect on measles incidence. Receiving VAS at birth was associated with an increased risk of rotavirus infection and rotavirus diarrhoea below 6 months of age in both sexes (205)(Chapter 3). There was no effect in older children. At the same time VAS at birth was associated with a decreased risk of non-rotavirus diarrhoea in boys below 6 months of age, but an increased risk in girls 6 months or older. Significant sex-differential effects, with a tendency for a negative effect on girls of vitamin A at birth, were also seen for measles infection. Hence, the morbidity findings supported the existence of sex-differential effects of VAS at birth. Furthermore, the diarrhoea subgroup study was conducted among 1-8- month-old children who almost exclusively had DTP vaccine as their most recent vaccine during the study period, and the negative effect of VAS on measles infection was seen among girls who had received DTP but not measles vaccine. Hence,



the finding also supported the hypothesis that the negative effect on girls could be due to a negative interaction between VAS and subsequent DTP vaccination in girls.

VAS, BCG, and vitamin A status. As a part of the trial we studied the effect of VAS on vitamin A status at 6 weeks of age and 4 months of age (179). Overall vitamin A status improved during this period. However, there was a significant inverse relationship between increase in vitamin A status and number of DTP vaccinations received in girls, which was particularly evident among VAS recipients. The finding underscored



the possibility of a negative interaction between VAS and subsequent DTP vaccinations in girls. *Discussion.* Five trials of neonatal VAS have now been published. Three trials from South Asia showed beneficial effects on mortality of neonatal VAS. Two trials from Africa found no overall beneficial effect, the estimates going in the other direction. Differences in vitamin A status do not seem to explain the divergent results. We have proposed that the divergent results may be explained by differences in vaccination intensity in the five trials (210). In our trial from Guinea-Bissau, all children received BCG at the same time as VAS or placebo (209). Having received VAS tended to be beneficial as long as BCG was the last vaccine to be received. However, once children received DTP vaccine, mortality in girls who had received VAS at birth was significantly 2-fold higher compared with girls who had received placebo at birth. Hence, in our experience neonatal VAS has a beneficial effect as long as BCG is the last vaccine but may have a negative effect on girls once they receive DTP. As a consequence the survival curves of vitamin A and placebo recipients should cross over once they start receiving DTP around two months of age if the coverage for DTP is high. This pattern is seen both in Guinea-Bissau and in the other African trial from Zimbabwe.

Such vitamin A-vaccine interactions could help explain the variation in trial results. Vaccination

intensity was high in Guinea-Bissau and probably also in Zimbabwe as judged by national coverage data. This was not the case in the trials from Asia. Furthermore, all the Asian studies were characterised by a high neonatal mortality, but low mortality in the months in which a negative interaction between vitamin A at birth and DTP vaccine would matter. Hence, existing data are compatible with the hypothesis that early DTP vaccination might interfere with the beneficial effect of neonatal VAS in girls.

Public health implications

A heated debate regarding a global or regional recommendation of neonatal VAS is ongoing (210, 215). A policy of providing VAS in South Asia has many advocates. If our hypothesis is correct and neonatal VAS is made a general policy in South Asia, the intervention may cease to be beneficial or even become detrimental as the DTP coverage increases and more children are vaccinated early in life, especially in populations in which mortality is not limited to the first months of life. However, there will be no way of knowing because it is considered unethical to conduct further trials once an intervention has become policy.

It will be up to the WHO to weigh the evidence for and against a neonatal VAS policy in South

Asia. So far there is limited scientific evidence for the interpretation that neonatal VAS is most beneficial in areas with highest degree of VAD and baseline mortality. We need better explanations for the contradictory results before we make subgroup policies.

References on vitamin A supplementation at birth: 26,140,173,174,179,184,205,209,210,215

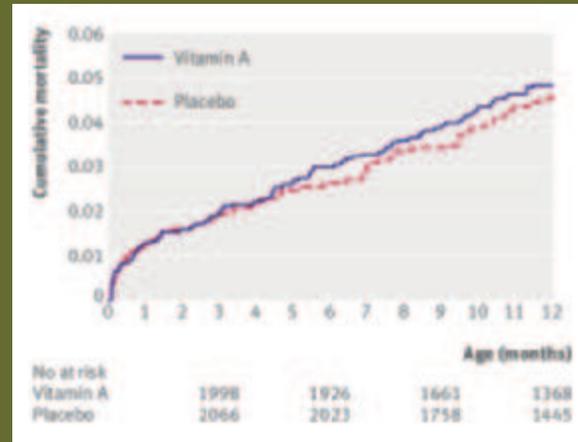


Figure 1. The effect of vitamin A supplementation at birth on overall mortality during the first year of life

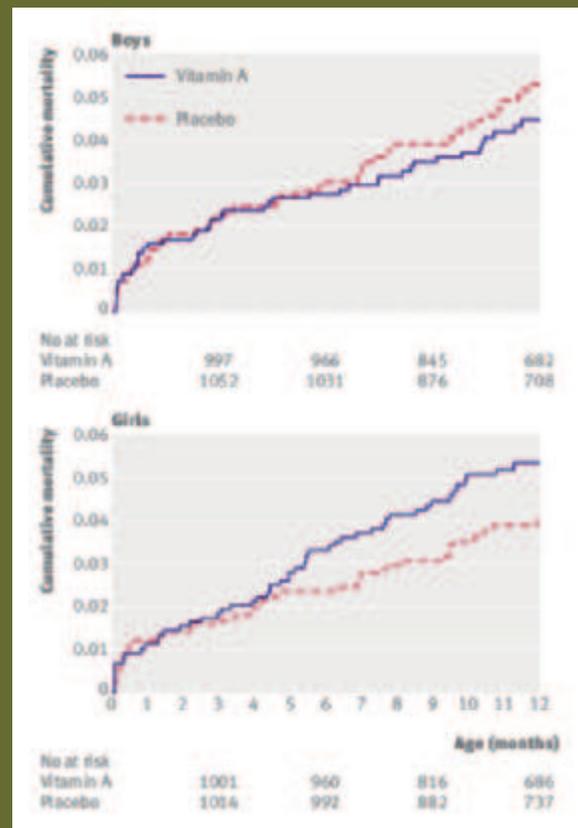


Figure 2. The effect of vitamin A supplementation at birth on overall mortality during the first year of life by sex



Redosing with vitamin A

Background

Vitamin A supplementation (VAS) continues to be one of the most important health interventions for children in low-income countries. WHO recommends supplementing children between 6 months and 5 years of age with 4 to 6-month-intervals. While studying the hypothesis that VAS and vaccines interact, we made observations which suggest that the effect of VAS may depend on prior dosing.

Results

Half versus recommended dose of VAS.

In recent years, children aged 6 months to 5 years have received VAS during annual campaigns in Guinea-Bissau. In 2002 and 2004 VAS was provided together with oral polio vaccine (OPV) at National Immunisation Days (NIDs). We studied the effect of different doses of VAS

on overall mortality. Children in the study area were randomized to receive the dose recommended by WHO or half this dose together with OPV and followed for mortality.

In the study from 2002 we found a significant beneficial effect of the lower dose compared with the WHO-recommended dose in girls (109). After 6 months of follow-up the mortality rate ratio (MRR) was 0.19 (0.06-0.66). However, this was not the case for boys. Overall mortality was lower among trial participants than among non-participants, and there was no indication that the high dose was associated with increased mortality; a smaller dose just seemed even more beneficial in girls.

Repeating the study in 2004 did not confirm these results. After 6 months and 12 months of follow-up there was no difference between the doses for either boys or girls. However, excluding the children who had previously received

VAS, the analysis produced results similar to the 2002 results (Table). Since NIDs with VAS were introduced in 2001, and maybe more importantly no children had received VAS at birth at that time, the children from the 2002 study were less likely to have received VAS previously than the children from the 2004 study. Hence, a possible beneficial effect of having received a priming dose of VAS on the subsequent response to a high dose of VAS would be less pronounced. This observation made us speculate that prior dosing with VAS may influence the effect of subsequent doses.

VAS or placebo at birth, followed by VAS at 12 months of age.

In the group of children participating in the randomised study of VAS with BCG at birth we intended to provide 100,000 IU of vitamin A (FU-VAS) to all children when they reached 12 months of age, irrespective of previous allocation. The effect of VAS at birth had not been beneficial during the first year of life (Chapter 26). However, among children who received FU-VAS after 12 months of age, it was strongly beneficial in the second and third year of life to have received VAS at birth compared with placebo at birth, the mortality rate ratio (MRR) being 0.53 (0.30-0.91). This difference was confined to girls who had a significant difference in mortality rate between 12 and 36 months of age depending on the randomisation group at birth ($p=0.03$) whereas there were no differences for boys (Figure).



Discussion.

The two studies differed considerably in design. However, they both indicate that VAS may have a priming effect on the effect of subsequent VAS on mortality, in particular for girls.

Public health implications

If VAS has a priming effect on the effect of subsequent VAS, implementing a policy may not achieve the full benefit in the short term and may depend on prior policies. The sex differences suggest that the overall impact of VAS could be optimized by having different recommendations for boys and girls.

Future perspectives

The current vitamin A policy makes it difficult to study the effect of re-dosing with VAS in randomised trials since depriving some children of a potentially beneficial vitamin A supplement would not be considered ethical. However, though we cannot conduct randomised trials, we can continue to pursue the observations using data from already conducted studies to evaluate the effect of repeated doses. The aim is to find the most beneficial dosing regime which will optimise the effect of VAS on overall mortality for both boys and girls.

References: 109, 209

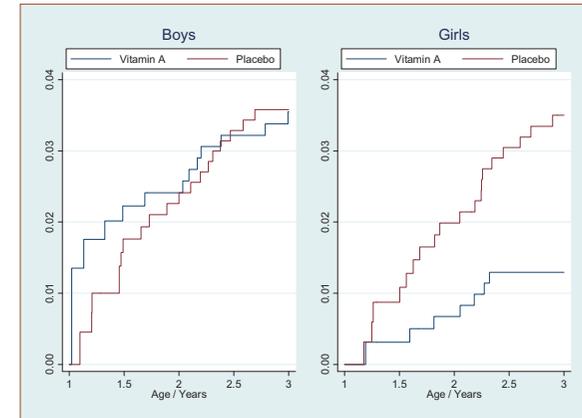


Figure. Effect of VAS / Placebo at birth on mortality after reception of FU-VAS

Trial year	Mortality rate ratios (95% CI) for a low versus a high dose		
	Boys	Girls	All
2002	1.98 (0.74-5.29)	0.19 (0.06-0.66)	0.69 (0.36-1.35)
2004	0.91 (0.26-3.13)	1.41 (0.57-3.45)	1.20 (0.58-2.47)
2004:No previous VAS	0.48 (0.09-2.63)	0.72 (0.23-2.26)	0.61 (0.24-1.58)
2004:Previous VAS	2.55 (0.27-25)	6.28 (0.77-51)	4.46 (0.97-20)

Table. The effect on mortality of receiving a low dose of vitamin A versus the WHO recommended dose

Immunological studies: Vitamin A and non-specific effects of vaccines

Background

A number of immunological studies have been initiated in order to address the mechanisms behind the findings of non-specific effects of vaccines and potential interactions with vitamin A supplementation (VAS). Some have focused on exploiting the often unique possibilities lying within the framework of the randomised studies conducted in Guinea-Bissau. Other studies have looked toward simplified systems such as dendritic cell cultures or animal studies. As we are able to measure an exponentially increasing number of immunological markers it is important not only to explore the effects of VAS and vaccines on various immunological markers, but also to establish the associations between such markers and subsequent mortality and morbidity. Hence, the work can be divided into two parts; first, to identify immunological markers, which are affected by VAS and vaccines,

second, to establish a correlation between immunological markers and mortality and morbidity.

Results

Exploring the immunological effects of VAS and vaccines

Human studies: Whole blood stimulations.

A methodological mainstay of the immunological approaches performed at the National Laboratory in Guinea-Bissau is *ex vivo* whole blood stimulations. The assay is designed to test different parts of the cellular immune system lending information of both innate and adaptive immune functions (Table 1). Currently the whole blood assay is used in the evaluation of the immunological effects of the following interventions: VAS and BCG at birth, early measles vaccine, VAS in combination with measles or DTP vaccinations, DTP booster vaccination, and OPV at birth



The cytokine response to the stimulations is measured by means of Luminex technique. The method allows simultaneous quantification of numerous cytokines on a very small volume of sample material. This is a major advantage when working with small children as a large volume of blood is neither available nor desirable.



Faced with the challenges of performing immunological studies in Guinea-Bissau we have first-hand experienced the value of critically reviewing the methods applied. In a study of the immunological effects of early measles vaccination, the goal was to obtain venous samples at 4.5 months of age and 6 weeks later. At the time of sampling this was, however, only possible for about half of the children. In the remaining children capillary blood samples were obtained instead. We tested if method of sampling influenced the cytokine levels. This turned out to be the case. The venous blood samples displayed lower production of $\text{TNF-}\alpha$ and Interleukin (IL)-10 than those obtained through capillary blood sampling (176).

The background for BHP's work on the immunology of VAS is the hypothesis that VAS has direct effects on the immune system and that it amplifies the non-specific effects of vaccines with which VAS is given (22). As a consequence

sex is taken into consideration in all analyses. An example of this is a study investigating the effect of VAS on the immune response to BCG vaccine when given simultaneously at birth. In the study approximately 2700 infants receiving BCG were randomised to either VAS or placebo. Skin reactions to PPD (purified protein derivative of *M. Tuberculosis*) were evaluated at 2 and 6 months of age. At 2 months of age the proportion of responders was lower among boys who received VAS than among boys in the placebo group. Capillary blood samples were collected from a subgroup of infants at 6 weeks of age. These samples were utilized to quantify the ex vivo response to PPD by means of whole blood stimulations. With regards to interferon- γ , a key marker of cellular immune response to TB, more boys who had received VAS were responsive than in the placebo group. No effect of VAS was seen among girls in either of the tests (184).

Dendritic cells cultures.

Dendritic cell cultures have been used to examine the immunological effects of BCG vaccine. Artificially matured dendritic cells were co-cultured in the presence of live BCG and their subsequent ability to stimulate naïve T cell development was measured. No Th1 or Th2 polarisation was observed but the BCG co-culture led to an increase in IL-10 producing dendritic cells which again primed naïve T cells to develop into IL-10 producing T cells (Figure). This cell-type is associated with the down-regulation of immune responses. The results suggest that BCG vaccination might result in the development of IL-10 producing dendritic cells as well as IL-10 produ-

cing T-cells that could contribute to restricting overt inflammation in infants exposed to pathogens and thus lead to lower infant mortality (163).

Animal models.

Animal models are well-suited to explore VAS/vaccine-interactions, in particular VAS/DTP interactions, which may be harmful for girls and unethical to conduct in humans. In a collaborative pilot study with a group at Stanford University, we investigated the effects of combined VAS/DTP-treatment on the outcome of influenza infection in mice. The study was not conclusive but produced interesting results which compelled us to go forward with another experimental model of infection. The model chosen was a well-established murine model of cerebral malaria and the studies were performed in collaboration with Department of Clinical Microbiology, University of Copenhagen. We found no effects on the main outcome, development of cerebral malaria, but repeated experiments showed that mice receiving the combination of VAS and DTP had higher levels of parasites in the blood.

Searching for immunological markers of mortality and morbidity

Several immunological markers of subsequent mortality and morbidity have been established. Thymus size, having a BCG scar and a positive delayed type hypersensitivity response to PPD have previously been identified as markers of

better survival, and this association was verified in recent studies (133,211). We aim to pool the data from the whole blood stimulation studies and link them to survival to identify cytokine markers of mortality or better survival as well.

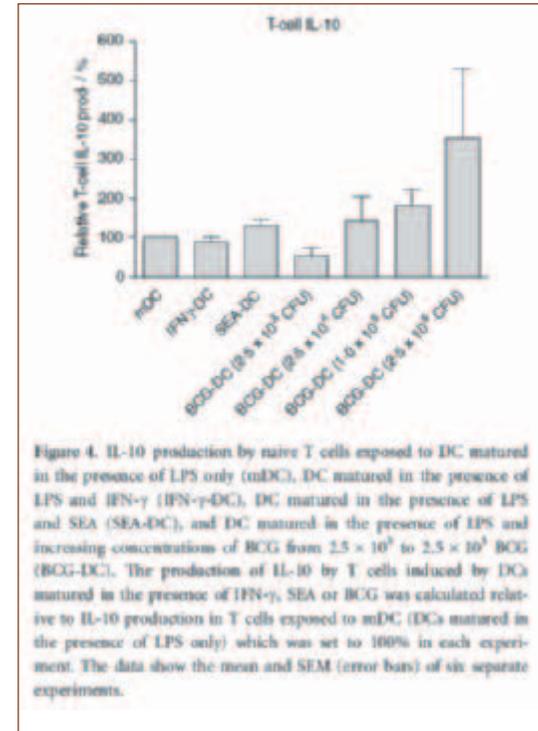
Future perspectives

The addition of immunological studies has the potential to substantiate some of the controversial findings by offering biologically plausible explanations. The studies have to a large extent been possible due to collaborations with other research groups, and we will continue to initiate and explore these possibilities through future studies. An example of this is within the field of lymphocyte homing. Dendritic cells from the intestinal mucosa have the ability to “instruct” lymphocytes to preferentially migrate to the gut. It appears that retinoic acid, a vitamin A metabolite produced by these dendritic cells, acts as the signal that induces the observed gut-specificity/preference. We are currently addressing this topic in collaboration with researchers at Lund University. This study will hopefully fill a current gap between in vitro and animal studies and the situation of children in Guinea-Bissau receiving VAS.

The magnitude and nature of data “produced” by modern immunological techniques when applied to large populations in immuno-epidemiological studies pose a number of analytical challenges. Repeated measurements of the same individual are performed and multiple parame-

ters are measured simultaneously creating large amounts of information. The parameters measured are often reflecting interacting processes but the extent of these interactions is less than straightforward and may not be similar across the groups investigated. Often distributions are not normal and have a large proportion of undetectable samples. These challenges combined with the sheer number of observations require more advanced statistical methods than often applied to exploit the full potential of the obtained information. In collaboration with Leiden University Medical Center, we are currently exploring and developing statistical methods which can deal with data from immuno-epidemiological studies.

References on immunology:
26, 133, 163, 176, 184, 211



Stimulant	Function tested
LPS	General recognition of gram-negative bacteria (innate – TLR4)
Pam3-cys	General recognition of gram-positive bacteria (innate – TLR2)
Poly I:C	General recognition of virus (innate – TLR3)
PHA	Unspecific T-cell activation
TT	Recall of tetanus toxoid part of DTP vaccine (adaptive)
PPD	Recall of BCG vaccine given at birth (adaptive)
OPV	Recall of polio vaccine (adaptive)

Table. Stimulants used in ex vivo whole blood stimulations

Re-introducing sex-differential treatment of children



Though it is well-accepted that boys and girls have different susceptibility to diseases in childhood there has been very little interest in investigating whether boys and girls benefit from the same health interventions. Gender has been an issue when it came to ensuring that boys did not receive preferential treatment, i.e. boys and girls should receive the same health intervention at the same time. It has not been considered that we may in fact be treating boys and girls differently giving them different survival probabilities, when we offer them the same health intervention at the same time.

We have consistently found that the major interventions to reduce morbidity and mortality in low-income countries have sex-differential effects. BCG and measles vaccine reduce overall mortality, and this is most pronounced in girls. DTP is associated with increased female mortality compared with male mortality (Figure).

OPV at birth may be associated with increased male mortality. Vitamin A supplementation (VAS) benefits boys more than girls.

In high-income countries there is an enormous focus on individualised prevention and treatment, including the prospect to target the intervention towards subgroups with different genetic profiles based on rapid genetic tests. It will take many years before that kind of genetic screening will be feasible in low-income countries. However, there is one genetic test which can be done without any remedies and at no cost: to determine the sex of a child. Based on our research, sex may be a very good determinant of the optimal health intervention programme.

In 10 years' time, we expect these ideas to be more generally accepted and many will be working to develop policies optimised for both

boys and girls. We have started gradually to examine whether major interventions should differ for boys and girls. Thus, we are testing whether MV given at 4½ months of age may reduce the negative effect of DTP for girls and whether girls might benefit from not receiving a booster dose of DTP but only OPV. We are also testing whether low- birth-weight infants may

benefit from a sex-differential treatment and the potential sex-differential effect of administering VAS with routine vaccinations as recommended by WHO.

References on sex-differential treatment:
139,140,177

Figure. Female-male mortality rate ratios among measles and DTP vaccinated children in all available studies

