Heterologous and sex differential effects of administering vitamin A supplementation with vaccines

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WHO recommends high-dose vitamin A supplementation (VAS) to children from 6 months to 5 years of age in low-income countries, in order to prevent and treat vitamin A deficiency-associated morbidity and mortality. The current policy does not discriminate this recommendation either by sex or vaccination status of the child. There is accumulating evidence that the effects of VAS on morbidity, mortality and immunological parameters depend on concomitant vaccination status. Moreover, these interactions may manifest differently in males and females. Certain vaccines administered through the Expanded Program on Immunization have been shown to alter all-cause mortality from infections other than the vaccine-targeted disease. This review summarizes the evidence from observational studies and randomized-controlled trials of the effects of VAS on these so-called heterologous or non-specific effects of vaccines, with a focus on sex differences. In general, VAS seems to enhance the heterologous effects of vaccines, particularly for diphtheria-tetanus-pertussis and live measles vaccines, where some studies, although not unanimously, show a stronger interaction between VAS and vaccination in females. We suggest that vaccination status and sex should be considered when evaluating the effects of VAS in early life.

Keywords: All-cause mortality, BCG vaccine, DTP vaccine, Measles vaccine, Sex, Vitamin A

Introduction

Vitamin A is derived from the diet either in the form of all-trans retinol, beta-carotene or retinyl esters, and is essential for the normal functioning of the immune system.[1] It is estimated that 190 million children under the age of 5, worldwide, are at risk of vitamin A deficiency (VAD), especially in Africa and southeast Asia.[2] VAD is principally associated with xerophthalmia and an increased risk of death from infectious diseases. It may be alleviated by vitamin A supplementation (VAS), usually as oral capsules of retinyl esters. WHO recommends VAS to children between 6 months and 5 years of age in low-income countries as a means of treating and preventing VAD;[3] this policy is partly based on a meta-analysis of 17 randomized-controlled trials (RCT) in Asia (11 trials), Africa (5 trials) and Brazil (1 trial), which found that VAS can reduce all-cause mortality by 24% (95% CI 17%–31%) in this age group.[4]

The meta-analysis was an extension of a former review, reporting a similar effect size.[5]

VAS before 6 months of age is not currently recommended by WHO,[6] due to a lack of convincing beneficial effects on mortality and morbidity as evaluated by several systematic reviews.[7–9] However, studies from different regions of the world are conflicting (Table 1). The provision of 50 000 IU neonatal VAS significantly reduced mortality in Bangladesh,[10] India[11] and Indonesia,[12] but not in Nepal.[13] By contrast, trials in Africa failed to demonstrate any effect on overall mortality in the 12-month follow-up. In more than 4000 normal birth weight newborns in Guinea-Bissau, mortality was comparable at 12 months for 50 000 IU VAS or placebo groups.[14] A smaller RCT in low-birth weight newborns of 25 000 IU versus placebo similarly showed no effect on mortality;[15] and there was no mortality difference in Zimbabwean infants receiving 50 000 IU VAS or placebo.[16]

Table 1. Summary of studies analyzing the effect of vitamin A supplementation to children less than 6 months of age

	Design	By vaccine	Mortality rate ratio (MRR)			Sex	Country	Reference
			All	Male	Female			
No vaccine information or independent of vaccine type	24 kIU at birth vs placebo twice. FU: 6 mo	Not reported	0.78 (0.63–0.96)	0.70 (0.52–0.94)	0.87 (0.65–1.17)	Sex#VAS: NA	South India	Rahmathullah 2003[11]
	50 kIU VAS vs placebo at birth. FU: 12 mo	Not reported	0.36 (0.16–0.87)	0.15 (0.03–0.68)	0.84 (0.26–2.77)	Sex#VAS: NA	Indonesia	Humphrey 1996[12]
	50 kIU/100kIU VAS vs placebo, aged 1–5 mo. FU: 4 mo	Not reported	1.11 (0.86–1.42)	1.24 (0.86–1.78)	0.98 (0.68–1.42)	Sex#VAS: NA	Nepal	West 1995[13]
	50 kIU VAS vs placebo at birth. FU: 12 mo	Not reported	1.18 (0.76–1.83)	NA	NA	Not analysed by sex	Zimbabwe	Malaba 2005[16]
	50 kIU VAS vs placebo at birth. FU: 24 wk	Not analyzed by vaccine. Vaccine coverage >70%	0.85 (0.73–1.00)	0.89 (0.72–1.10)	0.81 (0.65–1.00)	Sex#VAS: p=0.054	Bangladesh	Klemm 2008[10]
	50 kIU/25kIU vs placebo at birth. FU: 12 mo	Irrespective of vaccination.Vaccine type (BCG/DTP)#VAS: NS	1.28 (0.91–1.81)	1.35 (0.84–2.16)	1.21 (0.73–2.01)	Sex#VAS: NS	Guinea-Bissau	Benn 2014[22]
	2×2: 25 kIU vs placebo at birth and BCG at birth vs BCG later. FU: 12 mo	Irrespective of vaccination (with BCG or BCG later)	1.08 (0.79–1.47)	0.74 (0.45–1.22)	1.42 (0.94–2.15)	Sex#VAS: p=0.046	Guinea-Bissau	Benn 2010[15]
BCG	2×2: 25 kIU vs placebo at birth and BCG at birth vs BCG later. FU: 12 mo	Given with BCG. BCG timing#VAS: p=0.73	1.14 (0.71–1.84)	NA	NA	Sex#VAS:NA	Guinea-Bissau	Benn 2010[15]
	50 kIU VAS vs placebo with BCG at birth. FU: 12 mo	Given with BCG	1.07 (0.79–1.44)	0.84 (0.55–1.27)	1.39 (0.90–2.14)	Sex#VAS: p=0.10	Guinea-Bissau	Benn 2008[14]
	50 kIU VAS vs placebo with BCG at birth	BCG last vaccine (FU: 1.5 mo)	0.86 (0.48–1.54)	NA	NA	Sex#VAS: p=0.89	Guinea-Bissau	Benn 2009[24]
	50 kIU/25kIU vs placebo at birth	FU: BCG last vaccine	1.28 (0.72–2.29)	1.53 (0.69–3.40)	1.04 (0.45–2.41)	Sex#VAS: NS	Guinea-Bissau	Benn 2014[22]
DTP	25 kIU VAS vs placebo thrice aged 6,10,14 wk. FU: 9 mo	Given with DTP+OPV. Vaccine#VAS: NA	0.96 (0.73–1.27)	N/A	N/A	Not analysed by sex	Ghana, India, Peru	WHO 1998[17]
	50 kIU VAS vs placebo given with BCG at birth	DTP last vaccine (FU: 9 mo)	1.43 (0.88–2.32)	0.90 (0.44–1.82)	2.19 (1.09–4.38)	Sex#VAS: p=0.08	Guinea-Bissau	Benn 2009[24]
	50 kIU/25kIU vs placebo at birth	FU: DTP last vaccine	1.04 (0.62–1.72)	0.84 (0.42–1.69)	1.31 (0.61–2.81)	Sex#VAS: NS	Guinea-Bissau	Benn 2014[22]
MV	50 kIU or 25kIU vs placebo at birth. FU: 17 mo	MV aged 4.5 mo MV#VAS: p=0.008	5.39 (1.62–17.99)	11.31 (1.50–85.47)	2.46 (0.51–11.85)	Sex#VAS: NA	Guinea-Bissau	Benn 2014[28]
Repeated VAS	50 kIU VAS vs placebo at birth. FU: aged 3 yr	No FU-VAS aged 1 yr FU-VAS aged 1 yr	1.10 (0.64–1.90) 0.54 (0.31–0.94)	0.57 (0.23–1.42) 0.73 (0.35–1.51)	1.67 (0.81–3.42) 0.37 (0.16–0.89)	NVAS#FU-VAS: male: p=0.68, female: p=0.009; Sex#NVAS#FU-VAS: p=0.035	Guinea-Bissau	Fisker 2011[35]

The same paper may appear in more than one row. #: interaction analysis; 50 kIU: 50 000 international units of retinol; BCG: bacille Calmette-Guérin; DTP: diphtheria-tetanus-whole cell pertussis; FU: follow-up; FU-VAS: VAS at follow-up; mo: month(s); MRR: mortality rate ratio; MV: measles vaccination; NA: not analyzed; NS: not significant; NVAS: neonatal VAS; VAS: vitamin A supplementation; wk: weeks(s); yr: year(s).

A large multicentre trial of VAS with routine vaccines at 6–14 weeks in Ghana, India and Peru also failed to show an effect on mortality.[17] This discrepancy is yet to be resolved and some of the potential underlying factors will be discussed here.

For logistic reasons, VAS is often delivered in combination with routine vaccines included in the Expanded Program on Immunization (EPI) schedule recommended by WHO. Several studies including RCTs suggest an interaction between VAS and vaccines in early childhood on mortality, and these will be discussed in this review. In particular, VAS may augment the heterologous effects of vaccines, the effects whereby prior vaccination alters morbidity and mortality from infections other than the vaccine-targeted diseases.[18] A number of studies further suggest that the VAS effects and vaccine heterologous effects manifest differently in females and males. The administration of VAS with the EPI scheduled vaccines was not an evidence-based decision. Meanwhile, it is WHO opinion that the current evidence concerning the influence of sex or preceding vaccines on the effect of VAS are insufficient to lead to policy change.

This literature review explores the limited evidence for an effect of VAS in combination with childhood vaccines on morbidity and mortality or immunological endpoints, with an emphasis on sex differences. RCTs or observational studies of high-dose VAS in children <5 years of age reporting mortality or morbidity as an outcome available via PubMed were selected for the review. Additionally, immunological or anthropometric studies of VAS in children addressing sex and/or vaccination were included. The review will not cover animal studies, or studies in adults or children above 5 years of age.

The heterologous effects of vaccines on all-cause mortality

Accumulating data from observational studies and RCTs show that some vaccines affect resistance to infections other than the targeted diseases.[18] These effects are termed heterologous or non-specific effects of vaccines. Live attenuated vaccines such as bacille Calmette-Guérin (BCG) and measles vaccine (MV) have been associated with a reduction in mortality, exceeding the possible specific protection against TB and measles, respectively, as recently acknowledged by WHO following a systematic review of the literature.[19] In contrast, several studies have found that inactivated adjuvanted vaccines such as diphtheria-tetanus-whole cell pertussis (DTP) vaccine are associated with increased mortality.[20] Hence, the heterologous effects of vaccines may be beneficial or detrimental.[21] The heterologous effects may manifest differently in males and females; the beneficial heterologous effects of MV and the detrimental heterologous effects of DTP generally being greater in females.[18,20,21] The immunological mechanisms behind the heterologous effects are not understood, although some mechanisms are beginning to emerge.[18]

VAS interaction with BCG effects on all-cause mortality

In two RCTs of neonatal VAS conducted in Guinea-Bissau, VAS tended to be beneficial for males, but detrimental for females (interaction between VAS and sex: p=0.10 and p<0.05 for either trial).[14,15] The latter trial also randomized low-birth weight infants to BCG at birth versus administration according to local practice, usually at age 6 weeks, but found no interaction between BCG and VAS (Table 1).[15] Similarly, trials found no effect of neonatal VAS on mortality when given together with BCG[14] or when the analysis was restricted to the time when BCG was the last vaccine;[22] furthermore, no significant sex-differential effects of VAS were found (Figure 1).

In a RCT of a BCG booster at 19 months of age in Guinea-Bissau, a number of the participating children received VAS in national campaigns before or after randomization to BCG booster. Among infants not receiving VAS, there was no effect of the BCG booster on overall survival, whereas among VAS recipients, BCG was associated with a four times higher risk of dying than in the
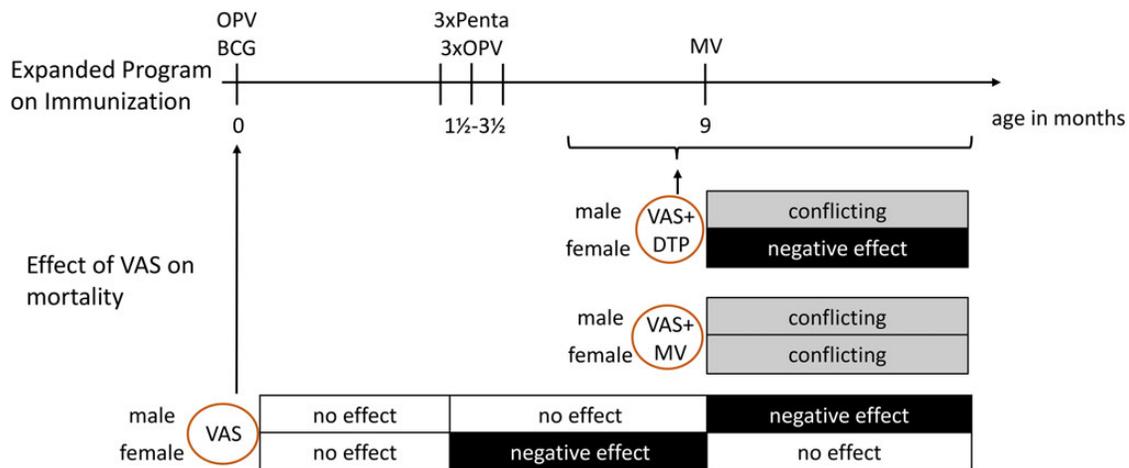

Figure 1. Diagram demonstrating the main effects of vitamin A supplementation (VAS) on all-cause mortality in males and females in the first year of life during the intensive phase of vaccination under the EPI. BCG: bacillus Calmette-Guérin; DTP: diphtheria-tetanus-whole cell pertussis vaccine; Hib: hepatitis B; MV: measles vaccine; OPV: oral polio vaccine; Penta: pentavalent vaccine consisting of DTP. This figure is available in black and white in print and in colour at Transactions online.

controls (hazard ratio 4.14 [1.17–14.7]). The effects were not reported by sex.[23]

VAS interaction with DTP vaccine effects on all-cause mortality

Previously given alone, DTP is now administered as part of the Pentavalent vaccine (DTP, Hepatitis B, *Haemophilus influenzae* type b) in much of the developing world at 6, 10 and 14 weeks of age, with a booster DTP at 18 months. Sixty percent of the infants in the 19-months BCG booster study discussed above had not received a DTP booster before enrolment, and many would have received it with VAS or during follow-up. Among those that received a DTP booster before enrolment there was a significantly beneficial effect of BCG, whereas those who had no DTP before BCG were significantly more likely to die; the interaction between DTP booster status and BCG being highly significant (p=0.006). The study suggested that BCG followed by DTP and/or VAS may have unfavourable effects in the children, but may also indicate that a subsequent BCG vaccine may alleviate the putative negative effect of a DTP booster. The analyses were not reported by sex.[23]

The above RCTs of neonatal VAS found that the interaction between sex and VAS was most evident after 3–4 months of age, during which infants usually receive multiple doses of DTP (Table 1).[14,15] A subsequent re-analysis of a neonatal VAS trial indicated an interaction between sex and VAS (p=0.08) for children with DTP as the most recent vaccine, with VAS being associated with a significantly higher mortality in females (mortality rate ratio [MRR] 2.19 {1.09–4.38}), with no such effect in males (Table 1).[24] A later trial of neonatal VAS in Guinea-Bissau did not find a differential effect of neonatal VAS for children having or not having received the first DTP vaccine; nor did the study find sex differences. However, the overall mortality was considerably lower than previous trials, thereby compromising the power of the subgroup analyses (Table 1).[22]

An interaction between VAS and concomitant or subsequent DTP on mortality was indicated in two other observational studies from West Africa. To explore the hypothesis that the effect of VAS may be modulated by vaccination, Benn et al. reanalyzed data from a RCT providing VAS to 6–90-months old children in rural Ghana.[22] Using health card information the authors found that VAS was associated with a beneficial effect in infants not vaccinated at the time of supplementation, with an overall MRR of 0.64 (0.47–0.88) for VAS versus placebo (Table 2). However, vaccination seemed to influence the VAS effect in females, which the authors ascribed to an effect of DTP. Those children who received MV before completing all the 3–4 scheduled doses of DTP were likely to receive additional doses of DTP during the 2-year follow-up; and among this group the effect of VAS was sex-differential, with a significantly negative impact on follow-up survival in females (MRR 2.60 [1.41–4.80]), but a borderline positive effect in males (MRR 0.50 [0.25–1.02]; p=0.0004 for interaction between VAS and sex). For children who had received all DTP doses before MV, there was no VAS effect overall or by sex (Table 2).[25] Taking advantage of a national campaign providing VAS together with any missing vaccines to children >6 months of age, the effect of VAS on mortality in an urban community in Guinea-Bissau was studied up to 18 months of age. Receiving VAS together with any DTP was associated with a higher mortality rate compared with receiving VAS alone (MRR 3.43 [1.36–8.61]); this effect was significant in males but not in females, hence contrasting the sex-differential effect in the former study from Ghana. By contrast, there were no deaths among children receiving VAS with MV. The overall interaction between VAS and vaccine type was highly significant (<p=0.001), and evident in both males (p=0.007) and females (p=0.03), and suggests that combining MV and VAS is safe, whereas VAS with DTP may be harmful (Table 2).[26]

VAS interaction with measles vaccination effects on all-cause mortality

A reanalysis combining three RCTs of neonatal VAS[14,15,22] and one RCT of an extra early dose of MV at age 4.5 months,[27] all taking place in Guinea-Bissau, found that among recipients of early MV, neonatal VAS compared with placebo was associated with a significantly higher mortality at 9 months of age (p=0.01), with a stronger effect in males (p=0.05) than females (p=0.12). There was no significant effect of VAS among infants not receiving early MV, resulting in a significant interaction between VAS and MV (p=0.03). At 17-month follow-up, the interaction between VAS and MV sustained significance (p=0.008), with an overall effect of VAS in the early MV recipients of MRR 5.39 (1.62–17.99), and still strongest in males (MRR 11.31 [1.50–85.47]). This indicates a long-lasting priming effect of neonatal VAS affecting subsequent vaccine responses, possibly stronger in males.[28]

The effect of neonatal VAS may differ from the effect exerted in older children. An observational study in Guinea-Bissau following children aged 6–35 months of age receiving VAS in campaigns found a significantly beneficial effect of VAS on mortality if the child had received MV as the last vaccine prior to the VAS campaign (MRR 0.34 [0.14–0.85]). The effect was significant for males but not for females, with no significant interaction between sex and VAS. The effect of VAS partially reverted, although not significantly, among children with DTP as the last vaccine, resulting in an interaction between vaccine type (MV vs DTP) and VAS (p=0.04).[29] An analysis combining data from a trial in Guinea-Bissau of MV versus inactivated polio vaccine (IPV) at 6 months and a trial of VAS versus placebo at 6 and 9 months found that vaccine type and VAS interacted (p=0.02) on 18-month follow-up mortality. Among MV-recipients, deaths only occurred in the placebo group, whereas among IPV-recipients, deaths only occurred in the VAS group.[30] The authors posited that the interaction with VAS might be ascribed to the nature of the vaccine as killed (IPV) or live (MV). However, overall the effect of VAS given together with or close to MV remains inconclusive, with studies indicating a detrimental effect of VAS given with MV on mortality in males[28,31] and females[25] in different studies.

VAS interaction with oral polio vaccine (OPV) effects on all-cause mortality

A temporary lack of oral polio vaccine (OPV) in Guinea-Bissau facilitated a 'natural experiment' to observe the effect of OPV at birth on infant mortality; 22% of the infants did not receive OPV at birth. The study found an association between increased mortality and OPV at birth. All the infants were also enrolled in a trial of

Table 2. Summary of studies analyzing the effect of vitamin A supplementation to children from 6 months to 5 years of age

	Design	By intervention	Mortality rate ratio (MRR)			Sex	Country	Reference
			All	Male	Female			
No vaccine information or independent of vaccine type	200 kIU VAS vs placebo twice, 12–72 mo age, (6 mo interval). FU: 12 mo	Not reported	0.74 (0.85–0.99)	0.59 (0.39–0.88)	0.92 (0.59–1.41)	Sex#VAS: NA	Indonesia	Sommer 1986[55]
	100 kIU/200kIU vs placebo, aged 6–72 mo. FU: 12 mo	Not reported	0.70 (0.56–0.88)	0.77 (0.55–1.09)	0.65 (0.48–0.89)	Sex#VAS: NA	Nepal	West 1991[56]
	50/100/200 kIU vs placebo, aged 1–59 mo. FU: 5 mo	Not reported	0.74 (0.55–0.99)	0.72 (0.48–1.08)	0.76 (0.48–1.19)	Sex#VAS: NA	Nepal	Daulaire 1992[57]
	200 kIU vs placebo, aged 9–72 mo. FU: 18 mo	Not reported	1.06 (0.82–1.37)	1.25 (0.85–1.83)	0.93 (0.66–1.31)	Sex#VAS: NA	Sudan	Herrera 1992[58]
	100/200 kIU vs placebo, aged 6–90 mo. FU: 2 yr	Not reported. Low vaccine coverage	0.81 (0.68–0.98)	0.73 (0.59–0.92)	0.90 (0.71–1.15)	Sex#VAS: p=0.3	Ghana	VAST 1993[59]
	100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo. FU: 6 mo	Irrespective of vaccine	0.91 (0.59–1.41)	1.92 (0.98–3.75)	0.45 (0.24–0.87)	Sex#VAS: p=0.003	Guinea-Bissau	Fisker 2014[31]
	100 kIU/200 kIU vs placebo with missing vaccines, aged 6–90 mo. FU: 2 yr	Not vaccinated / Vaccinated (any vaccine)	0.64 (0.47–0.88) / 0.95 (0.72–1.26)	0.68 (0.47–0.99) / 0.74 (0.51–1.08)	0.60 (0.40–0.92) / 1.18 (0.84–1.67)	Vaccinated#VAS: male: p=0.78, female: p=0.009; Vaccinated: Sex#VAS: p=0.046	Ghana	Benn 2009[25]
DTP	100 kIU/200 kIU with missing vaccine, aged 6–60 mo. FU: aged 18 mo	VAS in campaign vs no VAS	1.11 (0.59–2.08)	NA	NA	Sex#VAS: NA	Guinea-Bissau	Benn 2009[26]
		VAS+DTP vs nothing	3.04 (1.31–7.07)	NA	NA	Sex#VAS: NA		
		All VAS: DTP vs no DTP	3.43 (1.36–8.61)	4.71 (1.37–16)	2.21 (0.52–9.44)	Sex#VAS: NA		
		All VAS: MV vs DTP. Vaccine#VAS: p=0.0005	Deaths: 0/116 among MV; 8/136 among DTP			Vaccine#VAS: male: p=0.007, female: p=0.03		
DTP	100 kIU/200 kIU VAS in campaign vs not in campaign, aged 6–35 mo. FU: aged 3 yr	DTP last vaccine	1.29 (0.52–3.22)	1.37 (0.39–4.93)	1.21 (0.33–4.43)	Sex#VAS: NS	Guinea-Bissau	Fisker 2012[29]
	100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo. FU: 6 mo	Given with DTP	1.06 (0.51–2.20)	2.16 (0.67–7.03)	0.61 (0.22–1.69)	Sex#VAS: p=0.11	Guinea-Bissau	Fisker 2014[31]
MV	100 kIU VAS vs placebo, aged 6mo and/ or 9 mo. FU: aged 18 mo	Given with MV	0.46 (0.14–1.47)	NA	N/A	NA	Guinea-Bissau	Benn 2003[30]
		Given with MV	1.17 (0.84–1.64)	0.69 (0.41–1.16)	1.75 (1.11–2.76)	Sex#VAS: p=0.009	Ghana	

	Intervention	By intervention				Interaction	Country	Reference
	100 kIU/200 kIU vs placebo with missing vaccines, aged 6–90 mo. FU: 2 yr	Given with MV, incomplete DTP. Likely to receive DTP after MV	NA	0.50 (0.25–1.02)	2.60 (1.41–4.80)	Sex#VAS: p=0.0004		Benn 2009[25]
		Given with MV, complete DTP. Unlikely to receive DTP after MV	NA	0.90 (0.45–1.81)	1.09 (0.54–2.20)	Sex#VAS: p=0.704		
	100 kIU/200 kIU VAS in campaign vs not in campaign, aged 6–35 mo. FU: aged 3 yr	MV last vaccine. Vaccine type (DTP/MV)#VAS: p=0.04	0.34 (0.14–0.85)	0.31 (0.10–0.96)	0.41 (0.09–1.85)	Sex#VAS: NS	Guinea-Bissau	Fisker 2012[29]
	100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo. FU: 6 mo	Given with MV Given with MV+DTP	1.29 (0.51–3.28) 0.52 (0.22–1.21)	2.40 (0.62–9.29) 1.66 (0.47–5.89)	0.62 (0.15–2.61) 0.16 (0.04–0.73)	Sex#VAS: p=0.18 Sex#VAS: p=0.02	Guinea-Bissau	Fisker 2014[31]
	100 kIU/200 kIU VAS vs placebo, aged 6 mo. FU: aged 9 mo	Given with MV or IPV. Vaccine#VAS: p=0.02	Deaths: Among MV: 0/78 in VAS, 3/72 in placebo; among IPV: 3/76 in VAS, 0/74 in placebo			NA	Guinea-Bissau	Benn 2003[30]
Repeated VAS	100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo, FU: 6 mo	Previous VAS No previous VAS	0.80 (0.41–1.58) 0.99 (0.57–1.77)	5.98 (1.34–26.7) 1.19 (0.53–2.65)	0.18 (0.05–0.62) 0.82 (0.35–1.89)	Sex#VAS: p=0.0004 Sex#VAS: p=0.53; Sex#prev VAS#VAS: p=0.007	Guinea-Bissau	Fisker 2014[31]
	100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo, FU: 6 mo	VAS in previous campaign vs no VAS	0.34 (0.14–0.80)	0.59 (0.20–1.77)	0.14 (0.03–0.68)	Sex#VAS: p=0.14	Guinea-Bissau	Fisker 2012[29]

The same paper may appear in more than one row. The column 'By intervention' refers to the health intervention status of the participants at the time of randomization or follow-up; usually vaccination status or other vitamin A supplements. #: interaction analysis; 50 kIU: 50 000 international units of retinol; DTP: diphtheria-tetanus-whole cell pertussis; FU: follow-up; FU-VAS: VAS at follow-up; IPV: inactivated polio vaccine; mo: month(s); MRR: mortality rate ratio; MV: measles vaccination; NA: not analyzed; NS: not significant; NVAS: neonatal VAS; VAS: vitamin A supplementation; wk: weeks(s); yr: year(s).

neonatal VAS administered together with BCG versus placebo and BCG. Neonatal VAS, however, did not modify the association between OPV and mortality; the combined effect of VAS and sex was not addressed, probably prohibited by the limited sample size.[32]

Effect of different doses of VAS in early childhood

The dose of VAS recommended by WHO is 100 000 IU for children <1 year of age, and 200 000 IU for children above 1 year of age. This recommendation is somewhat arbitrary,[3] but has been tested in a few studies. Taking advantage of a combined OPV and VAS national campaign in Guinea-Bissau, almost 5000 children between 6 months and 5 years of age were randomized to receive the recommended dose, or half the recommended dose. Overall, the study indicated improved survival in the lower dose group at 6-months follow-up (MRR 0.69 [0.36–1.35]). In females, the association was significant (MRR 0.19 [0.06–0.66]), and the interaction between dose and sex was significant as well (p=0.004), suggesting that females benefit more from this intervention. At 9-months follow-up, the effects were still significant, albeit less so.[33] Two subsequent trials, one in neonates[22] and the other in infants aged 6–59 months[34] did not find any difference in mortality at 12-months follow-up between the standard dose (50 000 IU for infants <6 months; 100 000 IU for infants 6–12 months; 200 000 IU for infants ≥12 months) and the halved dose, overall or by sex.

Effect of repeated doses of VAS on all-cause mortality

A study comparing the different size of VAS dose in children aged 6–59 months in Guinea-Bissau, of whom a large fraction had previously participated in a trial of neonatal VAS,[15] did not find any significant interaction between dose of VAS (standard versus halved dose) and previous neonatal VAS, overall or in either sex; the MRR of low-dose versus high-dose campaign VAS was 1.50 (0.27–8.18) for those having previously received neonatal VAS and 0.18 (0.02–1.52) for neonatal placebo recipients.[34]

A subsequent study in Guinea-Bissau of a second VAS dose to children previously enrolled in a trial of neonatal VAS found that females who had received neonatal VAS benefited significantly from receiving high-dose VAS at age 1 year compared to females who received placebo at birth. This inferred a significant interaction between neonatal VAS and subsequent VAS in females (p=0.009), but not in males.[35] An observational study was conducted in children 6–35 months of age living in an area of Guinea-Bissau targeted for consecutive VAS campaigns over 2 years. Those that participated in a prior VAS campaign had lower mortality after a second VAS campaign compared with participants in the second campaign only (MRR 0.34 [0.14–0.80]), the association being significant in females but not in males (interaction between repeated VAS and sex: p=0.14).[29] These results suggest an immunological priming effect of neonatal VAS for the response to a secondary dose, and would imply that previous supplements should be considered in studies of VAS. The findings further imply a sex-differential priming effect, perhaps

pertaining to differences in the early state of the immune system in males and females. Of note, the effect of VAS may depend on the vitamin A status. A differential effect of VAS on cellular responses to vaccine antigens was found in Bangladeshi children, as VAS increased the frequency of responders among children with serum retinol levels ≥0.7 μmol/l (considered adequate) at the time of testing, but had no effect in children with low serum retinol levels.[36] Such differences in serum retinol levels, however, may not explain the effect of previous VAS, as the effects of VAS on serum retinol levels are only modest and rapidly wane.[37]

Effects of VAS on morbidity

A few studies have investigated the effect of VAS on morbidity in respect to sex. A trial in South Indian pre-school children receiving repeated high-dose VAS every 4 months found no benefit of VAS on 12 morbidity indicators (including incidence, number of days ill, and duration of respiratory illness, diarrhoea, skin infection and measles infection) during the 1-year follow-up, but a longer duration of diarrhoea episodes in females.[38]

There was no effect of neonatal VAS on the measles incidence rate at 12-months follow-up during a measles epidemic in Guinea-Bissau among MV-naïve infants. However, before age 6 months, VAS tended to protect males from measles, but increased the risk in females (interaction between VAS and sex: p=0.04). The same interaction between VAS and sex was observed for severe measles cases leading to hospitalization or death, although it was not significant (p=0.08).[39]

In Guinean infants <6 months of age, rotavirus infection and diarrhoea were more frequent in neonatal VAS recipients, with no difference by sex. The overall incidence rate ratio (IRR) for rotavirus infection was 1.72 (1.04–2.85), and 3.74 (1.40–9.98) for diarrhoea.[14] Also in Guinea-Bissau, VAS was associated with a lower incidence of non-rotavirus infections in males <6 months (IRR 0.51 [0.27–0.95]), with no difference for <6-month-old females; while above 6 months, VAS tended to cause a higher incidence in females, with no effect in males (interaction between VAS and sex: p=0.03).[40] In Indonesian pre-school children, VAS was associated with a significantly increased incidence of acute lower respiratory illness in females, but not in males (p=0.09 for interaction between sex and VAS).[41] In Indian 2–24 months old children admitted to hospital, there was no effect overall or by sex of a double dose of 30 000 IU VAS for 4 consecutive days on recovery rate from severe acute lower respiratory infections.[42]

Effect of VAS on immunological responses to vaccines

Antibody responses to vaccines

Savy et al. have comprehensively reviewed the literature addressing interactions between micronutrient status or supplementation and vaccine responses.[43] Overall, the review found no evidence for an effect of VAS on specific responses to diphtheria and tetanus, cholera, influenza, Haemophilus influenzae type b and pneumococcal vaccines. For antibody responses to MV, the review concluded that VAS may reduce seroconversion rates in children with high baseline antibody levels, but have no effect in those with low baseline antibody levels; and there is a suggestion

that VAS impairs specific responses to BCG measured by skin test, particularly in males.[43] There is, however, an almost complete lack of studies reporting the effects by sex. One exception is a study on the effect of VAS versus placebo together with MV at 6 and/or 9 months of age on measles-specific seroconversion rate in Guinea-Bissau. There was no overall effect on the antibody titres or seroconversion rate in infants receiving MV at 6 and 9 months; but for children receiving only a single MV at 9 months, VAS had an antibody enhancing effect in males, but not in females, leading to a significant interaction between VAS and sex (p=0.02). It should be noted, however, that the seroconversion rates were >90% in all subgroups.[44] When the infants were revisited at 6–8 years of age, the effect of VAS had waned as the frequency of protective titres was high in both supplemented and non-supplemented children, and sex differences were no longer apparent.[45]

Cellular responses to vaccines

The available data support sex differences in the effect of VAS on the cell-mediated immune response to BCG vaccination. At age 2 months in Guinea-Bissau, neonatal VAS together with BCG was associated with a lower prevalence of tuberculin skin test responders in males (prevalence ratio 0.79 [0.67–0.92]), but no such effect in females. The association was transient and gone by 6 months. At 6 weeks, VAS was associated with significantly higher interferon-γ responses to purified protein derivative (PPD) in males (geometric mean ratio [GMR] 1.07 {1.00–1.15}), but not in females; however, subtracting the baseline responses of non-stimulated cells, there was no sex-difference, but the overall effect of VAS remained significant (stimulation index 1.40 [1.03–1.91]).[46]

In Guinean infants, cytokine responses were sex-differentially affected 6 weeks after randomization to neonatal VAS. Baseline tumour necrosis factor (TNF) 24 hour-production by unstimulated cells (medium alone) was significantly lower in female VAS-recipients compared to non-supplemented females (GMR 0.41 [0.26–0.72]), with no significant difference in males. The TNF responses to lipopolysaccharide were lower in VAS males (GMR 0.68 [0.47–1.00]), but not females. Moreover, DTP-vaccinated VAS males had lower TNF and interleukin (IL)-10 released in unstimulated cells, as did non-DTP vaccinated VAS females, resulting in a significant three-way interaction between VAS, sex and DTP (p=0.03 and p=0.04 for TNF and IL-10, respectively). The VAS DTP-vaccinated males also had a higher IL-5 response to phytohaemagglutinin (PHA), and higher IL-13 to PPD suggesting enhanced type 2 immunity.[47] Albeit the study was exploratory, the findings support a sex-differential interaction between VAS and DTP.

By combining data from two RCTs in Guinea-Bissau, early MV and neonatal VAS, neonatal VAS was associated with a differential effect of early MV on immunological markers. Among VAS recipients, MV females but not males had increased plasma IL-1 receptor agonist and IL-8, an effect not present in infants without neonatal VAS. The MV group had a higher ratio of in vitro TNF:IL-10 to antigenic stimulation among children who had received neonatal VAS, with the opposite effect in non-VAS recipients; for PHA stimulation, the interaction between VAS and MV was p=0.02.[48]

The study suggested long-term immunomodulation by neonatal VAS with effects sustained at least up to 5 months after administration.

Neonatal VAS was associated with increased monocytes at 6 weeks of age in females, but not in males, and only among those who received DTP before follow-up, but not in the DTP non-vaccinated. Lymphocytes, granulocytes or eosinophils were not significantly affected.[47] There was no overall or sex-differential effect of VAS at 9 months of age on CD4 or CD8 T cell counts or the ratio between the two at 18 months of age if children had already received VAS at 6 months.[49] In summary, VAS may modify heterologous immunological effects of vaccines, in a sex-specific manner, although current evidence is too limited to infer mechanistic hypotheses.

Conclusions

The literature is inconsistent as to the effect of VAS on morbidity and mortality and reactivity to vaccines in early childhood. Some of the variation may be explained by factors that could modify the effect of VAS, and which may differ between the studied populations. These include nutritional factors, for example VAS in Indonesia enhanced the incidence of acute lower respiratory infections particularly in children with normal growth status, but less so in stunted children,[41] and the effect of VAS on cellular responses was differential in Bangladeshi children with adequate versus non-adequate serum retinol levels.[36] Vaccination coverage may play a role since VAS effects seem to depend on vaccination status;[50] or perhaps even genetic background, for example the effect of VAS on cytokine responses was only evident in homozygotic carriers of the G allele at the TNF-α locus -238.[51] Moreover, a number of the trials are observational in nature and therefore prone to many confounding factors. Despite these caveats a series of sex-specific differences have been described, mostly, but not exclusively supporting a more beneficial effect of VAS in males than females (Figure 1). Hence, a null-effect may comprise differential effects in opposite directions in males and females, or perhaps vaccinated and un-vaccinated groups. Unfortunately, data analysis by sex is not yet commonplace in study design and dissemination.[52] Many of the studies come from the same group in Guinea-Bissau, and there is an urgent need to extend these studies to other populations and geographical settings. In order to investigate this issue further, WHO has commissioned three large RCTs of neonatal VAS on all-cause mortality in India, Ghana and Tanzania.[53] The studies will address potential effect-modifiers including sex and vaccines. Moreover, two smaller human immunological studies in The Gambia[54] and Bangladesh are underway. Results from these studies will soon be available, and hopefully will resolve the question of the benefits or risks of neonatal VAS.

References

1 Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. Nat Rev Immunol 2008;8:685–98.

2 WHO. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. Geneva: World Health Organization; 2009.

3 WHO. Guideline: Vitamin A supplementation in infants and children 6–59 months of age. Geneva: World Health Organization; 2011.

4 Imdad A, Herzer K, Mayo-Wilson E et al. Vitamin A supplementation for preventing morbidity and mortality in children from 6 months to 5 years of age. Cochrane Database Syst Rev 2010:CD008524.

5 Beaton GH, Martorell R, Aronson KJ et al. Effectiveness of Vitamin A Supplementation in the control of young child morbidity and mortality in developing countries − Nutrition policy discussion paper No. 13. Toronto: University of Toronto; 1993.

6 WHO. Guideline: Neonatal vitamin A supplementation Geneva: World Health Organization; 2011.

7 Kirkwood B, Humphrey J, Moulton L, Martines J. Neonatal vitamin A supplementation and infant survival. Lancet 2010;376:1643–4.

8 Haider BA, Bhutta ZA. Neonatal vitamin A supplementation for the prevention of mortality and morbidity in term neonates in developing countries. Cochrane Database Syst Rev 2011:CD006980.

9 Gogia S, Sachdev HS. Vitamin A supplementation for the prevention of morbidity and mortality in infants six months of age or less. Cochrane Database Syst Rev 2011:CD007480.

10 Klemm RD, Labrique AB, Christian P et al. Newborn vitamin A supplementation reduced infant mortality in rural Bangladesh. Pediatrics 2008;122:e242–50.

11 Rahmathullah L, Tielsch JM, Thulasiraj RD et al. Impact of supplementing newborn infants with vitamin A on early infant mortality: community based randomised trial in southern India. BMJ 2003;327:254.

12 Humphrey JH, Agoestina T, Wu L et al. Impact of neonatal vitamin A supplementation on infant morbidity and mortality. J Pediatr 1996;128:489–96.

13 West KP Jr, Katz J, Shrestha SR et al. Mortality of infants < 6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. Am J Clin Nutr 1995;62:143–8.

14 Benn CS, Diness BR, Roth A et al. Effect of 50,000 IU vitamin A given with BCG vaccine on mortality in infants in Guinea-Bissau: randomised placebo controlled trial. BMJ 2008;336:1416–20.

15 Benn CS, Fisker AB, Napirna BM et al. Vitamin A supplementation and BCG vaccination at birth in low birthweight neonates: two by two factorial randomised controlled trial. BMJ 2010;340:c1101.

16 Malaba LC, Iliff PJ, Nathoo KJ et al. Effect of postpartum maternal or neonatal vitamin A supplementation on infant mortality among infants born to HIV-negative mothers in Zimbabwe. Am J Clin Nutr 2005;81:454–60.

17 Randomised trial to assess benefits and safety of vitamin A supplementation linked to immunisation in early infancy. WHO/CHD Immunisation-Linked Vitamin A Supplementation Study Group. Lancet 1998;352:1257–63.

18 Flanagan KL, van Crevel R, Curtis N et al. Heterologous ('nonspecific') and sex-differential effects of vaccines: epidemiology, clinical trials, and emerging immunologic mechanisms. Clin Infect Dis 2013; 57:283–9.

19 WHO. Meeting of the Stategic Advisory Group of Experts on immunization, April 2014 - conclusions and recommendations. Wkly Epidemiol Rec 2014;89:221–36.

20 Aaby P, Benn C, Nielsen J et al. Testing the hypothesis that diphtheria-tetanus-pertussis vaccine has negative non-specific and sex-differential effects on child survival in high-mortality countries. BMJ Open 2012;2:e000707.

21 Flanagan KL, Klein SL, Skakkebaek NE et al. Sex differences in the vaccine-specific and non-targeted effects of vaccines. Vaccine 2011;29:2349–54.

22 Benn CS, Diness BR, Balde I et al. Two different doses of supplemental vitamin A did not affect mortality of normal-birth-weight neonates in Guinea-Bissau in a randomized controlled trial. J Nutr 2014;144:1474–9.

23 Roth AE, Benn CS, Ravn H et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. BMJ 2010;340:c671.

24 Benn CS, Rodrigues A, Yazdanbakhsh M et al. The effect of high-dose vitamin A supplementation administered with BCG vaccine at birth may be modified by subsequent DTP vaccination. Vaccine 2009;27:2891–8.

25 Benn CS, Aaby P, Nielsen J et al. Does vitamin A supplementation interact with routine vaccinations? An analysis of the Ghana Vitamin A Supplementation Trial. Am J Clin Nutr 2009;90:629–39.

26 Benn CS, Martins C, Rodrigues A et al. The effect of vitamin A supplementation administered with missing vaccines during national immunization days in Guinea-Bissau. Int J Epidemiol 2009;38:304–11.

27 Aaby P, Martins CL, Garly ML et al. Non-specific effects of standard measles vaccine at 4.5 and 9 months of age on childhood mortality: randomised controlled trial. BMJ 2010;341:c6495.

28 Benn CS, Martins CL, Fisker AB et al. Interaction between neonatal vitamin A supplementation and timing of measles vaccination: a retrospective analysis of three randomized trials from Guinea-Bissau. Vaccine 2014;32:5468–74.

29 Fisker AB, Aaby P, Bale C et al. Does the effect of vitamin A supplements depend on vaccination status? An observational study from Guinea-Bissau. BMJ Open 2012;2:e000448.

30 Benn CS, Bale C, Sommerfelt H et al. Hypothesis: Vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines? Int J Epidemiol 2003;32:822–8.

31 Fisker AB, Bale C, Rodrigues A et al. High-dose vitamin A with vaccination after 6 months of age: a randomized trial. Pediatrics 2014;134:e739–48.

32 Benn CS, Fisker AB, Rodrigues A et al. Sex-differential effect on infant mortality of oral polio vaccine administered with BCG at birth in Guinea-Bissau. A natural experiment. PLoS One 2008;3:e4056.

33 Benn CS, Martins C, Rodrigues A et al. Randomised study of effect of different doses of vitamin A on childhood morbidity and mortality. BMJ 2005;331:1428–32.

34 Yakymenko D, Benn CS, Martins C et al. The impact of different doses of vitamin A supplementation on male and female mortality. A randomised trial from Guinea-Bissau. BMC Pediatr 2011;11:77.

35 Fisker AB, Aaby P, Rodrigues A et al. Vitamin A supplementation at birth might prime the response to subsequent vitamin A supplements in girls. Three year follow-up of a randomized trial. PLoS One 2011;6:e23265.

36 Rahman MM, Mahalanabis D, Alvarez JO et al. Effect of early vitamin A supplementation on cell-mediated immunity in infants younger than 6 mo. Am J Clin Nutr 1997;65:144–8.

37 Sommer A, West KP Jr. Impact of vitamin A on childhood mortality. Indian J Pediatr 1987;54:461–3.

38 Ramakrishnan U, Latham MC, Abel R et al. Vitamin A supplementation and morbidity among preschool children in south India. Am J Clin Nutr 1995;61:1295–303.

39 Diness BR, Martins CL, Bale C et al. The effect of high-dose vitamin A supplementation at birth on measles incidence during the first 12 months of life in boys and girls: an unplanned study within a randomised trial. Br J Nutr 2011;105:1819–22.

40 Diness BR, Christoffersen D, Pedersen UB et al. The effect of high-dose vitamin A supplementation given with bacille Calmette-Guerin vaccine at birth on infant rotavirus infection and diarrhea: a randomized prospective study from Guinea-Bissau. J Infect Dis 2010;202 Suppl:S243–51.

41 Dibley MJ, Sadjimin T, Kjolhede CL, Moulton LH. Vitamin A supplementation fails to reduce incidence of acute respiratory illness and diarrhea in preschool-age Indonesian children. J Nutr 1996; 126:434–42.

42 Mahalanabis D, Lahiri M, Paul D et al. Randomized, double-blind, placebo-controlled clinical trial of the efficacy of treatment with zinc or vitamin A in infants and young children with severe acute lower respiratory infection. Am J Clin Nutr 2004;79:430–6.

43 Savy M, Edmond K, Fine PE et al. Landscape analysis of interactions between nutrition and vaccine responses in children. J Nutr 2009;139:2154S-218S.

44 Benn CS, Aaby P, Bale C et al. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, west Africa. Lancet 1997;350:101–5.

45 Benn CS, Balde A, George E et al. Effect of vitamin A supplementation on measles-specific antibody levels in Guinea-Bissau. Lancet 2002; 359:1313–4.

46 Diness BR, Fisker AB, Roth A et al. Effect of high-dose vitamin A supplementation on the immune response to Bacille Calmette-Guerin vaccine. Am J Clin Nutr 2007;86:1152–9.

47 Jorgensen MJ, Fisker AB, Sartono E et al. The effect of at-birth vitamin A supplementation on differential leucocyte counts and in vitro cytokine production: an immunological study nested within a randomised trial in Guinea-Bissau. Br J Nutr 2013;109:467–77.

48 Jensen KJ, Sondergaard M, Andersen A et al. A randomized trial of an early measles vaccine at 4(1/2) months of age in Guinea-bissau: sex-differential immunological effects. PLoS One 2014;9:e97536.

49 Benn CS, Lisse IM, Bale C et al. No strong long-term effect of vitamin A supplementation in infancy on CD4 and CD8 T-cell subsets. A community study from Guinea-Bissau, West Africa. Ann Trop Paediatr 2000;20:259–64.

50 Benn CS, Fisker AB, Jorgensen MJ, Aaby P. Conflicting evidence for neonatal vitamin A supplementation. Vaccine 2008;26:4111–2.

51 Jorgensen MJ, Fisker AB, Erikstrup C et al. SNP may modify the effect of vitamin A supplementation at birth on cytokine production in a whole blood culture assay. Br J Nutr 2012;107:615–20.

52 Flanagan KL. Sexual dimorphism in biomedical research: a call to analyse by sex. Trans R Soc Trop Med Hyg 2014;108:385–7.

53 Group NSA, Bahl R, Bhandari N et al. Efficacy of early neonatal vitamin A supplementation in reducing mortality during infancy in Ghana, India and Tanzania: study protocol for a randomized controlled trial. Trials 2012;13:22.

54 McDonald SL, Savy M, Fulford AJ et al. A double blind randomized controlled trial in neonates to determine the effect of vitamin A supplementation on immune responses: The Gambia protocol. BMC Pediatr 2014;14:92.

55 Sommer A, Tarwotjo I, Djunaedi E et al. Impact of vitamin A supplementation on childhood mortality. A randomised controlled community trial. Lancet 1986;1:1169–73.

56 West KP Jr, Pokhrel RP, Katz J et al. Efficacy of vitamin A in reducing preschool child mortality in Nepal. Lancet 1991;338:67–71.

57 Daulaire NM, Starbuck ES, Houston RM et al. Childhood mortality after a high dose of vitamin A in a high risk population. BMJ 1992; 304:207–10.

58 Herrera MG, Nestel P, el Amin A et al. Vitamin A supplementation and child survival. Lancet 1992;340:267–71.

59 Ghana VAST Study Team. Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. Lancet 1993;342:7–12.